RESPONSE OF MAIZE TO PHOSPHORUS AND NITROGEN FERTILIZERS ON A SOIL WITH LOW PHOSPHORUS STATUS

by

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(2006008508)

Submitted in partial fulfilment for the requirements

of the degree Magister Scientiae Agriculturae

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BLOEMFONTEIN

2013

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ABSTRACT

Maize (*Zea mays* L.) is an important cereal crop not only in the world but more specifically in South Africa. Therefore, understanding maize's nutrient requirement becomes an importance factor especially during the vegetative growth period. Nitrogen (N) and phosphorus (P) are reported to be two essential nutrients for both accelerated vegetative growth and maximum yield. Addition of these two plant nutrients should include consideration of both form and total nutrient concentration, since these two factors determine availability and accessibility.

In order to evaluate the response of maize to P sources and P application rates as well as N sources a glasshouse experiment was conducted in 40.5 L pots filled with a dark brown sandy-loam topsoil pertaining a medium soil pH of 5.5. Treatments consisted of three main factor treatments *viz.* N source (urea and limestone ammonium nitrate - LAN), P source (monoammonium phosphate - MAP, nitrophosphate - NP and ammonium polyphosphate - APP) and P application rate (0, 10, 20, 30 and 40 kg P ha^{-1}). Treatments combinations were replicated three times and independently subjected to a randomized complete block design with a factorial combination. The experiment was repeated on two planting dates. Treatments and treatment combinations were band applied to dry soil in a single 0.34 m line, 50 mm below and 50 mm away from the maize seeds; which were planted with a between row spacing of 0.91 m, 50 mm below the soil surface. After planting the soil was watered and maintained at field capacity for a duration of five weeks after emergence. The aerial parameters of three plants per pot were measured on a weekly basis following emergence while the subsoil parameters were taken at the end of the five week vegetative growing period.

Both aerial and subsoil parameters showed responses to nitrogen source; which was strongly reflected during both plantings. Plants treated with LAN yielded both greater aerial and subsoil measurements compared to urea, primarily ascribed to immediate availability after application in addition to ease in uptake. Both aerial and subsoil parameter response to phosphorus source and P application rate, though apparent throughout both plantings, was more prominent during the first planting. Monoammonium phosphate and NP (orthophosphate sources) yielded greater aerial measurements compared to that of the APP (polyphosphate source). Subsoil parameter results comparing phosphorus sources were inconsistent. Subsoil parameters of the fertilized zone were significantly greater with the use of MAP (orthophosphate), while APP (polyphosphate) yielded significant greater subsoil parameters within the unfertilized

zone. Both aerial and subsoil parameter measurements taken either throughout or at the end of the vegetative growth period were overall significantly greater when P was applied at 40 kg P ha^{-1}. Subsoil parameter response in and away from the fertilizer band was however inconsistent.

The aerial dry plant material was analyzed (Omnia Nutriology®) to evaluate the effect of the three main treatments on the quantitative nutrient concentration as well as the uptake thereof. Nutrient concentration and uptake was used to determine the synergistic or antagonistic effect of treatments or treatment combinations.

Nutrient concentration measurements were inconsistent for N source, however total uptake proved to be more efficient with the application of LAN compared to urea. Both nutrient concentration and uptake was greater with the application of both the orthophosphate sources (MAP and NP) compared to the polyphosphate source (APP). The 40 kg P ha^{-1} application yielded a synergistic response to the total uptake of S, N, P, Ca and B, while a synergistic nutrient concentration response was found with the control treatment for N, Mg, Cu and Zn nutrients. Nutrient uptake was also stimulated by an increasing rate of P.

UITTREKSEL

Mielies (*Zea mays* L.) is 'n belangrike graangewas in die wêreld, maar meer spesifiek in Suid-Afrika. Daarom is dit belangrik om mielies se voedingsbehoefte te verstaan, veral gedurende die vegetatiewe groeiperiode. Stikstof (N) sowel as fosfor (P) word beskou as twee noodsaaklike nutriënte vir versnelde vegetatiewe groei, sowel as vir maksimum opbrengs. Wanneer hierdie twee nutriënte in 'n bemestingsprogram ingesluit word, moet beide vorm én die totale nutriëntkonsentrasie in ag geneem word, aangesien bogenoemde twee faktore die beskikbaarheid en toeganklikheid van nutriënte bepaal.

Ten einde die reaksie van mielies op P-bronne, P-toedieningspeile sowel as N-bronne te evalueer is 'n glashuisproef met 40.5 L potte uitgevoer. Potte is gevul met 'n donkerbruin sandleem bogrond met 'n medium pH van 5.5. Behandelings het bestaan uit drie hooffaktore nl. N-bronne (ureum en kalksteen ammonium nitraat - KAN), P-bronne (monoammoniumfosfaat - MAP, nitrofosfaat - NP en ammoniumpolifosfaat - APP) en P-toedieningspeile (0, 10, 20, 30 en 40 kg P ha^{-1}). Die proef is uitgelê as 'n volledig ewekansige blokontwerp met 'n faktoriaalreëling. Elke behandelingskombinasie is drie keer herhaal en die proef is twee keer herhaal. Behandelings en behandelingskombinasies is in 'n enkelry van 0.34 m lank, 50 mm onder en 50 mm weg van die mieliesade in droë grond gebandplaas. Die tussenry spasiëring was 0.91 m en die mieliesade is op 'n diepte van 50 mm geplant. Na plant is die grond natgemaak en by veldkapasiteit vir die tydperk van die proef (vyf weke na opkoms) gehou. Die bogrondse plantparameters van drie plante per pot is vanaf een week na opkoms op 'n weeklikse basis gemeet vir vyf weke. Ondergrondse plantparameters is aan die einde van die vyfweek vegetatiewe groeiperiode geneem.

Beide die bo- en ondergrondse plantparameters het gereageer op die N-bronne vir beide aanplantings. Bo- en ondergrondse parameters het beter op KAN as ureum gereageer. Laasgenoemde word primêr aan KAN se onmiddelike beskikbaarheid na toediening, sowel as die gemak in opneembaarheid daarvan toegeskryf. Beide bo- en ondergrondse parameters se reaksie op P-bronne en -toedieningspeile was meer sigbaar tydens die eerste plant datum.

Beide MAP en NP ortofosfaatbronne het betekenisvol beter resultate vir bogrondse plantparameters in vergelyking met die van APP (polifosfaat) gelewer. Fosfaatbronresultate van ondergrondse plantparameters se reaksie op P-bronne was deurgaans onkonsekwent. Ondergrondse plantparameterresultate van die bemeste wortelsone het beter op MAP (ortofosfaat) gereageer, terwyl APP (polifosfaat) beter

resultate in die onbemeste wortelsone gelewer het. Beide bo- en ondergrondse plantparameters het die beste resultate gelewer met die hoogste P-toedieningspeil van 40 kg P ha^{-1}. Ondergrondse parameterreaksies in en weg van die bemestingsband was nie konsekwent.

Die droë bogrondse plantmateriaal is ontleed (Omnia Nutriology®) om die kwantitatiewe nutriëntkonsentrasie, sowel as die opname te evalueer. Die nutriëntkonsentrasie en –opname is ook gebruik om die sinergistiese of antagonistiese effek van die behandelings of behandelingskombinansies te bepaal.

Nutriëntkonsentrasie in die plant het nie konsekwent op N-bronne gereageer nie, alhoewel die totale opname meer doeltreffend was vir KAN in vergelyking met ureum. Beide die nutriëntkonsentrasie en –opneembaarheid was beter met die toediening van enige van die ortofosfaatbronne (MAP en NP) in vergelyking met die polifosfaatbron (APP). Die 40 kg P ha^{-1} toediening het 'n sinergistiese reaksie tot die totale opneembaarheid van S, N, P, Ca en B gelewer, terwyl 'n sinergistiese nutriëntkonsentrasiereaksie gevind is met die kontrolebehandeling vir N, Mg, Cu en Zn. Nutrientopname is ook deur 'n verhoging in P-toedieningspeile gestimuleer.

Sleutelwoorde: ortofosfate, polifosfate, plantontwikkelingsparameters, makro- en mikronutriënte, opneembaarheid, konsentrasie, blaargetal, stam dikte, planthooghte, droëmassa, wortels

DECLARATION

I declare that this dissertation, hereby submitted for the Magister Scientiae Agriculturae degree at the University of the Free State, is my own independent work and has not previously been submitted to any other University. I furthermore cede copyright of this dissertation in favour of the University of the Free State.

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Pieter-Ernst Coetzee   Date

ACKNOWLEDGEMENTS

Ephesians 3:20   Now to Him who is able to do immeasurably more than all we ask or imagine, according to His power that is at work within us, to Him be the glory in the church and in Christ Jesus throughout all generations, for ever and ever! Amen.

CHAPTER 1

INTRODUCTION

Maize (*Zea mays* L.) is an extremely important cereal crop throughout the world. Following wheat and rice, maize is ranked the third most important cereal crop in the world (Van Rensburg, 1994). In South Africa, maize is however ranked the number one cereal crop followed by wheat, sunflower, soya bean and sorghum (National Agro-meteorological Committee (NAC), 2012). Even though maize is produced throughout South Africa, it's mainly cultivated in the Free State, North West and Mpumalanga provinces (Division of Planning and Statistics, 1993). Unfortunately its cultivation is limited by biotic and abiotic factors, of which low soil fertility is but one. Inorganic fertilizers have generally been used to increase or maintain soil fertility and enhance maize yields with great success (Jones & Wendt, 1994). Nitrogen (N) and phosphorus (P) occur in different forms with varying soil and crop reactions. These differences could be used to the advantage of both crop production and food security.

Direct comparisons between P fertilizers are complicated due to the fact that fertilizer differ not only in formulation (solid or liquid), but also in chemical form (orthophosphate or polyphosphate) (Ottman *et al.*, 2005). Most fertilizers such as, phosphoric acid (PA), monoammonium phosphate (MAP), diammonium phosphate (DAP), triple superphosphate (TSP) and nitrophosphate (NP) contain P as orthophosphate. Once orthophosphates are dissolved in the soil, orthophosphate ions are readily available for plant uptake as either a primary orthophosphate ion ($H_2PO_4^-$ at a soil pH < 7.0) or a secondary orthophosphate ion (HPO_4^{2-} at a soil pH > 7.0) (Noack *et al.*, 2010).

Ammonium polyphosphate (APP) contains about half of the P as polyphosphates (chains of orthophosphates) and the other half as orthophosphate (Rehm *et al.*, 1998). Ammonium polyphosphate is water soluble and consequently hydrolyzes into the simpler orthophosphate form, given enough water (Robertson, 2004). The time required for polyphosphate hydrolysis, varies with soil temperature (Anonymous, 2008) as well as soil acidity (Robertson, 2004). Temperature has the greatest effect on increasing the rate of hydrolysis with the amount of hydrolysis being 42, 63, and 84% after 72 hours, respectively, at 5, 20, and 35°C. However, under cool and/or dry conditions, hydrolysis may take longer (Robertson, 2004). The different forms of P as well as soil reactions could ultimately influence crop (maize) response.

Nitrogen fertilization is an expensive but necessary input in any agricultural system. Nitrogen fertilization furthermore enables farmers to achieve high yields that drive modern agriculture (Brady & Weil, 2008). At least eleven forms of nitrogen fertilizers are currently available (Jensen, 2006). The four most commonly used N fertilizers are; ammonium nitrate (NH_4NO_3), urea ($CO(HN_2)_2$), anhydrous ammonia (NH_3) and ammonium sulphate ($(NH_4)_2SO_4$) (Jensen, 2006).

According to Brady and Weil (2008), plants principally absorb N as both dissolved nitrate (NO_3^-) and ammonium (NH_4^+) ions. Nitrate sources are available immediately after application if sufficient water is available. In contrast, ammonium sources must first be oxidized to nitrite (NO_2^-), and then again to nitrate (NO_3^-). However, various factors (dissolved oxygen, pH, salinity and temperature) affect the nitrification process (Myrold, 1998).

Khalil *et al.* (2004) reported that the transformation of ammonium into nitrite and nitrate via nitrification took at least 14 days. However this process may even take as long as 21 days. Khalil *et al.* (2004) furthermore concluded that the higher the ammonium concentration added to the soil, the higher the nitrite and nitrate concentrations following nitrification. Transformation rate alone do not affect fertilization effectivity but the consideration of application method is also very important.

Applying P in a band near the developing roots is most effective since phosphates generally move short distances from their point of placement. Phosphorus fixation is reduced when the extent of contact between the phosphate and the soil fixing particles is reduced (Havlin *et al.*, 1999; Lafond *et al.*, 2003; Bouma & Scott, 2006). However, whether N is broadcast or band applied depends on soil conditions, climatic conditions, the cultivated crop as well as the selected N source. Nitrogen and P mixtures have been found to be an effective fertilization practice (Brady & Weil, 2008).

Two studies by Duncan & Ohlrogge (1958) and Miller & Ohlrogge (1958) concluded that N fertilization increased the uptake of P when applied in the form of a band as a N and P mixture. They stated that increased uptake was due to more extensive root development within the band. A study by Robertson *et al.* (1954) furthermore found that there is a significant interaction effect between N and P.

Under South African conditions the question still remains which phosphorus source is more effective, orthophosphate or polyphosphate? The latter question prompted this study and the main objectives of this study are therefore to:

1. Evaluate the quantitative growth parameters of maize response to different phosphorus sources *viz*. MAP, NP and APP as well as phosphorus application levels *viz*. 0, 10, 20, 30 and 40 kg P ha^{-1}, during the early growth (first 5 weeks) of maize.

2. Evaluate the quantitative growth parameters of maize response to different nitrogen sources *viz*. limestone ammonium nitrate (LAN) and urea, during the early growth (first 5 weeks) of maize.

3. Evaluate the quantitative growth parameter response of maize to the interaction of phosphorus sources and phosphorus application level as delineated in objective one when applied to the different nitrogen sources as delineated in objective two, during the early growth (first 5 weeks) of maize.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The primary objective of this study was to determine the response of maize to different phosphorus (P) sources at varying P application levels and secondarily to establish how maize respond to the aforementioned treatments when applied in combination with different nitrogen (N) sources. Therefore, this literature review has been divided into two sections. The first sections (Section 2.2) will aim to provide an amended understanding as to how P react in the soil-plant system (main objective), whilst the second section (Section 2.3) aims to achieve the same as the aforementioned however, this time with N as focus (secondary objective).

2.2 Phosphorus in the soil-plant system

2.2.1 Introduction

More than ten decades ago P has been recognized as an important nutrient required for plant growth and was regarded as an indispensable component of crop technology (Relwani, 1961). In order to ensure sustainable and profitable agriculture that has a minimal impact on the environment (Richardson *et al.*, 2009), the application of P-based fertilizers is routinely used to overcome soil deficiencies and to maintain the productivity of agricultural systems. Phosphorus fertilizers are primarily applied in 'water-soluble' forms, such as superphosphate (Richardson *et al.*, 2009), while poorly soluble P fertilizers, such as rock phosphates, are generally less effective in promoting plant growth on most soils (Bolland *et al.*, 1997).

Phosphorus is; [1]involved in photosynthesis, [2]energy transfer, [3]cell division and enlargement, [4]root formation and growth, [5]improves fruit and vegetable quality, [6]vital to seed formation, [7]improves water use and [8]helps hasten maturity (Roberts, 2010). For production to be sustainable it is important that P removed from the soil is balanced by a plant available form of P input. This is not always the case as there is often a net export of soil P from production systems, where P is either not supplied at rates and in forms to balance P removal by plant products or simply not applied (McLaughlin *et al.*, 1991; Oehl *et al.*, 2002; Burkitt *et al.*, 2007). Above mentioned agronomic practices are unsustainable and can be associated with declining yields over time depending on soil type (Richardson *et al.*, 2009).

Different soil types make it difficult in pre-determining the quantity of P needed to grow a cereal crop throughout a given growing season, but in general P is only added in the early stages of plant establishment. However, potentially high yielding crops can become P-deficient later in the growing season (Gray, 1977) and may show signs of stunted growth, a shorter period for grain filling, a reduction in the number of fertile tillers followed by a reduction in grain yield (Batten *et al.*, 1986; Elliott *et al.,* 1997). Applying foliar P during the early growth stages can increase the number of fertile tillers (Elliot *et al.,* 1997; Grant *et al.*, 2001). As a plant progresses from the vegetative stage into the reproductive stage its P requirement increases accordingly (Gray, 1977; Batten *et al.,* 1986). When root growth ceases, the nutrients required for seed growth must be translocated via the leaves to the seeds (Williams, 1955; Gray, 1977). Applying P to the leaves may lead to a significant grain yield response but may also result in an early dry matter response (Silbertstein & Wittwer, 1951). The efficiency of a foliar P fertilizer is a function of the available leaf area. Fertilizer use efficiency (FUE) of foliar applied P could equate to 50% (or may even be lower). During the early vegetative growth stage (Scotford & Miller, 2004) when the surface cover is less than halve of what it should be at flowering (Hedley & McLaughlin, 2005).

Whether granular or liquid P fertilizer is the most economical to use is not clear, but the cost of liquid P fertilizers can be 44% greater than granular formulations (Meister, 2004). The application of liquid P fertilizers through irrigation water is less expensive in comparison to top-dressed granular fertilizers. Direct comparisons between granular and liquid fertilizers are complicated due to the fact that these fertilizers differ not only in formulation (solid or liquid), but also in the chemical form of P (orthophosphate or polyphosphate) (Ottman *et al.*, 2005). Fertilizers (N:P:K) such as liquid phosphoric acid (PA, 0:24:0) and granular fertilizers such as monoammonium phosphate (MAP, 11:22:0), diammonium phosphate (DAP, 18:20:0), and triple superphosphate (TSP, 0:20:0) contain P as orthophosphate. In contrast, ammonium polyphosphate (APP, 14:31:0), a common liquid P fertilizer, contains about half of the P as polyphosphates (chains of orthophosphates) and the other half as orthophosphate (Rehm *et al.*, 1998). Hence water solubility, formulation and chemical composition of P fertilizers should be considered when comparing the two P fertilizer forms (Ottman *et al.*, 2005).

2.2.2 Production of phosphates

The manufacture of almost all commercial phosphate fertilizers starts with the production of phosphoric acid. The manufacturing process of various P fertilizers involve various steps (Figure 2.1).

Phosphoric acid can be produced either by a dry or wet process. During the dry process the rock phosphate is treated in an electric furnace which produces a very pure jet of white phosphoric acid. Such phosphoric acids are primarily used in the food and chemical industry. Fertilizers that make use of these white phosphoric acids as the P-source are generally more expensive due to the costly treatment process (Rehm *et al.*, 2002). The wet process on the other hand involves treating the rock phosphate with sulphuric acid in the presence of water (Anonymous, 2009). This process produces phosphoric acid as well as gypsum which is removed as a by-product. Either wet and/or dry treatment processes produce orthophosphoric acid, the form of phosphate that is taken up by plants (Rehm *et al.*, 2002).
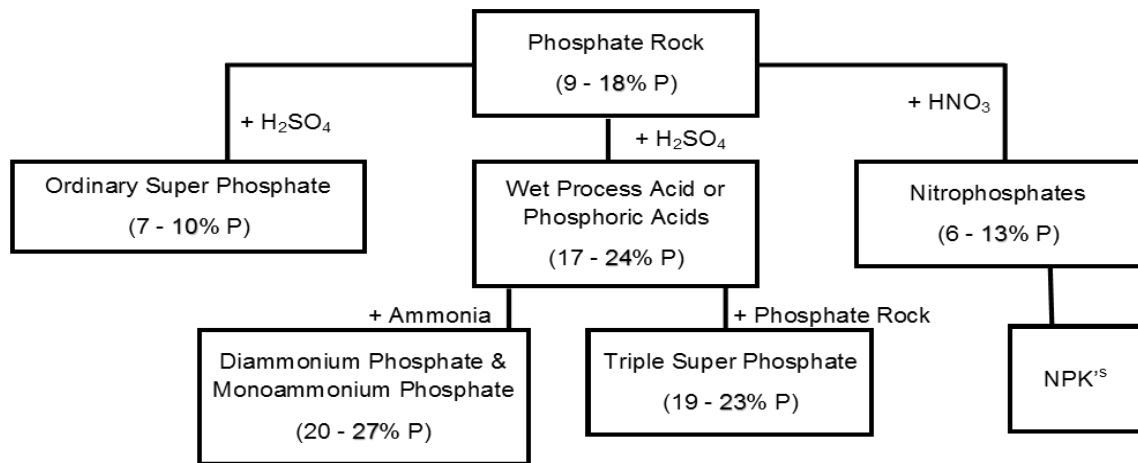
Figure 2.1   The production process of phosphate from rock phosphate (Rehm *et al.*, 2002).

When the phosphoric acid produced by either the wet or the dry process is heated, water is driven off and a superphosphoric acid is produced. The P concentration in superphosphoric acid normally varies between 31 and 32%. Phosphorus in phosphoric acid is either present as an orthophosphate or a polyphosphate. Polyphosphates consist of a series of orthophosphates chemically joined together which, upon contact with the soil, hydrolyzes back into the orthophosphate form (Rehm *et al.*, 2002).

When ammonia is added to unheated phosphoric acids, MAP (11:22:0) or DAP (18:20:0) is produced depending on the ratio of the mixture. Both aforementioned fertilizers contain P in the orthophosphate form. The cost of converting rock phosphates into these individual phosphate fertilizers are costly but varies depending on the process used. More important is to note that the conversion processes used have no effect on the availability of P to plants (Rehm *et al.*, 2002).

There are various kinds of phosphoric acids and phosphates. The simplest phosphoric acid series begins with monophosphoric (orthophosphoric) acid, continues with many oligophosphoric acids such as diphosphoric (pyrophosphoric) acid and ends in polyphosphoric acids (Robertson, 2004).

2.2.3 Orthophosphates

The simplest compound of a series of phosphoric acids is sometimes called by its common name, orthophosphoric acid (Figure 2.2) (Robertson, 2004).
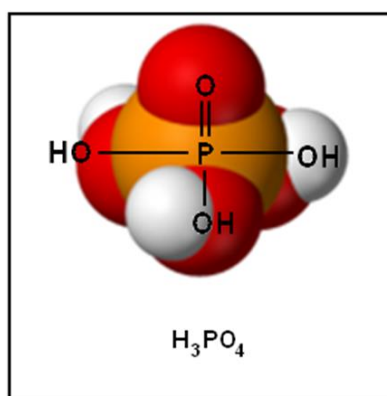
Figure 2.2   A generalized illustration of orthophosphoric acid (Robertson, 2004).

An orthophosphoric acid has three hydrogen atoms, each bonded to an oxygen atom in its structure. All three of these hydrogen atoms are acidic to varying degrees and may be lost from the molecule as H^+ ions. When these three H^+ ions are lost from the orthophosphoric acid, an orthophosphate ion (PO_4^{3-}) is formed (Robertson, 2004). Orthophosphorus is a very soluble reactive form of phosphorus and is readily available for biological uptake (Anonymous, 2004).

2.2.3.1 Orthophosphoric acid chemistry

Most people refer to orthophosphoric acid as *phosphoric acid*, which is the International Union of Pure Applied Chemistry (IUPAC) name for this compound. The prefix *ortho* is used to distinguish the acid from polyphosphoric acids. Orthophosphoric acids are non-toxic, inorganic and rather weak triprotic acids, which, when pure is a solid at room temperature and atmospheric pressure.

Orthophosphoric acids are very polar molecules and therefore highly soluble in water (Anonymous, 2011). The meaning of triprotic acid is that an orthophosphoric acid

molecule can dissociate up to three times, consequently giving up an H^+ each time, which typically combines with a water molecule, H_2O, as shown in the reactions below:

$$H_3PO_{4(s)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + H_2PO_4^-_{(aq)} \qquad K_{a1}= 7.25 \times 10^{-3} \qquad 2.1$$

$$H_2PO_4^-_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + HPO_4^{2-}_{(aq)} \qquad K_{a2}= 6.31 \times 10^{-8} \qquad 2.2$$

$$HPO_4^{2-}_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + PO_4^{3-}_{(aq)} \qquad K_{a3}= 3.98 \times 10^{-13} \qquad 2.3$$
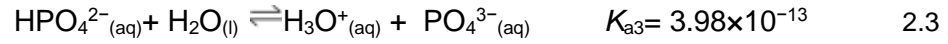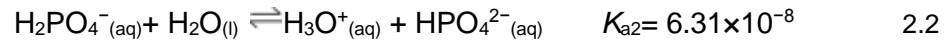
The anion after the first, second and third dissociations, namely $H_2PO_4^-$ (Equation 2.1), HPO_4^{2-} (Equation 2.2) and PO_4^{3-} (Equation 2.3) are known as the *dihydrogen phosphate* ($H_2PO_4^-$), *hydrogen phosphate* (HPO_4^{2-}) and *phosphate* or *orthophosphate* (PO_4^{3-}) anions, respectively. For each of the dissociation reactions shown above, there is a separate acid dissociation constant, called K_{a1}, K_{a2}, and K_{a3} given at 25°C. Even though all three hydrogen atoms are equivalent on an orthophosphoric acid molecule, the successive K_a values differ (Anonymous, 2011).

After heating an orthophosphoric acid, the phosphoric units can be induced by driving off the water formed from condensation. When one molecule of water has been removed for each two molecules of phosphoric acid, the result is pyrophosphoric acid ($H_4P_2O_7$). When an average of one molecule of water per phosphoric unit has been driven off, the resulting substance is a glassy solid (HPO_3) which is called metaphosphoric acid (Anonymous, 2011). Metaphosphoric acid is a singly anhydrous version of orthophosphoric acid. Further dehydration of metaphosphoric acid produces a phosphoric anhydride, which has an empirical formula P_2O_5 ($P_2O_5 \times 0.436 = P\%$), that is extremely soluble in water (Bonderud, 2010).

The initial orthophosphoric acid solution may contain 10 to 14% P, but can be concentrated by the evaporation of water to produce commercial phosphoric acids, which contains about 24% P. Further evaporation of water yields superphosphoric acid with a P concentration greater than 31% (Simplot, 2009). Phosphates such as DAP, TSP, NP and MAP are typical of orthophosphates.

2.2.3.2 Orthophosphate fertilizers and the soil

Commercial P fertilizers are highly (≥90%) water soluble. Once orthophosphates are dissolved in soils, orthophosphate ions are readily available for plant uptake as either a primary orthophosphate ion ($H_2PO_4^-$ with a soil pH < 7.0) or a secondary orthophosphate ion (HPO_4^{-2} with a soil pH > 7.0) (Noack *et al.*, 2010). Orthophosphate is a negatively charged anion, the form in which P is absorbed through the root system. Polyphosphate

can be thought of as a string of orthophosphate anions hooked together via chemical bonding (Rehm, 2010).

Two or more orthophosphoric acid molecules can be joined together by condensation into larger molecules by means of water elimination. In this way a series of polyphosphoric acids can be obtained (Robertson, 2004).

2.2.4 Polyphosphates

Ammonium polyphosphate fertilizers (APP) are excellent liquid fertilizers that are widely used in agriculture today (McBeath *et al.*, 2007a; McBeath *et al.*, 2009).

The starting material for most phosphate fertilizers is phosphoric acid, but the acidity and some of the chemical properties make this material difficult to use directly. The prefix *poly*, refers to multiple phosphate molecules linked in a chain. Each linkage of phosphate molecules has a name depending on its length. The most common APP fertilizers have a N:P:K composition of either 10:15:0 or 11:16:0. The advantages of polyphosphate fertilizers are [1]that these crystal-free fluid fertilizers are stable under a wide range of temperature, [2]has a high nutrient content and [3]has a long storage life. Another advantage is that a variety of other nutrients [4]mix well with polyphosphate fertilizers, therefore making them excellent carriers for micronutrients that may be needed by plants (Anonymous, 2010).

Between half and three-quarters of the P in polyphosphate fertilizers is present in chained polymers. These chains are then broken down to simpler phosphate molecules by enzymes produced by soil microorganisms and plant roots. Enzyme activity is much faster within a moist and warm soil. The remainder of the P (orthophosphate) is immediately available for plant uptake. Generally, half of the polyphosphate compounds will be converted to orthophosphates within a week or two, however under cool and dry conditions the conversion (hydrolysis) may take longer. Therefore, because polyphosphate fertilizers contain a combination of both orthophosphate and polyphosphate, plants are able to use this fertilizer form more effectively (Anonymous, 2010).

Ammonium polyphosphate fertilizers are gaining popularity in the agricultural industry due to its ease of application and yield benefits in calcareous soils (McBeath *et al.*, 2007a). Fluid fertilizers are convenient for farmers since they can be easily mixed with many other nutrients and each drop of fluid is exactly the same.

The decision whether to use dry or fluid fertilizers is mostly based on the price, handling preferences and field practices rather than significant agronomic differences (Anonymous, 2010). Polyphosphate fertilizers recently gained attention in Australian agricultural research. This is due to its significant yield increases with the application of liquid polyphosphate compared to granular orthophosphate fertilizers on highly P fixing soils (Holloway *et al.*, 2004; McBeath *et al.*, 2005). It is also necessary to understand the chemistry of polyphosphates as well as its behaviour within the soil (Blanchar & Hossner, 1969a; Hashimoto *et al.*, 1969; Mnkeni & MacKenzie, 1985; Al-Kanani & MacKenzie, 1991).

2.2.4.1 Polyphosphoric acid chemistry

Polyphosphates are polymeric oxyanion salts or esters formed from tetrahedral PO_4 structural units linked together by oxygen atoms. The polyphosphate has a linear chain- or cyclic ring structure (Robertson, 2004) when each P is linked to its neighbours' oxygen atoms (Niemeyer, 1999). The structure of tripolyphosphoric acid in Figure 2.3 illustrates the principles which define the structures of polyphosphates. It consists of three tetrahedral PO_4 units linked together by sharing oxygen atoms (Robertson, 2004).
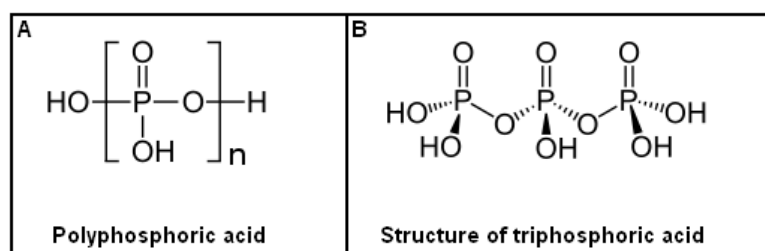
Figure 2.3   Structural differences between polyphosphoric acid (A) and triphosphoric acid (B) (Robertson, 2004).

The polymerization reaction can be seen as a condensation reaction. The process begins with two phosphate units coming together:

$$2\ PO_4^{3-} + 2\ H^+ \rightleftharpoons P_2O_7^{4-} + H_2O \qquad\qquad 2.4$$
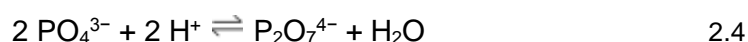
The polymerization reaction is shown as an equilibrium reaction as it can go in the reverse direction. This change in direction is known as a hydrolysis reaction because a water molecule is split. This process is able to continue in various steps. At each step another PO_3 unit is added to the chain. Ending condensation result in P_4O_{10}, where each tetrahedron shares three corners with the others (Robertson, 2004).

2.2.4.2 Hydrolysis of polyphosphates

Ammonium polyphosphate fertilizers are relatively soluble in water and in aqueous solutions; where water gradually hydrolyzes polyphosphates into simpler orthophosphates, given enough water (Robertson, 2004).

The time required for polyphosphate hydrolysis is soil temperature (Anonymous, 2008) and soil acidity dependent (Robertson, 2004). Temperature has the greatest effect on the rate of hydrolysis with the amount of hydrolysis being 42, 63, and 84% after 72 hours, at 5, 20, and 35°C respectively. However, under cool and/or dry conditions, hydrolysis may take longer. The efficiency of polyphosphates with more than 80% water solubility is considered to be equal to, but not better than, orthophosphates (Anonymous, 2008). Approximately 30% of applied phosphate is utilized by maize in the year of application regardless of source, however soil chemistry determines how much will be utilized. In calcareous soils, this percentage is lower (Rehm, 2010).

The amount of P in each P source as well as the form of P does not remain constant due to hydrolysis reactions, where more condensed P forms react with water to form less condensed forms of P. The most important hydrolysis reaction of polyphosphate fertilizer is the conversion of polyphosphates to orthophosphates (McBeath *et al.*, 2007b). Polyphosphate compounds are generally expected to be less reactive in soils than orthophosphates due to their chain or ring structure, which can increase soil P availability and plant P uptake (Philen & Lehr, 1967; Engelstad & Terman, 1980; Torres-Dorante *et al.*, 2006).

2.2.5 Polyphosphates versus orthophosphates

2.2.5.1 Sorption characteristics

Approximately 30 to 40% of the P fertilizer is present in the orthophosphate form while 50 to 55% is present as polyphosphate at the point of sale, and the remainder exists as tripolyphosphate and more condensed forms of P (McBeath *et al.*, 2007a & McBeath *et al.*, 2007b).

Torres-Dorante *et al.* (2006) stated that studies conducted by Blanchar and Hossner (1969b), Hashimoto *et al.* (1969), Mnkeni and MacKenzie (1985) as well as by Al-Kanani and MacKenzie (1991) to compare the soil sorption characteristics of orthophosphates and polyphosphates have concluded that the sorption capacity of soils for polyphosphates was greater than for orthophosphates. In another study conducted by McBeath *et al.* (2007b) on Australian soil types, polyphosphates showed a stronger sorption affinity compared to orthophosphates.

In general, the addition of polyphosphates to soil resulted in a decrease in Ca concentration and an increase in Fe concentration in a soil with a low pH. However, polyphosphates are expected to be less reactive in soils than their orthophosphate counterparts because of their chain or ring structure, which can increase soil P availability and plant P uptake (Torres-Dorante *et al.*, 2006).

2.2.5.2   Concentration in the soil

A low orthophosphate concentration in the soil solution is most likely to occur after the addition of polyphosphate fertilizers to soils. An equal orthophosphate concentration throughout the soil solution can only be expected if polyphosphate compounds are completely hydrolyzed (Torres-Dorante *et al.*, 2006).

Torres-Dorante *et al.* (2006) reported that 1 to 3 days after the application of polyphosphate compounds to sandy soils, the orthophosphate concentration in the soil solution was initially lower, but increased with time, reaching the same concentration as the orthophosphate treatment after 60 days. Torres-Dorante *et al.* (2006) also reported that in the silty-loam soil the orthophosphate concentration was unexpectedly high after one week with polyphosphate application, and remained at these high levels after 100 days.

In general, the rate of polyphosphate hydrolysis seemed to be faster or adsorption was stronger in the silty-loam soil than in the sandy soil. Dick and Tabatabai (1986) reported that phosphatase activity, which is involved in the hydrolysis of polyphosphates, therefore increasing the orthophosphate concentration, shows its optimum in neutral soils. Hons *et al.* (1986) suggested that the biological activity responsible for phosphatase production is higher in finer than in coarse-textured soils.

2.2.6   Phosphorus in the soil

Phosphorus' immobility is illustrated in Figure 2.4. The quantity of P that can be acquired by plants is determined by the amount of roots and the degree of P depletion at the root surface. In order to meet the plant's P demand the P must reach the root surface by either diffusion and/or mass flow in the soil solution (Jungk & Claassen, 1997; Claassen & Steingrobe, 1999).
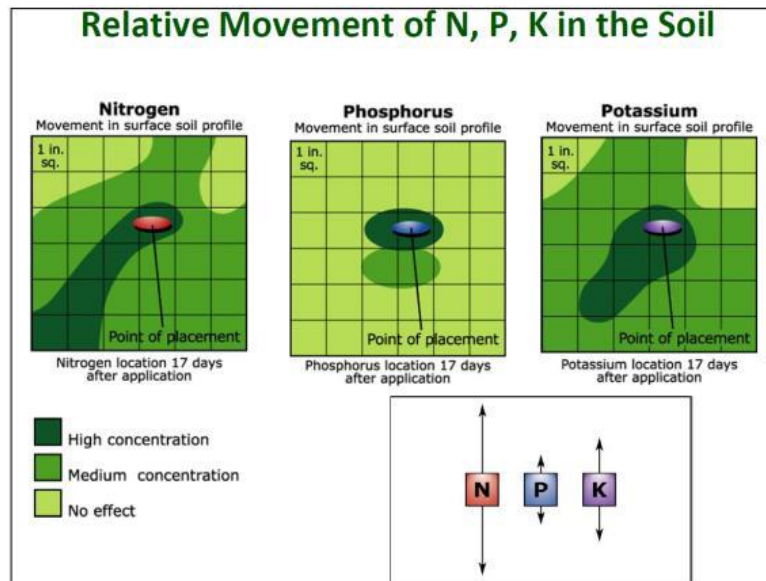
Figure 2.4   Differences in N, P and K fertilizer mobility in the soil (Roberts, 2010).

Forms of soil P may be divided into three groups:

Soil solution P

According to Havlin *et al.* (1999), the average P concentration in the soil solution is approximately 0.05 mg kg^{-1} but varies widely (0.003 to 0.3 mg kg^{-1}) depending on soil type, crop species, level of production and history of fertilization (Basu, 2011). The availability of $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} in the soil solution (Figure 2.5) is highly dependent on the pH of the soil solution. When the pH of the soil solution is equal to 7.2, approximately equal amounts of $H_2PO_4^-$ and HPO_4^{2-} occur in the soil (Annan, 2002). When the pH range drops below 7.2 the predominant P-form in the soil solution will be $H_2PO_4^-$, while at a soil solution pH above 7.2 the predominant P form will be HPO_4^{2-} (Haynes, 1982).

Organic soil P

Organic P (Figure 2.5) represents roughly 50% of the total soil solution P; nevertheless it may vary between 20 and 80% depending on soil type (Vlek *et al.*, 1997). Similar to organic matter, soil organic P decreases with soil depth but the amount of variation is also dependent on soil form. Thus, if the soil contains 4% organic matter in its surface (0 to 15 cm) then the following equation may be used to determine the amount of organic P in the soil solution:

$$2 \times 10^6 \text{ soil/ha}^{15cm} \times 0.01 \times 0.04 = 800 \text{ ha organic P/ha}^{15\,cm} \qquad 2.5$$

Therefore, the amount of organic P in the soil increases as the organic C and/or N in the soil increases; however soils have been characterized by their C:N:P:S ratio which has been found to be on average 140:10:1.3:1.3. Most of the characterized organic P compounds are esters of orthophosphoric acid ($H_2PO_4^-$) and have been identified primarily as inositol phosphates, nucleic acids and phospholipids (Havlin *et al.*, 1999). The approximate proportion of these compounds of organic P has been found to be:

Inositol phosphates	-	10 to 50%
Nucleic acids	-	0.2 to 2.5%
Phospholipids	-	1 to 5%

Inorganic soil P

When inorganic P (Figure 2.5) is added to the soil or when organic P is mineralized to inorganic P, it may become adsorbed to mineral surfaces or be precipitated as secondary P compounds. This is consequently termed P-fixation or P-retention and is however, very dependent upon soil pH (Brady & Weil, 2008). For the discussion on P-fixation, focus will be given to three types of soil conditions; [1]acidic soils, [2]neutral soils and [3]calcareous soils (Brady & Weil, 2008).

When the P concentration of the soil solution is low, adsorption predominates while precipitation will predominate when the P concentration of the soil solution exceeds that of the solubility product (K_{sp}). When water soluble P fertilizers are applied to the soil, the amount of P and accompanying cations instantly increase (Havlin *et al.*, 1999). Thus precipitation reactions will proceed. As the P concentration in solution decreases, P adsorption to reactive surface sites will continue. Regardless of precipitation or adsorption, understanding these fixation processes is important for optimum P-nutrition (Marschner, 1995).

$$[\text{High soil solution P}] \rightarrow [\text{Low soil solution P}] \qquad 2.6$$

[Precipitation] → [Adsorption]

In acidic soils, P either precipitates as Fe/Al-P secondary minerals and/or is adsorbed to surfaces of Fe/Al oxide and clay minerals. Therefore, Al^{3+} and Fe^{3+} oxides as well as hydroxide (OH) minerals are primarily involved in the adsorption of inorganic P (Haynes & Mikolobate, 2001). Due to the fact that the soil solution is acidic, the surface of these minerals has a positive net charge, and it is these positive charge sites that attract $H_2PO_4^-$

anions. When the $H_2PO_4^-$ orthophosphate ion is bonded with one Al-O-P bond, the $H_2PO_4^-$ is considered *labile-P*, which can then readily be desorbed from the mineral surface towards the soil solution. When the $H_2PO_4^-$ orthophosphate ion is bonded with two Al-O-P bonds, a stable six-member ring is formed. The desorption of $H_2PO_4^-$ from the mineral surface towards the soil solution is therefore more difficult and is then termed as *nonlabile-P* (Brady & Weil, 2008).

On the other hand, Havlin *et al.* (1999) reported that in neutral and calcareous soils, P either precipitates as Ca-P secondary minerals and/or is adsorbed to surfaces of $CaCO_3$ and clay minerals. In calcareous soils, small amounts of P can be adsorbed through the replacement of CO_3^{2-} on the surface of $CaCO_3$. Therefore, in soils pertaining low P concentrations, $CaCO_3$ surface adsorption predominates; while in soils with a high P concentration, Ca-P minerals precipitate on the surface of $CaCO_3$ (Wang, 2010).
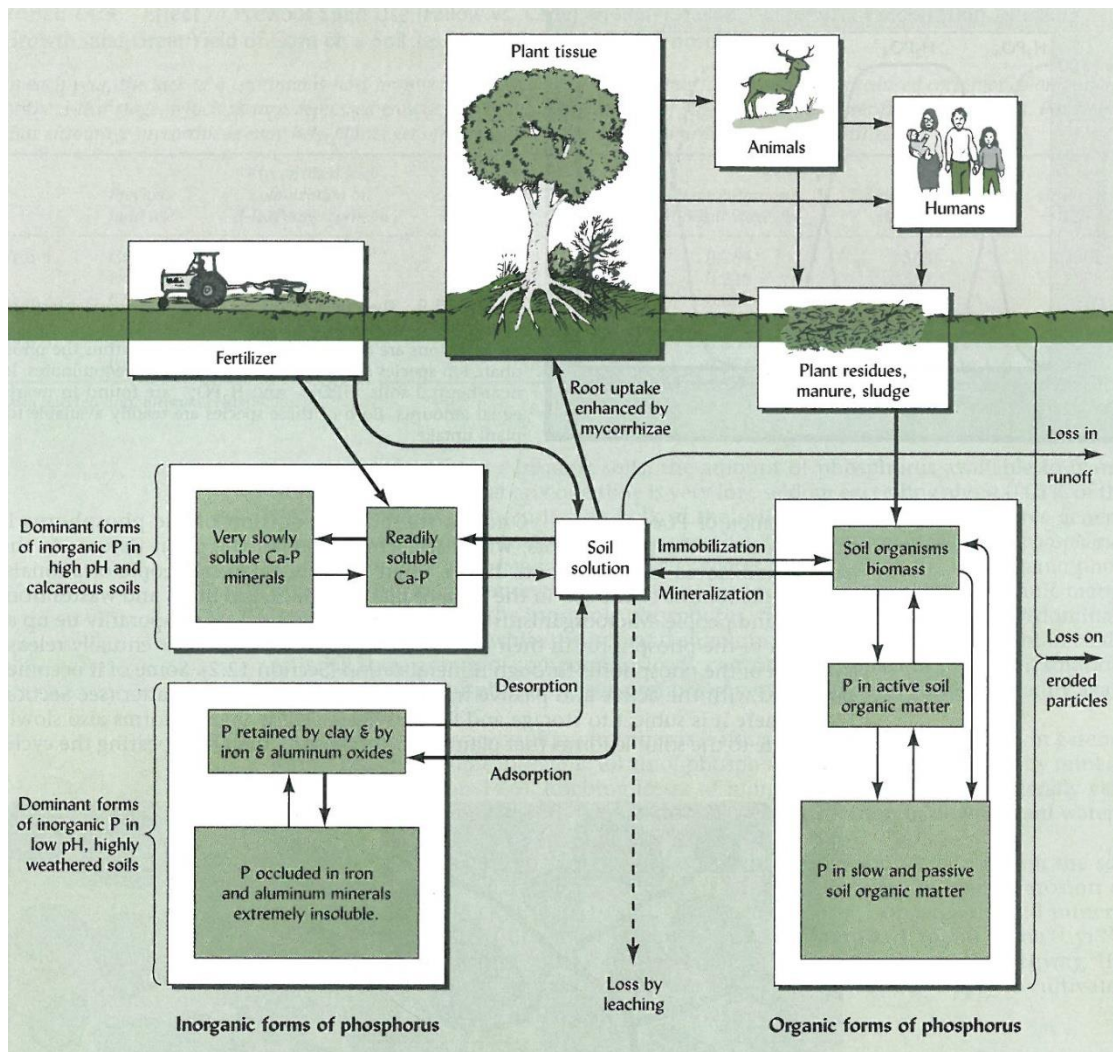
Figure 2.5   Phosphorus cycle in order to describe the interrelationship of the various forms and processes of P in the soil (Brady & Weil, 2008).

2.2.7 Phosphorus gains and losses

Phosphorus is essential for plant growth and therefore, P losses due to erosion, fixation and leaching are detrimental to agricultural production, in particular where fertilizers are unavailable or too expensive (Zöbisch *et al.*, 1994). However, to supplement the natural soil nutrient status, and in order to meet plant P demands of high yielding potential crops for an economically feasible yield, certain gains, such as phosphorus fertilizers are used (Johnston, 2000).

2.2.7.1 Gains

Considering the crop, the effectiveness of a P fertilizer depends mainly on its capacity to provide that crop with P over and above the amount which the plant can receive from unfertilized soil (Goswami *et al.*, 1990). The effectiveness is also further dependent on the rate of P supply in order to meet the requirement for optimum growth (Sharpley & Smith, 1992). A range of sources may be used in order to modify the P status of the soil, but detailed attention will be given referring to sources of phosphorus supply to the plant (Section 2.2.9.4).

2.2.7.2 Losses

Phosphorus losses, whether from surface runoff (erosion) or subsurface drainage (leaching), increase with the use of P fertilizers under either intensive pastoral or agricultural farming (Ward *et al.*, 1998). In several detailed studies, O'Conner (1968) demonstrated that both particulate P and dissolved inorganic P may be lost through surface runoff as well as through subsurface drainage. Another aspect of P loss from agricultural land due to erosion or leaching is the eutrophication of surface water (Zöbisch *et al.*, 1994).

Erosion

Soil erosion is a serious environmental problem in all areas of agriculture throughout the world (Hazarika & Honda, 1999), and thus one of the main factors limiting soil fertility and crop yields (Mati & Zöbisch, 1993). Fire and intense rain or irrigation alter soil properties (Andrue *et al.*, 1997) which ultimately increases soil erosion susceptibility; resulting in an increase of nutrient runoff accompanied by soil loss (Andrue *et al.*, 1996). These nutrient losses occur as organic matter and nutrients are transported away with the water (Gimeno-García *et al.*, 2000). When phosphorus fertilizers are surface applied to the soil, the fixing sites at the surface gradually becomes saturated which will increase

the concentration of dissolved P in the soil solution (Biggar & Corey, 1969). This ultimately increases the amount of P lost through erosion.

Appropriate crop rotation, cropping systems and management practices may help to diminish these losses (Breves & Schröder, 1991). However, certain crops such as maize, every so often encourage soil erosion. In areas where P loss is common an increase in soil pH will assist in raising the availability of P for plant uptake however, the reduction of soil erosion remains the most successful short-term solution in order to minimize P losses (Zöbisch *et al.*, 1994).

Chambers *et al.* (2008) stated that the use of minimum tillage techniques in which straw is left on the soil surface have been found to be effective in the reduction of erosion. In addition to that, avoiding fine rolled seedbeds in which slaking at the soil surface is minimized, thus helping to maintain water infiltration rates ultimately reduce soil erosion.

Leaching

Phosphorus leaching in most mineral soils is rarely viewed as an important environmental issue (Beauchemin *et al.*, 1997) however, P losses through leaching can be similar or even greater compared to losses through erosion (Ryden *et al.*, 1973). As P accumulates in the surface horizon of long-term fertilized soils, the downward movement of P may increase (Beauchemin *et al.*, 1997). The path of runoff water, whether along the surface or through the soil towards the subsurface has a great influence on the amount of P leached from the field (Bottcher *et al.*, 1980). In the case of deep infiltration, the slow movement of water through the subsoil, where the equilibrium concentrations tend to be lower, favors the sorption of dissolved P from the percolating waters (Sharpley & Syers, 1979). However, the P retention capacity of soil, particularly in the lower horizons, is great enough to retard the movement of even the greatest P-fertilized soils (Cisse & Amar, 1999).

It has been suggested by Tanton *et al.* (1988) that salts, transported via water during leaching, are rendered immobile when moving through the micro pores compared with salts passing through that of the macro pores as a result of adsorption. On the other hand, Turtola & Paajanen (1995) reported that on poorly drained clay soils, the leaching of dissolved orthophosphate phosphorus will decrease as the amount of surface runoff decreases by means of improved subsurface drainage. Phosphorus leaching from the soil have been found to increase under the following conditions; [1]by increasing he amount of water moving through the soil, [2]by increasing the concentration of the P in solution, [3]by decreasing the P buffer capacity and lastly [4]by increasing the P saturation

of the soil (Soon, 1985). Even though leaching may occur in peat soils as well as in very sandy soils (Mengel & Kirkby, 1987), the loss (0.44 kg P ha^{-1} year^{-1}) of P from most soils due to leaching have been found to be negligible (Ryden *et al.*, 1973),

2.2.8 Phosphorus transformation processes

Phosphorus mineralization and immobilization (Figure 2.6) occur through biological processes (Brady & Weil, 2008) and these processes occur simultaneously in soils (Sharpley & Smith, 1992; Addiscott & Thomas, 2000).
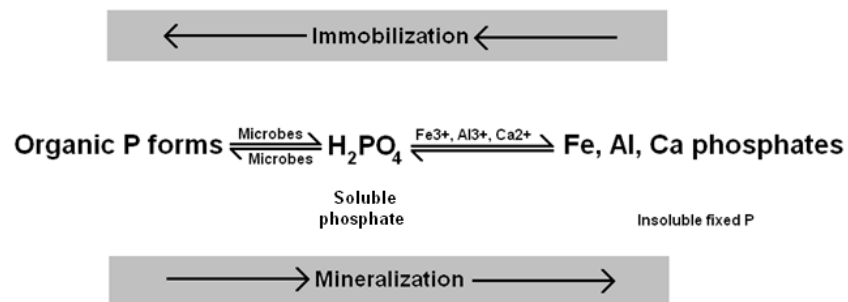
Figure 2.6   Phosphorus mineralization and immobilization as affected by soil microbes (Brady & Weil, 2008).

2.2.8.1 Mineralization

Organic phosphorus amounts to between 20 and 80% of total soil P; which is derived from the turnover of organic matter (OM) by microbes when animal manure and crop residues are added to soil (Tiessen *et al.,* 1994). The rate of turnover is however influenced by abiotic parameters such as soil texture, water content and temperature (Skopp *et al.*, 1990). In order to become plant available, organic phosphorus must be mineralized (Frossard *et al.*, 2011). The net organic phosphorus mineralization can be divided into three different processes; [1]basal mineralization, [2]flush effects, [3]and biological mineralization (Mary & Recous, 1994).

[1]Basal P mineralization can be defined as the mineralization of soil organic matter in a soil that has not received fresh organic matter inputs recently (Oehl *et al.*, 2001). [2]Flush effects are caused by sequences of drying–wetting or freezing–thawing (Mary & Recous, 1994) and are partly due to microbial death and subsequent decomposition of microbial cells. Gressel *et al.*, (1995) defined [3]biological mineralization as the release of phosphorus from organic materials at some stage in the oxidation of C by soil organisms, and that mineralization is driven by the search for energy and is thus closely linked to C

mineralization (McGill & Cole, 1981). As the organic C in the soil decrease, so does mineralization (C:P ratio <200:1 = Net mineralization) (Havlin *et al.*, 1999).

Phosphatase enzymes play a large role in the mineralization of organic phosphorus in the soil. Phosphatase activity in the soil will increase as the organic C content of the soil increases, but is however affected by soil pH, temperature and moisture. The pH influence has been found to be related to: [1]OH^- which competes with either $H_2PO_4^-$ or HPO_4^{2-} for bonding sites, [2]neutral pH soils having a greater microbial activity and [3]the fact that there is an increase in Ca-P minerals as the soil pH rises above 7 (Brady & Weil, 2008).

2.2.8.2 Immobilization

Immobilization increases with an increase in soil C (C:P ratio >300:1 = Net immobilization) (Havlin *et al.*, 1999). Biological immobilization (Figure 2.6) occurs when microorganisms acquire P from the residues metabolized. Hence, immobilization is the inverse response of mineralization (Brady & Weil, 2008).

Maximum immobilization has been noted to occur at a maximum soil temperature of 30°C but it will also proceed at soil temperatures as low as 5°C (Harrison, 1987). The immobilization of inorganic (applied) P occurs in most soils while the quantity (25 to 100%) thereof varies widely (Sharpley & Smith, 1992).

2.2.9 Phosphorus in the plant

2.2.9.1 Movement of phosphorus from soil to plant

The actively absorbing surface of the plant root occurs at young tissue near the root tips. Relatively high concentrations of P accumulate in the root tips, followed by a zone of lesser accumulation, where the root cells are elongated, and then by a second region of higher concentration, where the root hairs develop (Walker *et al.*, 2003). Therefore, rapid replenishment of the soil solution P is necessary where the roots are actively absorbing P (Walker *et al.*, 2003). The absorption of inorganic P from the soil solution is accomplished in three main ways namely, root interception, mass flow and diffusion (Havlin *et al.*, 1999).

Root interception

The importance of root interception as a mechanism for ion absorption is dependent and enhanced by the growth of new roots throughout the soil. As the roots develop and come

in contact with a greater soil volume, the root mass is exposed to a greater deal of soil solution ions. Thus the absorption of these ions occurs due to a contact mechanism (Havlin *et al.*, 1999; Brady & Weil, 2008). Ions such as H^+ which are attached to the surface of the root hairs, exchange with ions held in the soil solution when the oscillation volumes of the two ions overlap; such as Ca^{2+} held on the surface of clays and organic matter. Consequently, the quantities of nutrients that are absorbed by the plant depend on the volume and rate of root growth (Weisenseel *et al.*, 1979). Roots usually inhabit 1% or less of the soil, but may inhabit up to 3% depending on the porosity and nutrient content of the soil (Walker *et al.*, 2003; Brady & Weil, 2008).

Root interception can be enhanced using mycorrhizae, a symbiotic association between fungi and plant roots. The hyphal thread of the mycorrhizae fungi thus act as an extension of the plant root system, ultimately resulting in greater soil contact. The two major groups of mycorrhizae are known as ectomycorrhizae and endomycorrhizae, last of which is more widely spread (Havlin *et al.*, 1999). The roots of most agronomic crops have vesicular arbuscular mycorrhizae; which means that the fungus grows into the cortex of the root and transport nutrients into the arbuscules. This increased nutrient absorption is partly due to the larger nutrient absorption surface. Fungal hyphae extend up to 80 mm into the soil surrounding the roots, and the area of infected roots in the soil has been calculated to be up to 10 times that of uninfected roots (Havlin *et al.*, 1999).

Mass flow

Mass flow occurs when either the nutrient ions in the soil solution or other dissolved substances are transported within the flow of water towards the roots, which result from transpirational water uptake by the plant. However, the amount of nutrients reaching the roots as a result of mass flow are determined by the rate of water flow or the water consumption of plants, as well as the average nutrient concentration in the soil water solution. As the soil moisture tension increases (soil moisture reduces), water movement towards the root surfaces decreases. With decreasing atmospheric temperatures, the movement of nutrients by mass flow decreases due to a lower plant transpiration rate at lower temperatures (Havlin *et al.*, 1999; Brady & Weil, 2008).

Mass flow in low-P soils provides only a small portion of the P requirement. It is estimated that only 1% of P moves to the plant through mass flow, however in fertilized soils with a P solution of 0.05 mg kg^{-1}, mass flow contributes to 20% or less of the total amount of P transport to the root surface. Areas of high P concentration; such as around or near the fertilizer bands are expected to encourage P uptake through both mass flow and

diffusion, however it has been found that diffusion is the primary mechanism of P transport (Havlin *et al.*, 1999).

Diffusion

Diffusion occurs when ions in a soil solution move from an area of higher concentration towards an area of lower concentration. Consequently, a nutrient concentration gradient is established which causes ions to diffuse towards the plant roots. If the plant requirement is high then the concentration gradient is high; favoring a high rate of ion diffusion from the soil solution into the roots (Bistow, 2002). Many soil factors influence the diffusion of nutrients of which the magnitude/rate of the diffusion gradient is the most important (Havlin *et al.*, 1999). The diffusion rate is directly proportional to the diffusion coefficient (*De*) which controls how far nutrients can diffuse to the roots. *De* is described as follows:

$$De = D\omega\ \theta\ (1/T)\ (1/b) \hspace{3cm} 2.7$$

where: *Dω* = diffusion coefficient in the water

θ = volumetric soil water content

T = turtuosity factor

b = soil buffer capacity

This equation shows that as the soil moisture content (θ) increases the diffusion coefficient (*Dω*) increases, which results in an increased diffusion rate. However, according to Brady and Weil (2008), as the moisture content of the soil is lowered, the films around the soil particles become thinner while the diffusion of ions through these films becomes more tortuous. It has also been found that the transport of nutrients towards the root surface is most effective when the soil water content is near/close to with the field capacity.

The uptake of nutrients through diffusion is also strongly influenced by temperature. The range of best diffusion occurs between 10 and 30°C. An increase of 10°C is usually followed by an increased rate of ion absorption with a factor of 2 or even more. Furthermore, the rate of diffusion also depends on the distance between the nutrient and the root. The average distance for diffusion between the nutrient and the root has been found to be 10 mm for N, 0.2 mm for P and 2 mm for K. In conclusion 80% of all P moves through the soil towards the roots by means of diffusion (Havlin *et al.*, 1999; Brady & Weil, 2008).

2.2.9.2 Uptake and functions of phosphorus in the plant

Plants absorb P as orthophosphates, *viz.* $H_2PO_4^-$ which is absorbed greatest at low soil pH values (below pH 7.2), or HPO_4^{2-} which is absorbed greatest at high pH values (above pH 7.2). Plants may then also absorb certain soluble organic phosphates such as nucleic acids and phytin, both which is produced by the degradation of organic matter in the soil (Brady & Weil, 2008). Two of the most essential functions of phosphorus in plants are energy storage and energy transfer. Adenosine di- and triphosphates (ADP and ATP) are formed and regenerated in the presence of sufficient P. When the terminal P molecule from either ADP or ATP is split off, energy is formed. Nearly every metabolic reaction of any significance proceeds via phosphate derivatives. Furthermore, P also aid in structural integrity of nucleic acids, phosphoproteins, phospholipids and sugar phosphates (Marschner, 1995).

Adequate supply of P in the early life of a plant is essential for crop development and reproduction. A large quantity of P is found in the seed and fruit, and is considered essential for seed development (Wallace, 1943). A good supply of P is associated with increased root growth. It is also associated with early maturity of crops, especially grain crops. This is due to the fact that ample supply of P reduces the time required for grain ripening, improved straw strength of cereals, reduced cold damage and the improvement of root-rot disease tolerance (Haberle *et al.*, 2008).

2.2.9.3 Plant response to phosphorus deficiencies

Phosphorus deficiencies of grass species can easily be characterized by the purple discoloration of leaves or leaf edges. Phosphorus deficiency symptoms first appear in the older leaves and are also characterized by retarded plant growth (Marschner, 1995). In order to alleviate the above mentioned P deficiencies in a plant, either organic-P or inorganic-P sources may be used (Havlin *et al.*, 1999).

2.2.9.4 Sources of phosphorus supply to the plant

Approximately 98% of organic-P is applied in the form of organic manure and have been found to be more mobile in the soil compared to inorganic-P sources (Havlin *et al.*, 1999). The most widely used P sources in South Africa include rock phosphates (RP), phosphoric acid, superphosphates and ammonium phosphates (Havlin *et al.*, 1999). After several processing and purification steps RP contains between 11.5 and 17.5% P. None of its P is water soluble. Finely ground RP can be used directly as a P fertilizer, but is effective only in acidic soils (pH<6) and only when applied in quantities two to three

times that of the rate of superphosphates. Finely ground RP is also commonly used for the restoration of low-P soils, while environmental conditions such as long growing seasons, moist soils and warm climates increases the effectiveness of rock phosphates (Brady & Weil, 2008).

Phosphoric acid (H_3PO_4) is produced by treating raw RP with sulphuric acid (H_2SO_4), which is known as the wet process (Section 2.2.2). This process also produces gypsum ($CaSO_4 \cdot 2H_2O$). Agricultural-grade phosphoric acid contains between 17 to 24% P, and can be applied to the soil either by direct soil injection or through irrigation water; especially to alkaline and calcareous areas due to acidification affects (Havlin *et al.*, 1999). Haynes and Naidu (1998) stated that superphosphates are neutral fertilizers which do not affect soil pH, compared to phosphoric acid- and NH_4^+-containing fertilizers. Single superphosphate (SSP) is also manufactured by reacting RP with sulphuric acid:

$$[Ca_3(PO_4)_2]_3 \cdot CaF_2 + 7H_2SO_4 \rightarrow 3Ca(H_2PO_4)_2 + 7CaSO_4 + 2HF \qquad 2.8$$
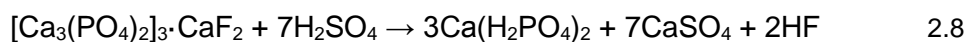
Rock phosphate + Sulphuric acid → Monocalcium phosphate + Gypsum + Hydrofluoric acid

Single superphosphate contain between 7 and 9.5% P, is 90% water soluble and essentially all is plant available. However, due to its low P analysis it is not commonly used (Marschner, 1995). For this very reason triple super phosphate (TSP), also known as concentrated superphosphate, is manufactured to increase the P content of SSP by reacting RP with phosphoric acid:

$$[Ca_3(PO_4)_2]_3 \cdot CaF_2 + 12H_3PO_4 + 9H_2O \rightarrow 9Ca(H_2PO_4)_2 + CaF_2 \qquad 2.9$$
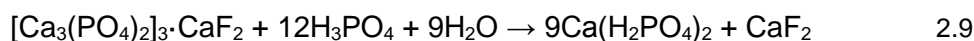
Rock phosphate + Phosphoric acid + Water → Monocalcium phosphate + Calcium fluoride

Triple super phosphate contains between 17 and 23% P, and due to its high P content is manufactured in a granular form, which is mixed and blended with other materials as well as used in direct soil applications. Single super phosphates and TSP can be ammoniated in order to produce MAP ($NH_4H_2PO_4$). The ammonization of superphosphates offer the advantage of inexpensive N but decreases the amount of water soluble P in the product (Havlin *et al.*, 1999).

Monoammonium phosphate is manufactured by reacting wet process phosphoric acid (H_3PO_4) with NH_3. The ammoniation of superphosphate reaction follows:

$$Ca(H_2PO_4)_2 + NH_3 \rightarrow CaHPO_4 + NH_4H_2PO_4 \qquad 2.10$$
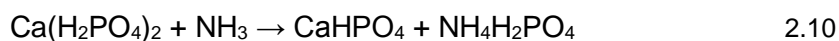
Monocalcium phosphate + Ammonia → Dicalcium phosphate + Monoammonium phosphate

Monoammonium phosphate contains between 11 to 13% N and about 21 to 24% P, while the most common grade is 11:22:0. Even though the use of MAP has increased significantly over the last decade, DAP is more widely used compared to any other P fertilizer (Beaton *et al.*, 1963; Havlin *et al.*, 1999).

$$2NH_3 + H_3PO_4 \rightarrow (NH_4)_2HPO_4 \qquad\qquad 2.11$$
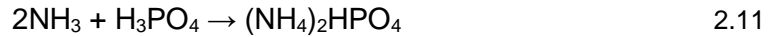
<div align="center">Ammonia + Orthophosphoric acid → Diammonium phosphate</div>

Both MAP and DAP are granular fertilizers which are completely water soluble. Ammonium phosphate fertilizers have the advantage of having a high plant-food content, which minimizes shipping, handling and storage costs (Beaton *et al.*, 1963).

Thus, according to Havlin *et al.* (1999), care must be taken when seeds are row-placed together with DAP since free NH_3 is produced; which is illustrated by the equation 2.12. The aforementioned may lead to seedling injury as well as inhibition of root growth.

$$(NH_4)_2HPO_4 \rightarrow 2NH_4^+ + HPO_4^{2-} \text{ (pH 8.5)} \qquad\qquad 2.12$$
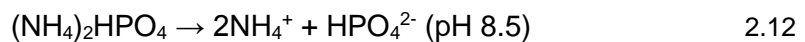
2.2.10 Crop response to phosphorus

Phosphorus is one of the macronutrients essential to plant growth and is critically needed to improve soil fertility for crop production. Even though plants need considerably less P compared to N, P is needed for energy production and transfer in young rapid growing plants. The use of P fertilizers becomes essential to successful crop production; since certain agricultural soils are low in both total and available P (Hammond *et al.*, 1990).

The placement of P is one of the most important factors to consider when studying/investigating at crop response. Phosphorus application before sowing is of negligible advantage to crops. Rapid P uptake takes place only three to four weeks after germination, due to a much higher demand for P at this time of growth as compared to other growth stages. Coincidentally, P forms that are water soluble release a large portion of their P immediately upon wetting (Lindsay & Stephenson, 1959). Therefore, P-fertilization at planting is reported to be more beneficial, compared to pre-plant broadcast incorporated applications. The common recommendation is to apply the full dose of phosphorus at planting (Hammond *et al.*, 1990).

Knowing P is not very mobile and may be susceptible to fixation depending on soil type, the best method of P-application is band placement. Broadcast-incorporated applications of P are less effective than banded applications, while broadcast application should be two to four times the recommended rates of band applications (Hammond *et al.*, 1990).

Band placement also increases the P concentration of the immediate soil area in contact with the developing root. Therefore, the effectiveness of a P fertilizer for a particular crop depends on its capacity to provide the crop with P over and above that which the plant can get from the unfertilized soil and at a rate to meet the crop's requirement for optimum growth (Hammond *et al.*, 1990). The P application rate for a specific crop depends on the nutrient status of the soil before planting. If the soil is deficient in P, the acceptable amount (depending on the soil analysis) should be put back into the soil before making a recommendation on the amount to be fertilized for optimum yields. The rate of application then also depends on the specific cultivar selected. If deficiencies were to occur during the growing season, then P may be applied as a foliar application (Havlin *et al.*, 1999; Lafond *et al.*, 2003).

According to Bhardwaj (1978) an application of 26 kg P ha^{-1} (combined with N) to wheat gave a 1.2 to 1.8 ton ha^{-1} yield increase over N alone. Data from coordinated wheat experiments showed that even at 35 kg P ha^{-1}, a response in the order of 26 to 35 kg grain per kg P can be expected. Tandon (1986) reported that P accounted for 35% of the total wheat yield increase brought about by NPK application, which was 10% more than the unfertilized control. Tandon (1987) concluded that wheat yield increases brought about by P application are widespread, significant and economically attractive. Yield responses to P have been obtained both at experimental stations and in numerous experiments on farmers' fields grown with irrigation or assured rainfall (Goswani *et al.*, 1990). It is important to know the quantity of P requirement by a crop to produce a unit yield. This provides an estimate of the net demand for P by the crop on the soil-fertilizer complex. For most grains, both straw and grain are removed from the field, which means the requirement of nutrients is about equal to the crop biomass removed from the field at harvest (Goswani *et al.*, 1990).

The texture of a soil is important when considering phosphorus application and crop reaction. Phosphorus applied to sandy calcareous soils has been found to be available to plants for a long period of time compared to phosphorus applied on clay soil, due to P-fixation (Havlin *et al.*, 1999). Since phosphates are relatively immobile in soil, it is rare that more than 25% of phosphates applied to a crop at planting, is used in the first year after application.

The type of root system is also the reason why there is a difference in the response to P between crops; e.g. wheat has an adventitious root system, and for this reason can only absorb P from the plough layer if sufficient P is available, while groundnuts has a taproot system which can absorb water insoluble P and P below the plough layer. It is for this reason that wheat responds better to newly applied P and why groundnuts responds well

on residual P, if sufficient P is available (Marschner, 1995; Havlin *et al.*, 1999; Brady & Weil, 2008).

2.2.11   Summary of phosphorus in the soil-plant system

Research has shown that various chemical forms of P (orthophosphates and polyphosphates) are all agronomically similar when applied by the same method of application at the same phosphate rate.

Approximately 30 to 40% of the P fertilizer is present in the orthophosphate form while 50 to 55% is present as polyphosphate. Orthophosphorus is a highly (≥90%) soluble reactive form of phosphorus and is readily available for biological uptake, while polyphosphate fertilizers are stable under a wide range of temperature and has a high nutrient content. Polyphosphate fertilizers contain a combination of both orthophosphate and polyphosphate and between half and three-quarters of the P in polyphosphate fertilizers is present in chained polymers. These chains are then broken down to simpler orthophosphate molecules by enzymes while enzyme activity is much faster within moist and warm soils. The remainder of the P (orthophosphate) is immediately available for plant uptake. Generally half of the polyphosphate compounds will be converted to orthophosphates within a week or two, however under cool and dry conditions, hydrolysis may take longer.

Polyphosphate based fertilizers are worldwide in use, and their effect on crop yield is often reported to be similar to orthophosphate products. The rates of hydrolysis of solid and liquid APP on alluvial, sodic and laterite soils under aerobic and anaerobic conditions show that hydrolysis is rapid in the beginning and slows down with time of incubation, irrespective of the soil type. However, the rate of hydrolysis was faster under anaerobic conditions, much more so when the fertilizer was liquid. Half-life values for polyphosphates ranged from 1.6 to 9.2 days under anaerobic conditions compared to 5.2 to 27 days under aerobic conditions. The half-life values for orthophosphate formation under aerobic conditions ranged from 9 to 25 days.

The rate of polyphosphate hydrolysis seems to be faster in a silty-loam soil than in a sandy soil. Phosphatase shows its optimum activity in neutral soils. In loamy soils, polyphosphates results in higher yields and higher levels of P uptake compared to orthophosphates. Orthophosphates also yields higher dry-matter compared to polyphosphates. Fluid APP was superior to granular fertilizers when applied in a subsurface band. However, granular fertilizers were superior to fluid APP when broadcast. Similar yields were obtained with APP irrespective of applications as a liquid

or solid. When fertilizer was applied in irrigation water, APP moved much deeper and was more available than orthophosphate fertilizer in sandy soils. Both NP and DAP fertilizers were equally effective when compared with plant yield and P content of wheat crops.

The possible incorporation of APP in fertilizer programs under South African conditions necessitates the response of maize to different phosphorus sources. These sources include MAP, NP and APP. Standardizing the N application of the different P sources meant that additional N had to be applied. It was recommended that urea was to be used to do the calibration, but this meant that some of the P sources had the advantage of having either ammonium (NH_4^+) or nitrate (NO_3^-); which could favour crop reaction and therefore it was decided to also include limestone ammonium nitrate (LAN) to calibrate the N applications. As a result of this the literature will also include a section on N and will specifically focus on the use of LAN and urea.

2.3 Nitrogen in the soil-plant system

2.3.1 Introduction

Nitrogen is the element most frequent deficient in crops (Havlin *et al.* 1999), however, according to Olsen and Sander (1988) the use of N fertilizers have resulted in yield increases by a factor of three since 1945.

Nitrogen fertilization is an expensive but often necessary input in agricultural systems (Brady & Weil, 2008), while N fertilization enable farmers to achieve high yields that drive modern agriculture. The use of N fertilizers will however continue to increase as the global population and food requirement increases (Bouwman & Booji, 1998). This increased protein requirement will require either more N inputs to produce increased grains and forage, or N-use efficiency by crops need to be increased (Alva *et al.*, 2010).

Managing farming systems to reduce nitrate losses has been the focus of several studies throughout the world. A study by Oenema and Pietrzak (2002) concluded that N management is the most important factor in determining the economic and environmental performance of agro-ecosystems. They also found that N management accounted for more than 50% of the variance in nutrient costs and input-output ratios among farms. Nutrient management begins where decisions are made about the amount and timing of nutrient application. Management of N on the farm, to increase efficiency of N use in maize, has proven to be complex because of the spatial and temporal variation in yields (Hatfield & Prueger, 2004).

At least eleven forms of nitrogen fertilizers are globally available (Jensen, 2006). The use of such fertilizers has increased the N in the soil solution as well as caused a quadrupling average maize yield in the USA (Olsen & Sander, 1988). When a plant is over supplied with nitrogen, excessive vegetative growth occurs. This causes a plant to become top-heavy and ultimately prone to falling over (lodging) with heavy rain and wind (Brady & Weil, 2008). Excessive supply of nitrogen to a crop degrades the colour and flavour of most fruits as well as decreases the sugar and vitamin levels of certain vegetables (Brady & Weil, 2008). Power (1980) furthermore reported that only between 30 to 70% of applied N is effectively used by plants; the rest is lost through various mechanisms.

Of all nutrient elements, nitrogen is by far the most mobile and therefore highly subject to losses due to both volatilization and leaching (Follet *et al.*, 1981). Leaching losses of N are mostly described to nitrate leaching which is a result of the nitrification of urea, ammonia and ammonium salts (Du Preez, 1983). Nitrate sources are more mobile in the soil compared to that of ammonium sources (Follet *et al.*, 1993). This is because ammonium sources are positively charged and thus become adsorbed to soil colloids, while nitrate sources, for all practical reasons, are not adsorbed by the soil (Du Preez, 1983). Nitrogen may also be lost through ammonia volatilization. This occurs when N fertilizers containing ammonia or ammonia forms are surface applied to alkaline or calcareous soils (Sheibani & Ghardiri, 2012). However, proper placement may reduce the extent of such losses (Follet *et al.*, 1981).

A portion of N for maize production is applied as a mixed fertilizer before or at planting while the remainder N is either top- or side-dressed 3 to 6 weeks after emergence of seedlings (Martin *et al.*, 1976). This method of application (split application) increases N use efficiency (Sheibani & Ghardiri, 2012). Split application further ensures that after an active root system has been established, the time for losses due to leaching and volatilization is reduced. It also ensures that enough N is available for both vegetative growth and grain formation (Olsen & Sander 1988). Urea fertilizers, when incorporated at planting, are more efficient due to decreased volatilization of ammonia (Maddux *et al.*, 1991). Nitrogen must be placed at a distance of 50 mm below and away from the maize seed or seedling in order to reduce the possibility of N toxicity (Tisdale *et al.*, 1993). More time and money are spent on the management of nitrogen compared to any other nutrient element (Brady & Weil, 2008).

2.3.2 Nitrogen in the plant

Because nitrogen is an integral component for many plant components such as amino acids, enzymes, nucleic acid and chlorophyll, ample supply is necessary (Marschner, 1995).

2.3.2.1 Uptake by the roots

According to Brady and Weil (2008), plants contain between 1 and 5% N by weight and principally absorb N as both dissolved nitrate (NO_3^-) and ammonium (NH_4^+) ions. Both these ions move towards the plant root through mass flow and diffusion. Both these mechanisms have been discussed in detail under P uptake mechanisms (Section 2.2.9.1).

In warm, moist and well-aerated soil, NO_3^- occurs in higher concentrations, while uptake is also favoured by low soil pH conditions. As NO_3^- uptake increases, an increase in organic anion synthesis within the plant have been found (Havlin *et al.*, 1999).

According to Havlin *et al.* (1999), NH_4^+ is the preferred N source for plants since energy will be saved when it is used for energy synthesis of proteins. Compared to NO_3^-, plants supplied with NH_4^+ show an increase in both carbohydrate and protein levels. NH_4^+ uptake is best under neutral pH soils and is however depressed as soil acidity increases. Rhizosphere pH decreases when plants receive NH_4^+. This reduces the uptake of Ca^{2+}, Mg^{2+} and K^+ whilst increasing $H_2PO_4^-$, SO_4^{2-} and Cl^- uptake. Ammonium uptake have also been found to increase the tillering capacity of plants, but can however retard growth (Havlin *et al.*, 1999). Whether the plant has the preference to use either dissolved nitrate or ammonium ions, is determined by [1]the age of the plant, [2]the type of crop and [3]the environment (Brady & Weil, 2008).

2.3.2.2 Response to deficiencies

Crops deficient in N are stunted and tend to turn yellow (chlorotic). Chlorosis first appears in the older (lower) leaves, while younger leaves stay green (Havlin *et al.*, 1999). Plants with deficiencies also tend to be tiny with spindly stems (Brady & Weil, 2008). Where severe deficiencies prevail, leaves turn necrotic (brown); which start at the leaf tip and progresses along the mid-rib until the entire leaf ultimately die (Havlin *et al.*, 1999).

2.3.3 Nitrogen transformation processes

According to Havlin *et al.* (1999), the N cycle (Figure 2.7) may be divided into N inputs (gains), N outputs (losses) as well as N cycling in the soil; where N is neither gained nor lost.

The most vital source of N used by plants is N_2 gas; which constitutes 78% of the earth's atmosphere. Higher plants however, cannot metabolize N_2 into protein and must be converted in the soil into plant available N forms. In addition, a portion of N_2 in the atmosphere is returned to the soil through rainfall as NH_4^+, NO_3^- and NO_2^-. This conversion is done by [1]microorganisms which live on the roots of certain legume and non-legume plants, [2]non-symbiotic or free-living microorganisms in the soil or ultimately [3]by adding inorganic N fertilizers to the soil (Havlin *et al.*, 1999).

2.3.3.1 Mineralization and immobilization

Mineralization

Inorganic N, mostly ammonium and nitrate, is readily available to plants. Before organic N can be taken up it must first be converted to inorganic forms. This process (where organic N is converted to plant-available inorganic forms), completed by soil microbes as a by-product of organic matter decomposition; is called mineralization (Crohn, 2004). Factors affecting the mineralization process include soil temperature and moisture, amount and type of clay as well as the C:N ratio (Havlin *et al*, 1999).

Soil temperature and moisture content have a strong effect on N mineralization reactions. Mineralization is limited at soil temperature near freezing and increases with rising soil temperature (Havlin *et al*, 1999). Maximum N mineralization occurs when the soil temperature reaches 30 to 35°C. In dry soils, N mineralization is low because soil microorganism activity is limited by water availability. In saturated soils, lack of oxygen limits N mineralization because only soil microorganisms that can survive under anaerobic conditions are active (Deenik, 2006).

The amount and type of clay in a soil also affects N mineralization reactions. Mineralization tends to be greater in coarse-textured soils low in clay and decreases as the soil clay content increases. Soils dominated by clay minerals that shrink and swell with fluctuations in soil moisture, such as montmorillonite as well as volcanic ash soils rich in organic matter, tend to have higher N mineralization rates than those containing clays that do not shrink and swell, such as kaolinite (Deenik, 2006). Decreasing the C:N ratio to below 15:1 increases the N mineralization rate (Van Kessel *et al.*, 2000).

Compared to soil texture, the effects of soil mineralogy on N mineralization are less clear (Deenik, 2006).
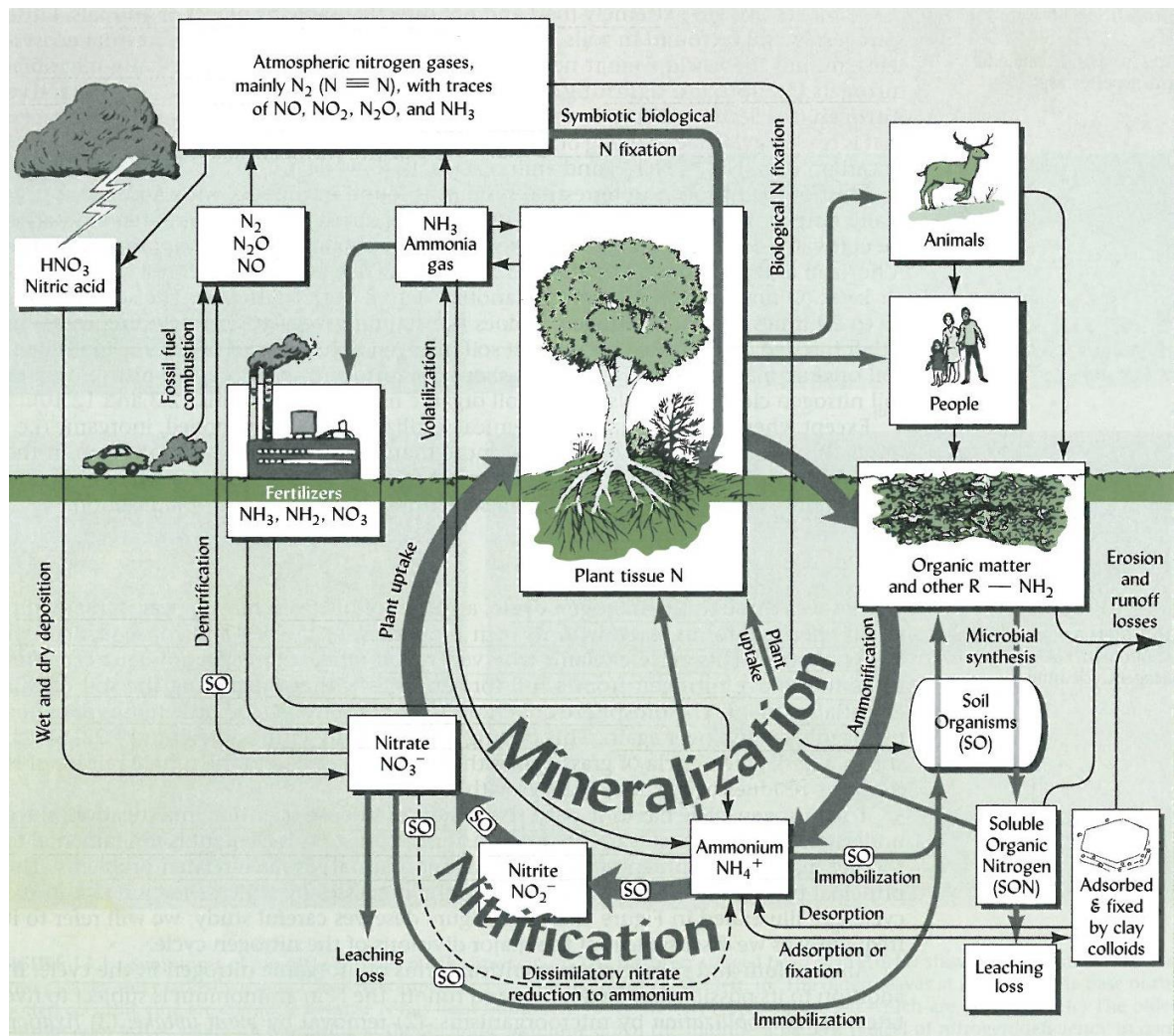
Figure 2.7   Nitrogen cycle in order to describe the interrelationship of the various forms and processes of N in the soil (Brady & Weil, 2008).

Immobilization

Immobilization is the reverse of mineralization. All living things require N therefore, microorganisms in the soil compete with crops for N. Immobilization refers to the process in which nitrate and ammonium are taken up by soil organisms and therefore become unavailable to crops (Hungate, 2006). However, immobilization only locks N temporarily. When the microorganisms die, the organic N contained in their cells is converted by mineralization and nitrification to plant available nitrate (Hungate, 2006).

Immobilization is affected by environmental conditions, such as soil temperature, moisture content, and pH (Mulvaney, 1993). In contrast with mineralization, when the C:N ratio is greater than 30:1, an initial temporary decrease in available N upon decomposition is likely. This is termed immobilization (N negative period), and occurs because the microbes need additional N to utilize all the carbon in the organic matter. Barker (2011) explains that when wheat straw or chaff is incorporated into the soil, immobilization can occur. Nevertheless, after a few weeks the immobilized N in microbial bodies can be mineralized back into the inorganic form and become available again for plant uptake. (Barker, 2011).

2.3.3.2 Nitrification and denitrification

Nitrification

According to Brady and Weil (2008), nitrification can be defined as the biological conversion (oxidation) of ammonia to nitrate by means of a two-step process:

$$NH_3 + 1.5\ O_2 + nitrosomonas \rightarrow NO_2^- + H_2O \qquad 2.13$$

$$NO_2^- + 0.5\ O_2 + nitrobacter \rightarrow NO_3^- \qquad 2.14$$
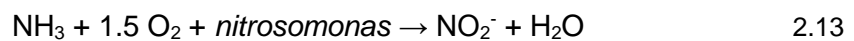
Bacteria known as *nitrosomonas* convert (oxidize) ammonia and ammonium to nitrite (NO_2^-). Next, bacteria called *nitrobacter* finish the conversion (oxidation) of nitrite to nitrate (NO_3^-). There are various factors affecting the nitrification process, including dissolved oxygen, pH, salinity and temperature (Myrold, 1998). The desired nitrification performance depends on the careful control of these various factors (Brady & Weil, 2008).

These bacteria known as nitrifiers are strict aerobes, meaning they must have free dissolved oxygen to perform their work. Thus nitrification occurs only under aerobic conditions at dissolved oxygen (DO) levels of 1.0 mg L^{-1} or more. At dissolved oxygen concentrations below 0.5 mg L^{-1}, the growth rate of nitrifying bacteria is minimal (Marschner, 1995).

The nitrification process also produces acid. This acid formation lowers the pH of the biological population in the soil and can cause a reduction of the growth rate of nitrifying bacteria. Leggett and Iskandar (1980) showed that the optimum pH for *nitrosomonas* and nitrobacter ranges between 7.5 and 8.5. Nitrification is effective at a soil pH ranging between 6.5 to 7.0, and stops at a soil pH below 6.0 (Havlin *et al.*, 1999).

The nitrification reaction consumes 7.1 mg L^{-1} alkalinity as $CaCO_3$ for each mg L^{-1} of ammonia nitrogen oxidized. An alkalinity of no less than 50 to 100 mg L^{-1} is required to insure adequate buffering. Water temperature also affects the rate of nitrification. Nitrification reaches a maximum rate at temperatures between 30 and 35°C (Marschner, 1995; Havlin *et al.*, 1999).

Denitrification

Denitrification is defined as the biological reduction of nitrate to nitrogen gas (N_2) by facultative heterotrophic bacteria. Heterotrophic bacteria need carbon as food source to survive, while facultative bacteria can get their oxygen by taking dissolved oxygen out of the water or by taking it off of nitrate molecules (Brady & Weil, 2008). Thus, denitrification may occur when oxygen levels are depleted and/or when nitrate become the primary oxygen source for microorganisms. There are various factors affecting the denitrification process, including dissolved oxygen, pH, organic matter and temperature (Myrold, 1998).

The process is performed under anaerobic conditions, when the dissolved oxygen concentration drops below 0.5 mg L^{-1}, ideally less than 0.2 (Marschner, 1995). When bacteria break nitrate apart to gain the oxygen, the nitrate is reduced to nitrous oxide (N_2O) and in turn into nitrogen gas (N_2). Since nitrogen gas has low water solubility, it escapes (volatilizes) into the atmosphere (Brady & Weil, 2008).

The optimum soil pH for denitrification ranges between 7.0 and 8.5. Denitrification is an alkaline producing process. Approximately 1.4 to 1.6 kg of alkalinity (as $CaCO_3$) is produced per kg nitrate, thus partially mitigating the lowered soil pH caused by nitrification in the soil solution (Brady & Weil, 2008). Soil temperature also affects the growth rate of denitrifying organisms, with greater growth rate at higher temperatures. Denitrification can occur between 5 and 30°C and these rates increase with temperature and type of organic source present (Havlin *et al.*, 1999).

2.3.4   Nitrogen gains and losses

The goal of N fertilization is to add N-containing fertilizers in a form and a specific location in order to minimize losses and maximize plant uptake (Jensen, 2006).

2.3.4.1   Gains

It is useful to look at the most general available N fertilizers with reference to the N-cycle to help understand how to maximize plant N uptake (Jensen, 2006). Therefore a short discussion on the two selected N fertilizers will be given:

Ammonium nitrate

Ammonium nitrate contains 34% N. Half of this N is in the nitrate form. Nitrate is subjected to denitrification losses immediately after irrigation or if waterlogged conditions are to be expected. Therefore ammonium nitrate is applied close to the time period to be used by a crop. Ammonium nitrate is commonly used as a surface broadcast application in the spring for grass crops. It is however not subjected to ammonia volatilization losses unless applied to soils exceeding a pH of 8.5 (Havlin *et al.*, 1999; Jensen, 2006).

Urea

Urea has the highest analysis of N of all granular fertilizers, namely 46%. It is hydrolyzed by urease enzymes in the soil in order to form free ammonia. Surface applied N from urea may be lost in the form of ammonia volatilization. However, volatilization losses under cool conditions are minimal compared to applications under warm conditions; where losses may range between 15 and 30% if irrigation does not follow soon after applications. Once irrigated with sufficient water, urea hydrolyzes into ammonia and then into ammonium. This minimizes N losses in the soil and enhances N uptake. The two ammonium ions, resulting from the urea molecule, are then nitrified to nitrate ions. Urea is in general applied either as a broadcast or incorporated application (Marschner, 1995; Havlin *et al.*, 1999; Jensen, 2006).

2.3.4.2 Losses

The most common losses of nitrogen from the soil have been found to be a result of ammonia volatilization, leaching and ammonium fixation. The prospect for ammonia volatilization is greatest when ammonium (urea) fertilizer is surface-applied where soil conditions promote rapid granular dissolution, but restricts the movement of ammonium into soil (McInnes *et al.*, 1986; Clay *et al.*, 1990), especially to alkaline and calcareous soils (Al-Kanani & McKenzie, 1996). Conditions that affect ammonia volatilization include physical and chemical soil properties as well as the temperature and moisture regime (Hargrove, 1988; Kissel & Cabrera, 1988). Ammonia volatilization substantially reduces the amount of nitrogen available to the growing crop and may contribute to both localized and long-range pollution (Rawluk *et al.*, 2001). Ammonia volatilization is also more pronounced at high pH soils (Brady & Weil, 2008).

By applying excess amounts of ammonium fertilizer to the soil to compensate for possible ammonia volatilization losses, results in an increase of N_2O in the soil as well as nitrate leaching to groundwater (Rawluk *et al.*, 2001). Nitrate is the primary form of nitrogen that

is leached from the soil. Nitrates originate from manures, the decay of plants and other organic materials or from fertilizers. Nitrate is however very mobile and is easily moved by water in the soil solution. It also has a direct impact on water quality. This is because heavy rains can cause nitrates to leach downward in the soil below the root zone (Killpack & Buchholz, 1993).

Ammonium may also be lost due to fixation to clay minerals. The positively charged ammonium ions are attracted to the negatively charged surfaces clays and humus. Here these ions are held in an exchangeable form for uptake by plants, but partially protected from leaching. However, due to the particular size of ammonium ions, it may become trapped within the structure of 2:1 type clays, such as vermiculite. Ammonium fixation by clay minerals is in general greater in the subsoil compared to the topsoil. This is due to the high clay content in clay subsoils. Ammonium fixation accounts for 5 to 10% of the nitrogen found in the surface soil and 20 to 40% of the nitrogen in the subsoil (Brady & Weil, 2008).

2.3.5 Summary of nitrogen in the soil-plant system

From all the nitrogen fertilizers globally available, ammonium nitrate, urea, anhydrous ammonia and ammonium sulphate are most widely used. Nitrate sources are available immediately after application if sufficient water is available whereas ammonium sources must first be nitrified to nitrate. The nitrification process of ammonium into nitrate takes at least 14 days, however this process may even take as long as 21 days. Plants absorb N as both dissolved NO_3^- and NH_4^+ ions. The preferred choice of source however depends on the crop. Furthermore, plants supplied with the NH_4^+ have been found to show an increase in both carbohydrate and protein levels, thus NH_4^+ is the preferred N source for plants.

Nitrate is more mobile compared to ammonium. Ultimately, N may be lost through leaching and ammonia volatilization as it moves through the soil cycle, but proper placement may reduce the extent of such losses. Proper placement of N fertilizers at a distance of 50 mm below and away from the maize seed decreases the possibility of N toxicity. Over supply of N during vegetative growth may cause a plant to become top-heavy and ultimately prone to falling over. Excessive supply of N also degrades the quality of most fruits and vegetables. Ultimately only 30 to 70% of applied N is effectively used by the plant.

2.4 Conclusion

Nitrogen and phosphorus fertilizers are essential nutrients for both accelerated vegetative growth and maximum yield. An important advantage of commercial N and P fertilizers is that these nutrients are already in a form that can be absorbed by the plant immediately after fertilization. However, accelerated input of these two nutrients can result in major ecological ecosystem problems. Decision on these nutrients should include the consideration of both form and total nutrient concentration, since these two factors determine availability and also accessibility. Phosphorus balances are also less complex compared to N balances. This is attributed to the fact that P lacks a significant gaseous phase, is less mobile than N, and P fluxes are dominated by physical rather than biological processes. These fundamental differences between N and P have important implications for management efforts.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site, design and layout

3.1.1 Experimental site

The study was conducted under controlled conditions in a glasshouse at the University of the Free State, Bloemfontein, South Africa.

3.1.2 Experimental design

Two pot experiments were conducted during the 2012/2013 maize growing season. The first one was planted on 3 January 2013 (first planting) and the second on 13 March 2013 (second planting). Both experiments were independently subjected to a three-factor randomized complete block design (RCBD) with a factorial combination (Figure 3.1). The three factors included two nitrogen sources *viz.* urea and LAN, three phosphorus sources *viz.* monoammonium phosphate (MAP), nitrophosphate (NP) and ammonium polyphosphate (APP) at five different application levels *viz.* (0, 10, 20, 30 and 40 kg P ha^{-1}), replicated three times. Urea and LAN were used to compensate for the N in the P sources when applied at different levels.

3.1.3 Experimental layout

Refer to Figure 3.1.

3.2 Agronomic practices

3.2.1 Pots and soil

A total of 90 polyethylene pots (Figure 3.2 a) were used for each planting, *viz.* 45 pots at the urea- and LAN-based experiments, respectively. The pot dimensions were 0.34 x 0.34 m (length x width) with a height of 0.35 m (40.5 L).

Each pot had three openings on opposite sides (Figure 3.2 a). Each opening was 51 mm in cross section and was placed in the following manner: The centre of the first opening was 80 mm from the brim in the middle of the pot, while the two underlying openings were each 50 mm away and to the side of the centre of the first opening. The above mentioned spacing ensured that root core samples could be taken within the fertilized

Urea-based experiment

Block 1

10 kg P ha^{-1} MAP	30 kg P ha^{-1} APP	40 kg P ha^{-1} NP
0 kg P ha^{-1} MAP	10 kg P ha^{-1} NP	20 kg P ha^{-1} NP
20 kg P ha^{-1} APP	40 kg P ha^{-1} MAP	10 kg P ha^{-1} APP
30 kg P ha^{-1} MAP	0 kg P ha^{-1} NP	0 kg P ha^{-1} APP
40 kg P ha^{-1} APP	20 kg P ha^{-1} MAP	30 kg P ha^{-1} NP

Block 2

20 kg P ha^{-1} MAP	30 kg P ha^{-1} MAP	0 kg P ha^{-1} NP
10 kg P ha^{-1} APP	40 kg P ha^{-1} NP	20 kg P ha^{-1} APP
30 kg P ha^{-1} NP	10 kg P ha^{-1} MAP	30 kg P ha^{-1} APP
40 kg P ha^{-1} APP	20 kg P ha^{-1} NP	10 kg P ha^{-1} NP
0 kg P ha^{-1} MAP	40 kg P ha^{-1} MAP	0 kg P ha^{-1} APP

Block 3

10 kg P ha^{-1} NP	40 kg P ha^{-1} MAP	20 kg P ha^{-1} APP
0 kg P ha^{-1} APP	30 kg P ha^{-1} NP	40 kg P ha^{-1} APP
30 kg P ha^{-1} MAP	20 kg P ha^{-1} NP	0 kg P ha^{-1} MAP
40 kg P ha^{-1} NP	10 kg P ha^{-1} APP	30 kg P ha^{-1} APP
20 kg P ha^{-1} MAP	0 kg P ha^{-1} NP	10 kg P ha^{-1} MAP

LAN-based experiment

Block 1

10 kg P ha^{-1} MAP	30 kg P ha^{-1} APP	40 kg P ha^{-1} NP
0 kg P ha^{-1} MAP	10 kg P ha^{-1} NP	20 kg P ha^{-1} NP
20 kg P ha^{-1} APP	40 kg P ha^{-1} MAP	10 kg P ha^{-1} APP
30 kg P ha^{-1} MAP	0 kg P ha^{-1} NP	0 kg P ha^{-1} APP
40 kg P ha^{-1} APP	20 kg P ha^{-1} MAP	30 kg P ha^{-1} NP

Block 2

20 kg P ha^{-1} MAP	30 kg P ha^{-1} MAP	0 kg P ha^{-1} NP
10 kg P ha^{-1} APP	40 kg P ha^{-1} NP	20 kg P ha^{-1} APP
30 kg P ha^{-1} NP	10 kg P ha^{-1} MAP	30 kg P ha^{-1} APP
40 kg P ha^{-1} APP	20 kg P ha^{-1} NP	10 kg P ha^{-1} NP
0 kg P ha^{-1} MAP	40 kg P ha^{-1} MAP	0 kg P ha^{-1} APP

Block 3

10 kg P ha^{-1} NP	40 kg P ha^{-1} MAP	20 kg P ha^{-1} APP
0 kg P ha^{-1} APP	30 kg P ha^{-1} NP	40 kg P ha^{-1} APP
30 kg P ha^{-1} MAP	20 kg P ha^{-1} NP	0 kg P ha^{-1} MAP
40 kg P ha^{-1} NP	10 kg P ha^{-1} APP	30 kg P ha^{-1} APP
20 kg P ha^{-1} MAP	0 kg P ha^{-1} NP	10 kg P ha^{-1} MAP

Figure 3.1 Experimental layout consisting of a factorial combination (Three P sources: ammonium polyphosphate (APP), monoammonium phosphate (MAP) and nitrophosphate (NP) by P five P levels: 0, 10, 20, 30 and 40 kg P ha^{-1}) laid out as a randomized complete block design for each of the two N sources (urea(46) and LAN(28)).

band as well as to the side of the band, using a stainless steel-tube and guiding frame (Figure 3.2 b and c). These openings were closed during the duration of the experiment.

A topsoil was collected from a farm in the Bainsvlei region (-29.074712, 26.119389) and allowed to be air dried. The air dried topsoil was then sieved through a 2 mm screen to remove debris and weed seeds before placement in the polyethylene pots. A representative sample of the sieved soil were sent for analysis of pH (KCl), extractable P and S, and extractable Ca, Mg, K and N (Table 3.1).
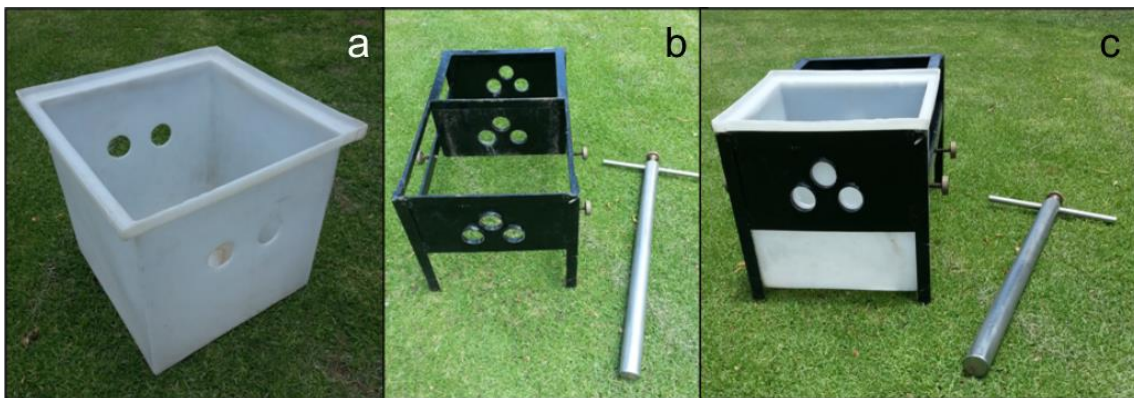
Figure 3.2   Delineation of a) the polyethylene pot, b) the stainless steel-tube and guiding frame and c) the pot within the guiding frame.

3.2.2 Fertilizer sources and treatments

Monoammonium phosphate (MAP (33)) and nitrophosphate (NP (30)) were used as orthophosphate sources and ammonium polyphosphate (APP (44)) as the polyphosphate source. Each of the three phosphorus sources was applied at five fertilization levels: 0, 10, 20, 30 and 40 kg P ha^{-1}. The relevant amounts were applied uniformly in a band at planting. Each source and level was calibrated against the highest nitrophosphate level of 40 kg P ha^{-1} to ensure that the nitrogen application stayed constant at 120 kg N ha^{-1}. The additional nitrogen needed for the calibration was uniformly band applied using urea(46) or LAN(28). The amounts of urea or LAN that were applied with MAP (Table 3.2), NP (Table 3.3) and APP (Table 3.4) to calibrate a constant N application at the five P levels are given in the mentioned tables. The certificates of analysis for ammonium polyphosphate (Table 3.5), nitrophosphate (Table 3.6) and monoammonium phosphate (Table 3.7) are also provided.

Table 3.1   Some properties of the topsoil used for the pot experiments

Soil sample (Lab number: 1757)	
Crop	Maize
Sample depth	Top-soil (50–150 mm) of a Bainsvlei form
Soil colour	Dark Brown
Sand (%)	78
Silt (%)	11
Clay (%)	11
Bulk density (kg.m^{-3})	1213
pH (KCl)	5.5 (M)
S (mg.kg^{-1})	5 (L)
P (mg.kg^{-1})	28 (M)
K (mg.kg^{-1})	150 (H)
K(% of ECEC)	15
Ca (mg.kg^{-1})	302 (L)
Ca(% of ECEC)	58
Mg (mg.kg^{-1})	38 (M)
Mg(% of ECEC)	26
Na (mg.kg^{-1})	5
Na(% of ECEC)	1
ECEC (cmol$_c$.kg^{-1}) Calculated	2.6
Ca / Mg	2.2
Mg / K	1.8
(Ca + Mg) / K	6
Zn (mg.kg^{-1})	0.7 (M)
Mn (mg.kg^{-1})	32.46 (VH)
Fe (mg.kg^{-1})	18 (VH)
Cu (mg.kg^{-1})	1 (H)
B (mg.kg^{-1})	0.17 (VL)
Organic C (% mm)	0.48

VL = Very Low   L = Low   M = Medium/Average   H = High

Extraction Methods: P – Bray I   Cations – NH$_4$OA$_c$

S – 0.1 N K$_2$SO$_4$ Fe,Mn,Zn,Cu – DTPA

B – Warm water extract   Organic C – Walkley-Black method

Table 3.2 Application rates for monoammonium phosphate (MAP) when calibrated with either urea or limestone ammonium nitrate (LAN)

Level of application (Treatments)	Source	Per hectare (kg ha^{-1})	Per pot (g 0.34 m^{-1} row)
MAP 0 kg P ha^{-1}	MAP	0	0
	Urea	261	8.137
MAP 10 kg P ha^{-1}	MAP	45	1.4178
	Urea	250	7.7982
MAP 20 kg P ha^{-1}	MAP	91	2.8357
	Urea	239	7.4591
MAP 30 kg P ha^{-1}	MAP	136	4.2535
	Urea	228	7.1201
MAP 40 kg P ha^{-1}	MAP	182	5.6714
	Urea	217	6.7810
MAP 0 kg P ha^{-1}	MAP	0	0
	LAN	429	13.3683
MAP 10 kg P ha^{-1}	MAP	45	1.4178
	LAN	411	12.8113
MAP 20 kg P ha^{-1}	MAP	91	2.8357
	LAN	393	12.2543
MAP 30 kg P ha^{-1}	MAP	136	4.2535
	LAN	375	11.6972
MAP 40 kg P ha^{-1}	MAP	182	5.6714
	LAN	357	11.1402

Table 3.3   Application rates for nitrophosphate (NP) when calibrated with either urea or limestone ammonium nitrate (LAN)

Level of application (Treatments)	Source	Per hectare (kg ha^{-1})	Per pot (g 0.34 m^{-1} row)
NP 0 kg P ha^{-1}	NP	0	0
	Urea	261	8.1372
NP 10 kg P ha^{-1}	NP	133	4.1590
	Urea	196	6.1029
NP 20 kg P ha^{-1}	NP	267	3.3180
	Urea	130	4.0686
NP 30 kg P ha^{-1}	NP	400	12.4771
	Urea	65	2.0343
NP 40 kg P ha^{-1}	NP	533	16.6361
	Urea	0	0
NP 0 kg P ha^{-1}	NP	0	0
	LAN	429	13.3683
NP 10 kg P ha^{-1}	NP	133	4.1590
	LAN	321	10.0262
NP 20 kg P ha^{-1}	NP	267	8.3180
	LAN	214	6.6841
NP 30 kg P ha^{-1}	NP	400	12.4771
	LAN	107	3.3421
NP 40 kg P ha^{-1}	NP	533	16.6361
	LAN	0	0

Table 3.4   Application rates for ammonium polyphosphate (APP) when calibrated with either urea or limestone ammonium nitrate (LAN)

Level of application (Treatments)	Source	Per hectare (kg ha^{-1})	Per pot (g 0.34 m^{-1} row)
APP 0 kg P ha^{-1}	APP	0	0
	Urea	260.87	8.1372
APP 10 kg P ha^{-1}	APP	31.35	0.9778
	Urea	251.06	7.8311
APP 20 kg P ha^{-1}	APP	62.70	1.9557
	Urea	241.24	7.5250
APP 30 kg P ha^{-1}	APP	94.04	2.9335
	Urea	231.43	7.2189
APP 40 kg P ha^{-1}	APP	125	3.9113
	Urea	221.62	6.9128
APP 0 kg P ha^{-1}	APP	0	0
	LAN	428.57	13.3683
APP 10 kg P ha^{-1}	APP	31.35	0.9778
	LAN	412.45	12.8654
APP 20 kg P ha^{-1}	APP	62.70	1.9557
	LAN	396.33	12.3625
APP 30 kg P ha^{-1}	APP	94.04	2.9335
	LAN	380.21	11.8596
APP 40 kg P ha^{-1}	APP	125.39	3.9113
	LAN	364.08	11.3568

Table 3.5   Ammonium polyphosphate analysis

Name of item: Ammonium polyphosphate   **Formula**: $(NH_4PO_3)n$

Molecular weight: Varying, 97 (Number average)   **CAS NO.**: 68333-79-9

Type of test	Standard	Observed
Description	White powder	White powder
Phosphate content	31.0 to 32%	31.5%
Nitrogen content	14%	14.4%
pH Value (10% suspended liquid)	5.5 to 7.0	6.2
Moisture content	< 0.25%	0.2%
Density	Approx. 1.9	Approx. 1.9
Melting point	>270°C	>270°C
Velocity (mpas)	100	100
Water solubility (g/100^3 water)	Approx. 0.5	Passes Test
Average particle size	Approx. 0.5	0.5

Table 3.6   Nitrophosphate analysis

Name of item: Nitrophosphate   **Formula**: Not provided

Molecular weight: Not provided   **CAS NO.**: Not provided

Type of test	Standard	Observed
Description	White powder	White powder
Phosphate content	7.5%	7.5%
Nitrogen content – NH_4	≥ 12.4%	12.36%
Nitrogen content – NO_3	≥ 6.5%	6.49%
Calcium content	Not provided	3.92%
Sulphate content	Not provided	6.88%
pH Value	Not provided	3.6
Average particle size	2 to 4 mm: ≥90%	0.64 mm

Table 3.7   Monoammonium phosphate analysis

Name of item: Monoammonium phosphate   **Formula**: $(NH_4H_2PO_4)$

Molecular weight: Varying, 115.03 (Number average)   **CAS NO.**: 7722-76-1

Type of test	Standard	Observed
Description	Granular	Granular
Phosphate content	≥ 51%	52%
Nitrogen content	≥ 11%	11%
pH Value (20% suspended liquid)	4.4	4.4
Moisture content	< 0.15%	0.15%
Density	Approx. 1.803	Approx. 1.803
Melting point	>180°C	>180°C
Water solubility (g/100^3 water)	Approx. 0.1	Passes Test
Average particle size	2 to 4 mm: ≥90%	0.64 mm

3.2.3   Seed and fertilizer placement

Each polyethylene pot was filled with soil to the level of fertilization (130 mm below the brim). The fertilizer was then band applied in a single 0.34 m line; 130 mm below and 50 mm away from in the middle of the pot. The latter was done to reduce the risk of burning the seeds and/or the seedlings. After the fertilizer was placed, 50 mm of soil was added to planting depth. Six maize seeds (cultivar; PAN 6126) per pot were then planted 80 mm below the pot brim and consequently in the middle of the pot. Another 50 mm of soil was then added on top of the seeds as to allow for a 30 mm clearing from the top of the pot brim; which allowed for sufficient water application.

The amount of fertilizer placed simulated maize planted with a between row spacing of 0.91 m (109 rows ha^{-1}). After emergence (ten days after planting) the maize seedlings were thinned to a total of three plants per pot.

3.2.4   Irrigation scheduling

The drained upper limit (ƟDUL) of the soil in the pots was determined to serve as basis for irrigation during the experiments. This was done by weighing six pots with the required amount of dry sieved soil. The pots were then saturated with water and left to stand for 48 hours. The pots were then again weighed in order to obtain a predetermined ƟDUL.

The treated pots were weighed every three/four days throughout the experiments, followed by the application of the necessary amount of distilled water to maintain the soil

at the predetermined ϴDUL. Distilled water was used in order to avoid the mineral content of treated municipal water.

3.2.5 Glasshouse temperatures

Glasshouse temperatures were maintained at 25°C (±1°C) during the day and 18°C (±1°C) during the night to ensure optimum growth conditions. The optimum temperatures for germination, seedling, leaf, stem and root development ranges between 24 and 27°C (Olsen & Sander, 1988). Disease and insect outbreak was visually monitored during the five week (8 leaf stage) vegetative growth period.

3.3 Measurements and analysis
3.3.1 Morphological parameters

All the above and below ground parameters listed below, were measured during the first five weeks (after emergence) of the plant's vegetative growth.

3.3.1.1 Aerial plant parameters

Aerial plant parameters included amount of leaves, stem thickness, plant height, leaf area and dry mass.

Amount of leaves: Only the fully developed leaves were counted weekly. Leaves were considered fully developed when the ligule/leaf collar was easily recognizable.

Stem thickness: Stem thickness was measured 10 mm above the soil surface, perpendicular to the main vein of the outer leaf sheath; on a weekly basis in millimetres (mm).

Plant height: Was measured from the soil surface up to the highest point of the maize plant; on a weekly basis in millimetres (mm).

Leaf area: At termination of an experiment all photosynthetic active leaves were removed from the stems by cutting the leaf behind the leaf collar. The leaves were then put through a LICOR 3000 leaf area meter, determining each plant's leaf area in cm².

Dry mass: After determining the leaf area, each individual plant's leaves together with its stems was placed in a paper bag and dried in an oven for 1 week at 60°C. After the drying cycle, each plant was

individually weighed to obtain its dry mass in grams (g). After the dry mass was determined, the plant material was finely ground in preparation for plant nutrient analysis.

3.3.1.2 Subsoil plant parameters

Following removal of the aerial plant organs, subsoil parameters such as root length and root mass were determined (Emmanuel, 2000). For this root core samples were taken using a stainless steel-sampling tube. Samples were taken, within the fertilized zone as well as the unfertilized zone, through the two bottom 51 mm openings of the pots. The remainder of the roots was measured separately. The roots were separated from the soil by washing it with water over a 0.5 mm sieve.

Root length: A modified infrared root line intersection counter was used to determine the length of the roots in mm.

Dry root mass: After roots were dried at 60°C for 48 hours in an oven, root mass (g) was obtained through weighing.

3.3.2 Plant nutrient analysis

Nitrogen in the aerial plant parts was determined by combustion of the grounded samples in a Leco TruSpec® CN analyser. For the determination of P, K, Ca, Mg, Cu, Fe, Mn, Zn, B and Mo the grounded plant samples were incinerated at 500°C for at least 3 hours, allowed to cool before wetted with concentrated nitric acid and then incinerated for another hour. After cooling, 10 ml of a 1:2 distilled water to nitric acid solution was added to the silica crucibles, heated on a sand bath and when warm enough washed over into a 100 ml volumetric flask with distilled water. The P in this solution was determined colorimetrically while the other nutrients were determined with atomic absorption spectrometry, as prescribed by manufacturers (Handbook of Standard Soil Testing Methods for Advisory Purposes, 1990).

3.4 Statistical analysis

Data was subjected to statistical analyses using analytical software SAS version 9.2® for Windows. A three-factor randomized complete block design with a factorial combination was employed for analysis of variance (ANOVA). The minimum significant difference (MSD) was calculated at $P \leq 0.05$. This allowed for treatment means to be compared using the Tukey-Kramer multiple comparison test (SAS Institute, 2000).

CHAPTER 4

INFLUENCE OF PHOSPHORUS AND NITROGEN SOURCES ON AERIAL- AND SUBSOIL PARAMETERS DURING THE EARLY GROWTH AND DEVELOPMENT OF MAIZE (*Zea mays* L.)

4.1 Introduction

Maize cultivated under irrigation contributed 47.2% of the total (9.7 million tons on average) maize produced during the 2010/2011 production season in South Africa (National Department of Agriculture and Forestry, 2011; South African Government Information, 2012). To sustain this biological productivity the use of inorganic nitrogen and phosphorus sources is inevitable (Boyhan *et al.*, 2007). Therefore, special attention should be given to the relative proportion and form of nutrient application for plant uptake during initial fertilization (Emmanuel, 2000).

Nitrogen plays a critical role during the vegetative growth of maize (Haynes, 1986). This is because N is a primary building block of plant compounds such as chlorophyll, nucleotides, proteins and amino acids (Havlin *et al.*, 1999). Nitrogen is also vital for plant cell integrity as well as for plant metabolic processes (Haynes, 1986; Havlin *et al.*, 1999). Proper N management is therefore critical in ensuring optimum vegetative growth especially where leaching of N is a major concern (Ranthamane, 2001).

Nitrogen is absorbed by plants in both ammonium and nitrate forms and may be a major limiting factor in many agricultural soils (Meisinger *et al.*, 1992; Bennett, 1993). When N availability is limited the general vegetative growth of the plant will be limited (Balko & Russel, 1980).

Phosphorus, similar to nitrogen play a vital role in the life cycle of plants and is important for both the vegetative and reproductive growth of maize; which is evident in the contribution of P to yield increases (35%) when N and P are simultaneously applied (Smith, 1976; Bennett, 1993). Phosphorus is also vital in various biological processes and best known for processes of energy transfer and storage via ADP and ATP compounds (Steward, 1990).

The P fraction within the orthophosphates (MAP and NP) vary between approximately 30 to 40% while the P fraction within the polyphosphate (APP) fraction vary between 50 and 55%. Polyphosphate forms are usually hydrolysed into orthophosphate forms.

Uptake at a low soil pH will primarily occur as an orthophosphate ion, HPO_4^{-2} (Noack *et al.*, 2010). Since P is so immobile and prevalent to fixation and precipitation in low pH soils, a major limitation to the growth of maize is consequently the inadequate supply of P (Larsen, 1967). Increased P application rates, via band placement, may assist in overcoming the above mentioned problems encountered with P availability within a low pH soil (Havlin *et al.*, 1999).

According to Kosugi *et al.* (1964) cited by Engelbrecht *et al.* (2007), the interaction between N and P fertilization and nutrient uptake may influence the overall growth of plants. This is attributed to either the antagonistic or synergistic response between the two nutrients. The interaction between N and P is ultimately dependent on various soil and crop conditions (Engelbrecht *et al.*, 2010).

Taking all factors ascribed to nitrogen and phosphorus into consideration an investigation into the response of maize to the various N and P forms is justified. The objective of this study is to evaluate the quantitative maize growth parameters' response to different nitrogen and phosphorus sources as well as different P application rates during the early growth (5 weeks) of maize.

4.2 Materials and methods

Refer to Chapter 3, Sections 3.1, 3.2 and 3.3.

4.3 Results and discussion

4.3.1 Aerial plant parameters

Only the aerial plant parameters that were significantly influenced by nitrogen source, phosphorus source and phosphorus application rate will be included in the discussion. A summary on the analysis of variance evaluating the effect of treatment factors, *viz.* N source, P source and P application rate on the aerial plant parameters is presented in Table 4.1.

The overall significant response to nitrogen source on the aerial parameters measured is stronger reflected during the first than the second planting (Table 4.1). The aerial response to phosphorus source, though apparent throughout both plantings, appear to have had a greater influence during the first planting, while the same trend is true for phosphorus application rate (Table 4.1).

The coefficient of variation for aerial parameters varies between 4.2 to 11.8%.

Table 4.1   Aerial growth indicators as affected by nitrogen source, phosphorus source and phosphorus application rate for the first and second planting

	Leaf count					Stem thickness					Plant height					Leaf area	Dry mass
Week	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	5	5
First planting																	
N Source (N)	ns	*	*	*	*	*	*	*	*	*	ns	*	*	*	*	*	*
P Source (P)	ns	ns	ns	ns	ns	ns	*	*	*	*	ns	*	*	ns	ns	*	*
P Rate (PR)	ns	*	*	ns	*	ns	*	*	*	*	ns	*	*	*	*	*	*
N×P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×PR	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns
P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
N×P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Second planting																	
N Source (N)	ns	*	ns	*	ns	*	ns	*	*	ns	ns	*	ns	*	*	*	*
P Source (P)	ns	ns	*	ns	ns	*	ns	*	*	ns	ns	ns	ns	ns	ns	*	*
P Rate (PR)	ns	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns	*	ns
N×P	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*	ns
N×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*= significant (P≤0.05), ns = not significant.

4.3.1.1 Leaf count

Leaf count is an indication of both crop growth and development. Generally, every third to fourth day of growth one fully developed leaf appear (Warrington & Kanemasu, 1983). However, ultra-fast maize hybrids like PAN6126 produce a lower amount of leaves compared to medium-long and long season growing cultivars (Almeida *et al.*, 2000; Sangoi, 2001). The cultivation of an ultra-fast hybrid maize plant with fewer, however more erect leaves ultimately increases radiation use efficiency (Loomis & Connor, 1992); this is because the level of interference between each individual plant and leaf is lower (Sangoi & Salvador, 1998). Though fewer leaves, leaf count can also be used for ultra-fast hybrids to compare crop growth and development response.

Nitrogen source significantly influenced leaf count during both plantings (first planting $p < 0.0001$ and second planting $p = 0.0261$). Leaf count, with the exception of week one, was greater with LAN compared to urea-fertilized plants, throughout both plantings

(Figure 4.1 a,b). Leaf count during the first planting was significantly greater from week two to week five with LAN (Figure 4.1 a). However, during the second planting leaf count was only significantly greater when LAN was used compared to urea for week two and four (Figure 4.1 b).
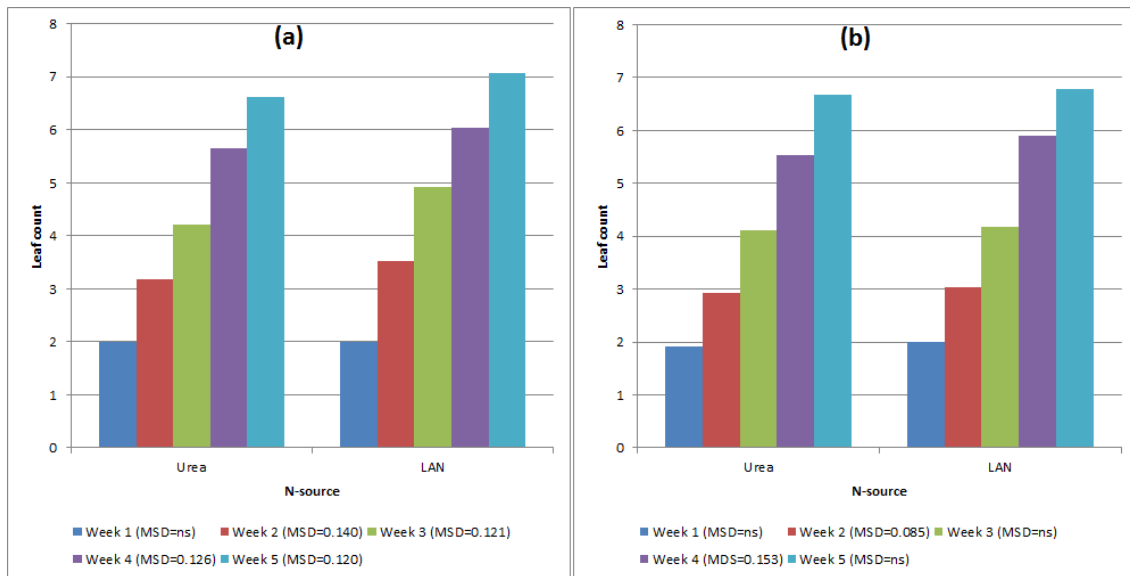

Figure 4.1   Leaf count as affected by nitrogen source during a) the first and b) the second planting.

Phosphorus source significantly influenced leaf count only during the third week of the second planting (p = 0.0306) (Table 4.1). Mono-ammonium phosphate yielded a significant higher leaf count compared to APP, while NP did not significantly differ from either the MAP or APP sources (Figure 4.2 b).

Phosphorus application rate influenced leaf count during the second (p = 0.0178), third (p = 0.0155) and fifth (p = 0.0263) week of the first planting only (Table 4.1). The amount of leaves for 0 kg P ha^{-1} (week 2) was significantly lower compared to that of 40 kg P ha^{-1}. The number of leaves for 10, 20 and 30 kg P ha^{-1} treated plants did not differ significantly from the control (0 kg P ha^{-1}) or from the 40 kg P ha^{-1} application treatment. This result was similar for week 3 and 5 (Figure 4.3).

Leaf count was significantly affected by the nitrogen by phosphorus source interaction (p = 0.0274) during the fifth week of the second planting (Table 4.1). The urea by APP treatment combination yielded a significantly lower leaf count compared to all other nitrogen by phosphorus source treatment combinations (Table 4.2).
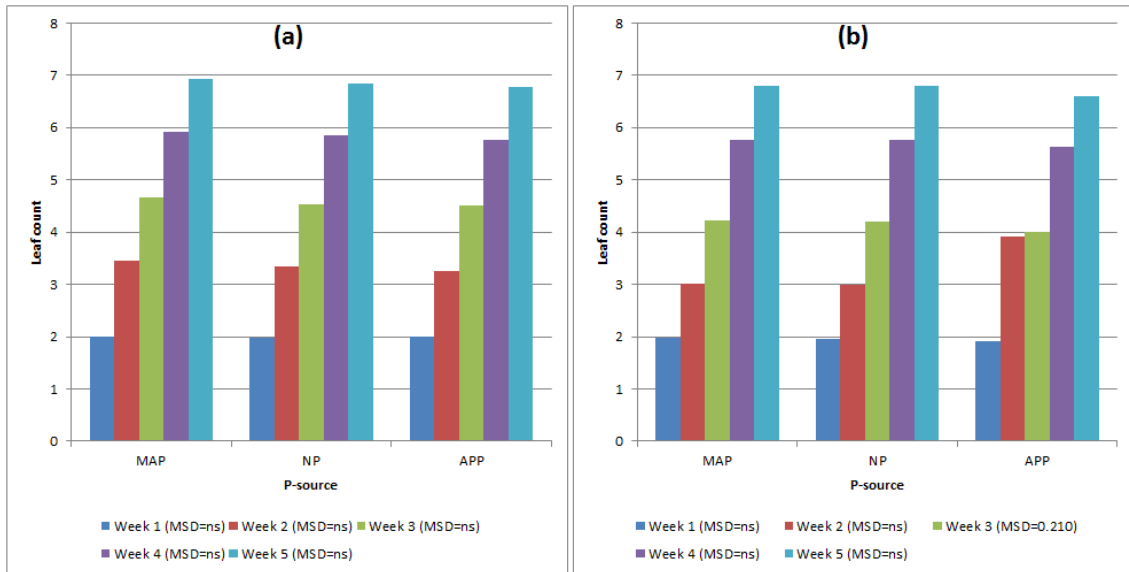
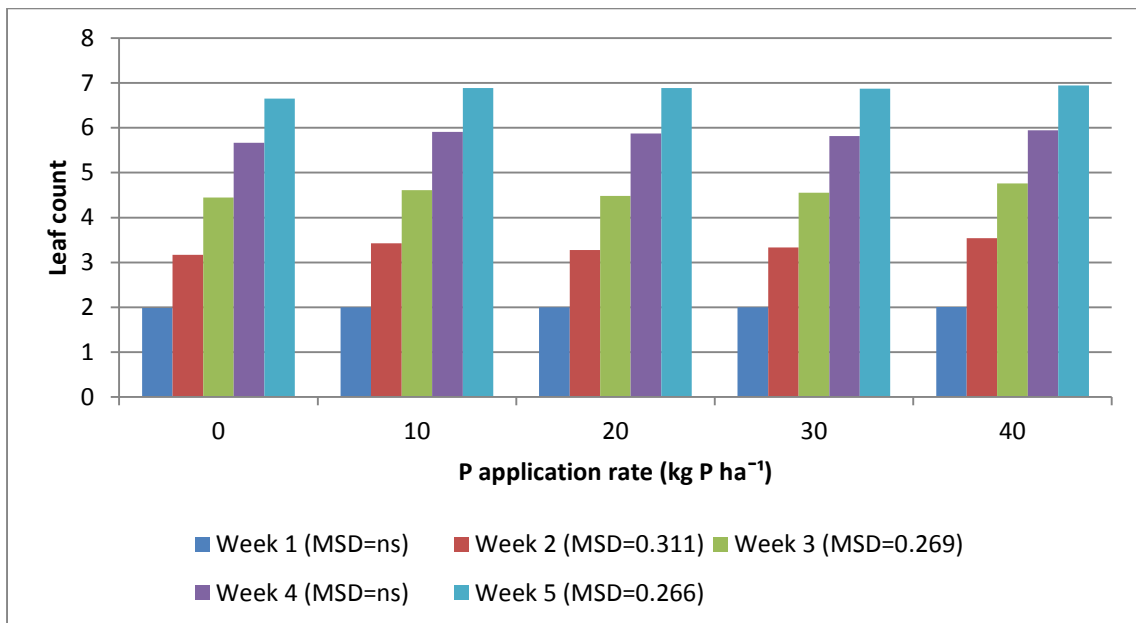**Figure 4.2**   Leaf count as affected by phosphorus source during a) the first and b) the second planting.

Figure 4.3   Leaf count as affected by phosphorus application rate during the first planting.

Table 4.2: Leaf count as affected by the nitrogen by phosphorus source interaction during week 5 of plant development for the second planting

			Nitrogen source (N)		Average (P)
			Urea	LAN	
Phosphorus source (P)		MAP	6.821$_a$	6.778$_a$	6.800
		NP	6.822$_a$	6.779$_a$	6.801
		APP	6.400$_b$	6.801$_a$	6.600
MSD$_{(0.05)}$ (N x P)			0.376		
Average (N)			6.681	6.786	

*Means followed by the same letter are not significantly different (MDS = 0.05)

An important aspect contributing to respectable early vegetative growth is having soil N in forms that are readily available for uptake and that are easily metabolized by the plant (Havlin *et al.*, 1999). Plants are able to absorb both nitrate and ammonium N forms during the early vegetative growth stage. The benefits of ammonium N forms depend on the chemistry in the soil as well as in the plant's metabolism (Bais *et al.*, 2006), however less energy is needed for the absorption of the nitrate form (Havlin e*t al.*, 1999). Inside the plant, the nitrate is transformed back into ammonium through energy provided by photosynthesis (Hodge & Fitter, 2010). The fact that LAN (Figure 4.1) plays such a major role in the rate of leaf development may be attributed to the ease of nitrate availability and absorption by root tissues via passive water uptake, assuring constant nitrogen supply in all but extremely dry soil conditions (Novoa & Loomis, 1981).

The increase in the importance of P on leaf development three weeks after emergence during the second planting (Figure 4.2 b) may be attributed to the important role (due to P demand) of P in cell division, cell expansion and enlargement during this time; which affects the vegetative development of maize (Amanullah *et al.*, 2009). The results of Figure 4.2 b, are explanatory in that ammonium polyphosphates are significantly different in agronomic performance to ammonium orthophosphates when applied at the same rates in a similar manner (Sharma, 1995). Phosphorus in soil solution should typically be similar for both P sources shortly after application since a major portion of polyphosphate hydrolysis is completed within 48 hours, however, the length of the orthophosphate hydrolysis process is still under serious discussion (Arrhenius *et al.*, 1997).

Two consequences of low P availability in the soil solution (0 kg P ha^{-1}) are: [1]a loss of cell integrity as well as [2]reduced energy charge within the plant cell (Sa & Israel, 1991).

Inorganic P is therefore extremely essential to the development of the leaves since it is vital in the generation of energy (Bieleski, 1973). The central role of P in the energy transfer processes therefore hardly allows for a decrease in the rate of P application (Vadez *et al.*, 1999) since low P availability will delay crop vigour and maturity (Havlin *et al.*, 1999). It appears that P application rates ranging between 10 and 40 kg P ha^{-1} are similar in the development rate of ultra-fast maize hybrid leaves (Figure 4.3) up to five weeks after emergence.

The interaction response of leaf development to the application of both nitrogen and phosphorus fertilizers (Table 4.2) varies depending on cultivar, time of planting, soil pH, availability of the nutrients and more importantly the growth stage (Vadez *et al.*, 1999). Chemical reactions in the soil therefore become especially important where inaccessible nutrients are converted into forms that are easily absorbed and translocated through the plant (Havlin *et al.*, 1999). As the plant's leafs increases so does the requirement for nutrients and the importance of soil chemistry (Davies *et al.*, 2002).

The results in Table 4.2 revealed that the urea and APP treatment combination resulted in a significantly lower leaf count than all other fertilizer source combinations. Both ammonium N and ammonium polyphosphate forms have to undergo oxidation which is pH dependent. The initial medium soil pH of 5.5 could have inhibited the hydrolysis/oxidation rate of both urea and APP, rendering nutrients available at lower concentrations compared to all other treatment combinations. In addition, the oxidation of ammonium into nitrate further decreases the soil pH, which in turn further reduces the conversion of ammonium into readily available forms (Havlin *et al.*, 1999). The reduction in soil pH in turn causes ammonium to be adsorbed onto the soil colloids, thus decreasing its availability even more (Havlin *et al.*, 1999).

4.3.1.2 Stem thickness

The stem has two functions; [1]to support the leaves and to [2]translocate water and nutrients to the necessary plant organs. As the stem expands and elongates the requirement for N and P availability increases accordingly (Gheysari *et al.*, 2009). Nutrient deficiencies will consequently result in a plant with a thinner stem and a slow initial growth rate (Tucker, 1999).

Nitrogen source, phosphorus source and phosphorus application rate, with the exception of week two and five of the second planting, significantly influenced stem thickness during both plantings (Table 4.1). It has to be noted that some of the main

effects/treatments are involved in interaction effects and will therefore be omitted during the main effect discussions (Table 4.1).

Nitrogen source significantly influenced the first planting's stem thickness (Table 4.1, Figure 4.4 a) throughout week one (p = 0.0113), two (p < 0.0001), three (p < 0.0001), four (p < 0.0001) and five (p < 0.0001). The second planting's stem thickness (Table 4.1, Figure 4.4 b) was significantly influenced by nitrogen source during week one (p = 0.0097), three (p < 0.0001) and four (p < 0.0001). Stem thickness, with the exception of the first week of vegetative growth, was significantly superior with LAN compared to urea-fertilized plants, throughout both plantings (Figure 4.4 a,b).
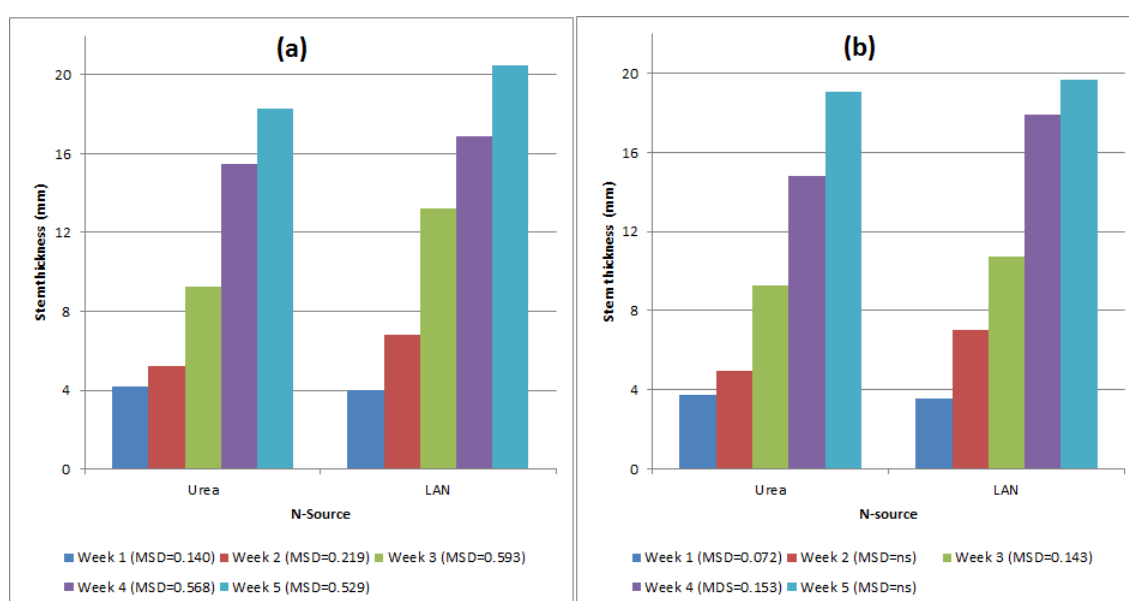

Figure 4.4   Stem thickness as affected by nitrogen source during a) the first and b) the second planting.

Phosphorus source significantly influenced stem thickness during both plantings (Table 4.1). More specifically, phosphorus source significantly influenced the first planting's stem thickness during week two (p = 0.0007), three (p = 0.0149), four (p = 0.0024) and five (p = 0.0097). The second planting's stem thickness was significantly influenced during week one (p = 0.0198), three (p = 0.0022) and four (p = 0.0052). Monoammonium phosphate plants yielded a significantly thicker stem when compared to the APP treated plants during both plantings (Figure 4.5 a,b). Nitrophosphate did not significantly differ from either the MAP or APP source during the first planting from week two onwards (Figure 4.5 a). During the second planting the stems of plants treated with NP was

significantly thicker than that of APP fertilized plants but not than that of MAP fertilized plants (Figure 4.5 b).
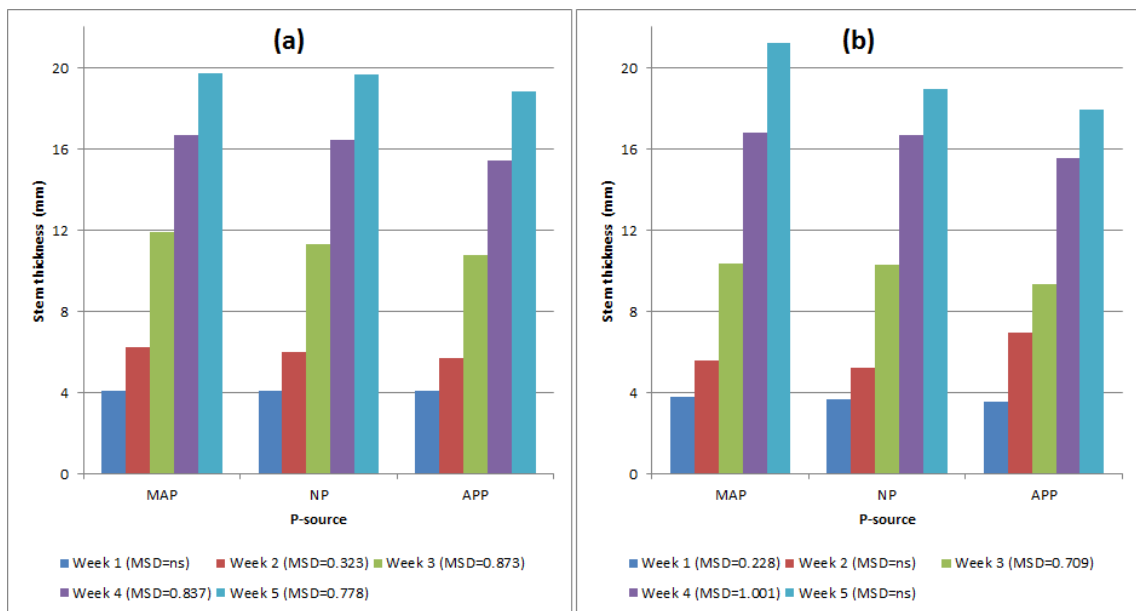

Figure 4.5   Stem thickness as affected by phosphorus source during a) the first and b) the second planting.

Phosphorus application rate significantly influenced stem thickness during the second (p = 0.0002), third (p < 0.0001), fourth (p < 0.0001) and fifth (p < 0.0001) week of the first planting (Table 4.1, Figure 4.6 a), while the second planting demonstrated that stem thickness was significantly influenced during week three (p = 0.0218) and four (p = 0.0068) of the vegetative growth period (Table 4.1, Figure 4.6 b).

The stems of plants during the first planting with 0 kg P ha^{-1} (week 2 and 4) was significantly thinner compared to that of plants fertilized at 40 kg P ha^{-1}. The stems of plants fertilized with P at rates of 10, 20 and 30 kg P ha^{-1} was neither significantly thicker than that of the control (0 kg P ha^{-1}) nor significantly thinner than that of the 40 kg P ha^{-1} application (Figure 4.6 a). This result was similar for week 3 and 4 of the second planting (Figure 4.6 b)
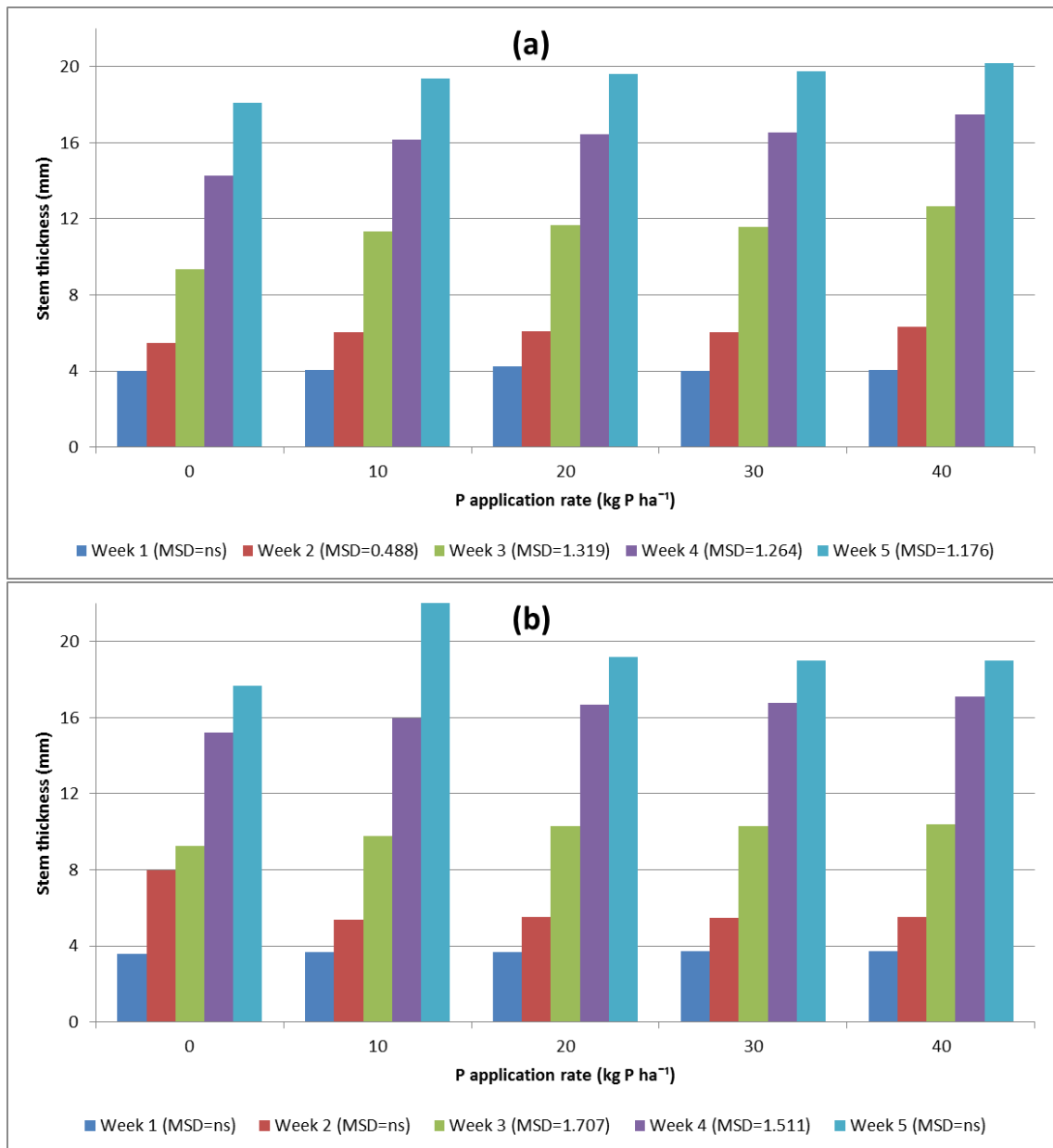
Figure 4.6 Stem thickness as affected by phosphorus application rate during a) the first and b) the second planting.

During week 3 and of the first planting the thickest stems were recorder at phosphorus applications of 20, 30 and 40 kg P ha^{-1} with LAN. The stems of all other treatment combinations were significantly thinner than the aforementioned treatment combinations (Table 4.3). The 40 kg P ha^{-1} application rate combined with LAN yielded overall the thickest plant stems (Table 4.3).

Table 4.3: Stem thickness (mm) as affected by a nitrogen source by phosphorus application rate interaction during week 3 of the vegetative growth period of the first planting

| | | Phosphorus application rate (kg P ha^{-1}) (PR) | | | | | Average (N) |
		0	10	20	30	40	
Nitrogen source (N)	Urea	8.256$_c$	9.407$_c$	9.162$_c$	9.419$_c$	10.861$_{bc}$	9.421
	LAN	10.413$_c$	13.291$_{ab}$	14.204$_a$	13.759$_{ab}$	14.414$_a$	13.216
MSD$_{(0.05)}$ (N x PR)		2.924					
Average (PR)		9.334	11.349	11.683	11.589	12.638	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Generally, the thickest stems were obtained with MAP and NP applied at rates ranging between 10 and 40 kg P ha^{-1} (Table 4.4). Although the thickest stems during week 5 for the first planting were recorded with NP at a rate of 40 kg P ha^{-1} it was not significantly thicker than the stems recorded at 10 to 40 kg P ha^{-1} for MAP and NP (Table 4.4).

Table 4.4: Stem thickness (mm) as affected by a phosphorus source by phosphorus application rate during week 5 of the vegetative growth period of the first planting

| | | Phosphorus application rate (kg P ha^{-1}) (PR) | | | | | Average (P) |
		0	10	20	30	40	
Phosphorus source (P)	MAP	18.600$_{bc}$	20.425$_{ab}$	20.402$_{ab}$	19.377$_{abc}$	19.720$_{abc}$	19.705
	NP	17.385$_c$	19.548$_{abc}$	19.350$_{abc}$	20.263$_{ab}$	21.898$_a$	19.689
	APP	18.270$_{bc}$	18.078$_{bc}$	19.073$_{bc}$	19.678$_{abc}$	18.942$_{bc}$	18.808
MSD$_{(0.05)}$ (P x PR)		2.557					
Average (PR)		18.085	19.351	19.608	19.773	20.187	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Nitrogen is needed for vigorous vegetative stem growth; even though the vast majority of N is only required after the eighth leaf has fully developed. The reason for LAN's superiority over urea in stem growth during the vegetative stage (Figure 4.4 a,b), may be ascribed to the ease of N uptake and availability. The nitrate portion (50%) within the LAN is immediately available after application whilst the ammonium portion is oxidized

(Havlin *et al.*, 1999). The significant response of maize stem thickness to N is attributed to the availability of P; if the P availability and uptake is optimised then the uptake of N increases accordingly (Gheysari *et al.*, 2009). The synergetic response between the two nutrients will consequently result in a plant with a thicker stem (Pagliari *et al.*, 2010).

Inorganic phosphorus is one of the least available plant nutrients in soils, even though it is essential for plant growth and development (Vance *et al.* 2003). However, once in the plant, P become very mobile and important for strong and thick stem development. Phosphorus stimulates early stem growth in this study (Figure 4.5 a,b). Tucker (1999) also reported that a deficiency can delay crop maturity and stem thickness. The reduction in growth can be attributed to the alteration of the cell division or cell elongation parameters (Vasellati *et al.*, 2001). It seems apparent to state that, even though phosphorus source did play a significant role in stem thickness (Figure 4.5 a,b) during both plantings, the concentration at which the source is available is of greater importance. Therefore, the potential of P in solution is determined by the rate of P absorption by plant roots, which is linearly correlated to increased P concentration (Havlin *et al.*, 1999).

Plants exposed to low P availability (Table 4.4) have been found to have thinner stems (Vasellati *et al.*, 2001). Plants suffering from phosphorus deficiency (Figure 4.6 a,b) showed retarded growth (McMurtrey, 1938; Alsaedi & Elprince, 2000) and a decrease in stem diameter (Lyon & Liang, 1944). This is because both the phloem and vascular bundles, important for nutrient transport, are smaller in size. Low phosphorus accessibility therefore stimulates a series of morphological, anatomical and physiological responses that maximize phosphorus needs (Raghothama, 1999), which is a result of internal phosphorus homeostasis (Ticconi & Abel, 2004). The interaction between P concentration and N source (Table 4.3) seem to be in accordance with the findings of Nissen (1974), where a decrease in available N (due to hydrolysis) limits the uptake of P. Phosphorus application level therefore becomes a mediating factor when applied with different sources of nitrogen (Kuiper *et al.*, 1988). This statement is also supported by the results of the third (Table 4.3) and fifth (Table 4.4) week of stem development during the first planting.

4.3.1.3 Plant height

Plant height is a well-established indicator of crop growth and a directly contributing parameter to biomass production (Wasaya *et al.*, 2012). However, maize hybrids have been found to differ in plant height compared to older cultivars used in the past (Stewart

& Dwyer, 1993). Plant height not only play an important role in plant lodging resistance, but is also closely correlated with grain yield, leaf number and flowering time (Troyer & Larkins, 1985; Mengel & Kirkby, 1987). Height differences is also related to nutrition competition (Schwinning & Weiner, 1998; Weiner, 1990). Therefore, adequate fertilization especially N and P is of vital importance (Agber & Ali, 2012).

Nitrogen source significantly influenced plant height during both plantings except during week one in both plantings and week three during the second planting (Table 4.1). Nitrogen significantly influenced plant height during week two ($p < 0.0001$), three ($p < 0.0001$), four ($p = 0.0008$) and five ($p < 0.0001$) of the first planting (Figure 4.7 a), while the second planting's plant height (Figure 4.7 b) was significantly influenced during week two ($p = 0.0149$), four ($p < 0.0001$) and five ($p < 0.0001$). Plant height, with the exception of week one, was greater with LAN compared to urea (Figure 4.7 a,b).
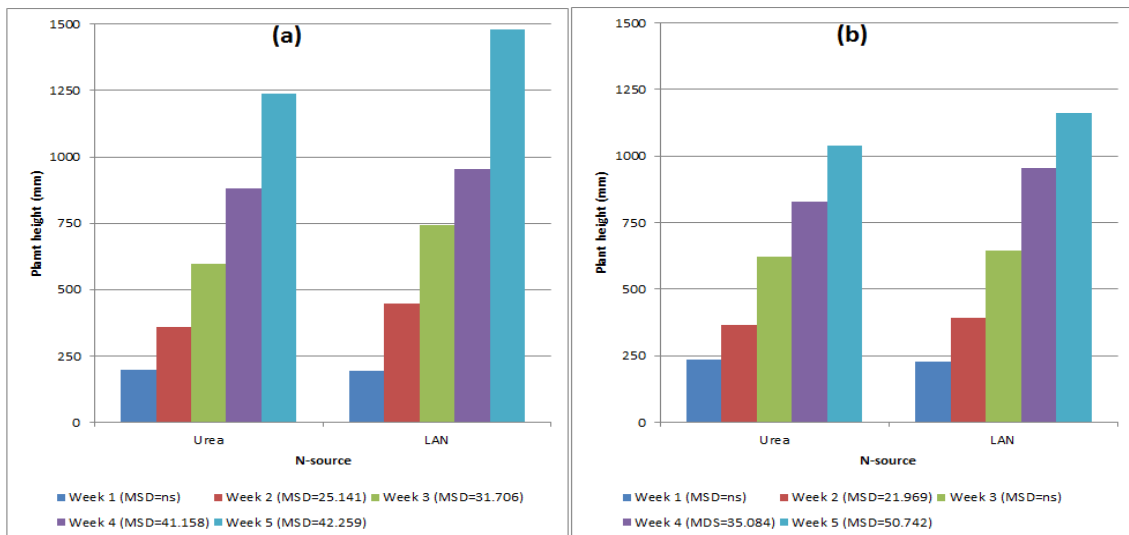

Figure 4.7   Plant height as affected by nitrogen source during a) the first and b) the second planting.

Phosphorus source significantly influenced plant height during week two ($p = 0.0144$) and three ($p = 0.0047$) of the first planting only (Table 4.1, Figure 4.8). MAP yielded a significantly taller plant in comparison to APP, while the plant height of NP did not significantly differ from that of either MAP or APP sources (Figure 4.8).

Phosphorus application rate significantly influence plant height during week two ($p = 0.0037$), three ($p < 0.0001$), four ($p < 0.0001$) and five ($p < 0.0001$) of the first planting only (Table 4.1). Plant height from week 2 to 5 of the first planting with 0 kg P ha^{-1} was significantly lower compared to that of 40 kg P ha^{-1}. Plant height of plants treated with

10, 20 and 30 kg P ha^{-1} was not significantly different than the control (0 kg P ha^{-1}) or the 40 kg P ha^{-1} treated plants (Figure 4.9).
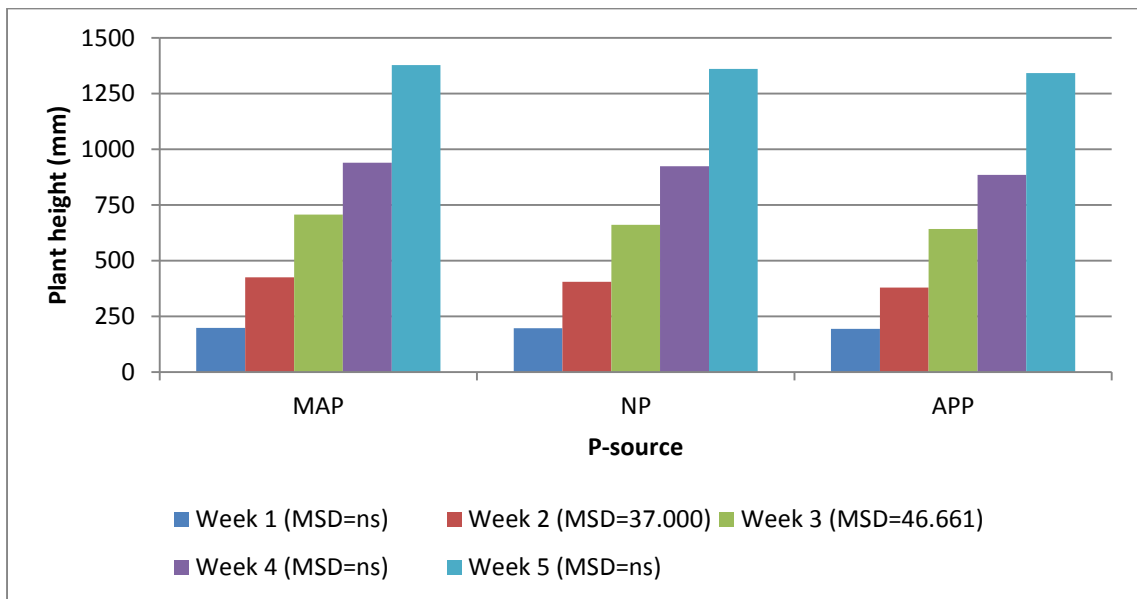

Figure 4.8   Plant height as affected by phosphorus source during the first planting.
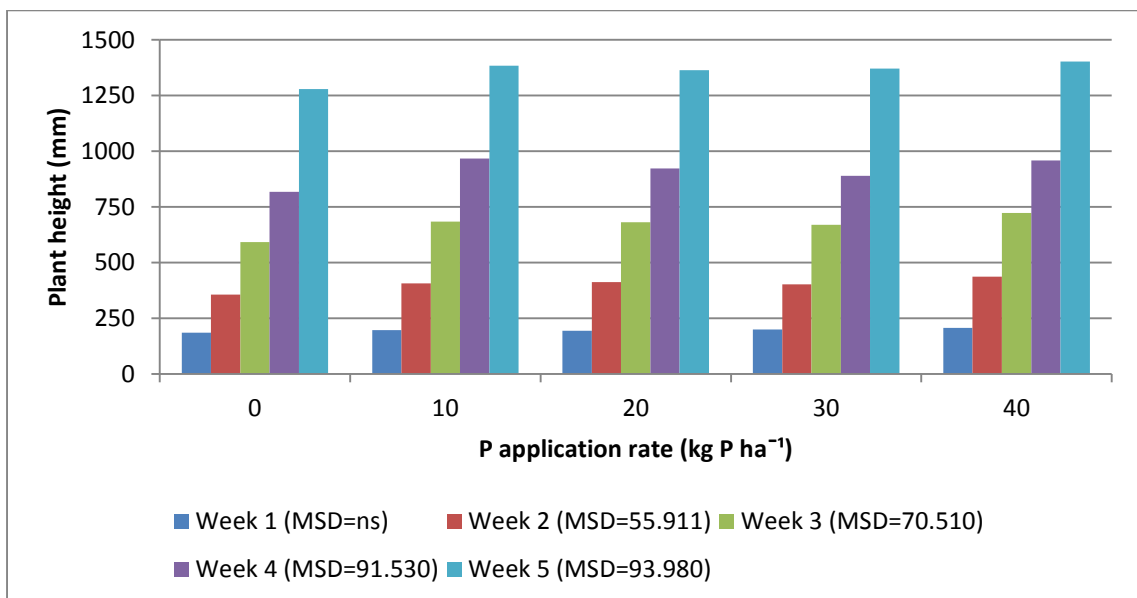

Figure 4.9   Plant height as affected by phosphorus application rate during the first planting.

During the fourth week of the first planting plant height was greater for all LAN by phosphorus application rate treatments compared to that of the plants for urea by phosphorus application rate treatments (Table 4.5). The tallest plants were recorded with

the LAN by 10 kg P ha^{-1} treatment combination. These plants were not significantly taller than that of the LAN by 20, 30 and 40 kg P ha^{-1} as well as urea by 40 kg P ha^{-1} combination. The shortest plants were recorded for both urea and LAN with 0 kg P ha^{-1} (Table 4.5).

Table 4.5: Plant height (cm) as affected by a nitrogen source by phosphorus application rate interaction during the fourth week of the vegetative growth period of the first planting

| | | Phosphorus application rate (kg P ha^{-1}) (PR) | | | | | Average (N) |
		0	10	20	30	40	
Nitrogen source (N)	Urea	814.814$_c$	865.184$_{bc}$	893.147$_{bc}$	847.295$_{bc}$	978.703$_{ab}$	879.930
	LAN	821.666$_c$	1069.167$_a$	950.926$_{abc}$	931.481$_{abc}$	991.295$_{ab}$	952.910
MSD$_{(0.05)}$ (N x PR)		151.174					
Average (PR)		818.240	967.180	922.040	889.390	985.000	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Plant height was only significantly affected by the interaction of N and P source treatments during the third week of the first planting (Table 4.6). The tallest plants were recorded for both the urea by MAP and LAN by APP treatments. Although these plant heights were the tallest, it did not differ significantly from any of the other treatment combinations with the exception of the urea by APP treatment (Table 4.6). It can therefore, with regard to plant height, be concluded that any of the selected N by P source combinations could be used, except for the urea by APP treatments in this instance (Table 4.6).

Nitrogen availability is important for the vegetative elongation of maize (Marschner, 1995). Maize plants can utilize either ammonium or nitrate as N source however, the degree of effectiveness between the two forms on plant height may be cultivar specific (Errebhi & Wilcox, 1990). The superiority of LAN may be attributed to the nitrate fraction that requires less energy for absorption (Hodge & Fitter, 2010). Retarded plant height with urea compared to LAN-fertilized plants (Figure 4.7) may be attributed to reduced water use efficiency within the maize plant after the passive absorption of ammonium; which directly reduces the uptake efficiency of nutrients (Goyal & Huffaker, 1984).

The total photosynthesis rate of nitrate-fed plants has been found to be higher compared to that of ammonium-fed plants (Cramer & Lewis, 1993). Decreased photosynthesis (as

affected by N source applied) rate may decrease the uptake of nutrients via the roots as well as the translocation of assimilates through the plant (Cramer & Lewis, 1993). The early growth and development of maize is less affected by nitrate N sources compared to ammonium N sources (Errebhi & Wilcox, 1990), which is in accordance with the results of Figure 4.7.

Table 4.6: Plant height (cm) as affected by a phosphorus source by phosphorus application rate during the third week of the vegetative growth period of the first planting

			Nitrogen source (N)		Average (P)
			Urea	LAN	
Phosphorus source (P)		**MAP**	662.001$_a$	646.333$_{ab}$	654.170
		NP	640.999$_{ab}$	630.889$_{ab}$	635.940
		APP	562.222$_b$	661.668$_a$	611.950
$_{MSD(0.05)}$ **(N x P)**			85.432		
Average (N)			621.740	646.300	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Phosphorus rank second to nitrogen as limiting factor among nutrient elements for plant height (Al Sharif *et al.*, 2004), but the application of P fertilizers has proven to be essential for the improvement of the vegetative elongation of maize (Rashid & Memon, 2001; Withers *et al.*, 2001). Plant height variation between the P sources were small even though MAP, which does not differ significantly from NP, yielded significant taller plants compared to that of APP (Figure 4.8). It appears that the significance of P is centred in its availability and concentration.

Maize is known to require large quantities of both N and P for superior growth with reference to height; therefore understanding the interaction between these two nutrients in Table 4.4 become of utmost importance (Agber & Ali, 2012). When N is amply supplied (in the case of the NP by urea interaction), then the plant height of ultra-fast maize hybrids increases as P availability increases (Sangoi & Salvador, 1998; Mahmood *et al.*, 2001; Ayub *et al.*, 2002; Aikins & Afukwa, 2010). Height increases are also due to increased proliferation of roots, which significantly increases the uptake of moisture and P (Aikins & Afukwa, 2010). Therefore, plant height is to a certain extend mediated by N availability. Low N availability reduces the uptake of P (Table 4.5) which has been found to reduce plant growth with up to 74% (De Groot *et al.*, 2003).

Limestone ammonium nitrate and urea should be equally efficient when band applied up to rates of 90 kg N ha^{-1} however, LAN becomes more efficient than urea when band applied at rates higher than 90 kg N ha^{-1} (Adriaanse & Human, 1993). Differences between LAN and urea may be ascribed to ammonium toxicity as a consequence of nitrification inhibition when band applied (Adriaanse & Human, 1993; Britto & Kronzucker, 2002). Phosphorus deficiencies, due to reduced N uptake using urea (Table 4.6), influence the plant's metabolism in a more direct manner (Schlüter et al., 2012). Decreased N and P uptake (Table 4.6) consequently negatively influence photosynthesis, protein synthesis (Simpson et al., 1982), cytokinin levels which will inhibit cell expansion (Rayle et al., 1982), energy availability needed for growth (De Groot et al., 2003), carbohydrates and related metabolites as well as important energy processes (Schlüter et al., 2012). Therefore, N availability in LAN for crop uptake compared to N availability in urea directly after application (Adriaanse & Human, 1993) has a great influence on how the plant will respond to varying levels of P application during the early vegetative growth period (Onasanya et al., 2009; Agber & Ali, 2012).

4.3.1.4 Leaf area

Leaf area is used to describe a plant's canopy size and consequently its photosynthetic capacity; two variables important in determining plant growth (Wasaya et al., 2012). Leaf area is therefore representative of the photosynthetic effectiveness in which carbon dioxide is assimilated and stored as photoassimilates (Plènet et al., 2000). Leaf area increases significantly by the application of inorganic nutrients (Biradar et al., 1994). During the vegetative growing period leaf area response is sink limited; meaning leaf area is determined by the number of cells capable of expansion and the rate of cell expansion when applied with both N and P (Plènet et al., 2000).

Nitrogen source significantly influenced leaf area during both the first (p < 0.0001) and second (p < 0.0001) planting. Leaf area was significantly greater with LAN compared to urea-fertilized plants when measured at the end of the fifth week of the first planting (Figure 4.10).

Phosphorus source significantly influenced leaf area during the first (p = 0.0095) and second (p = 0.0089) planting (Table 4.1). Once more only the first planting's results will be discussed as a result of the second planting's interaction (N by P) effect. MAP yielded a significant greater leaf area compared to APP, while NP did not significantly differ from either the MAP or APP source (Figure 4.11).
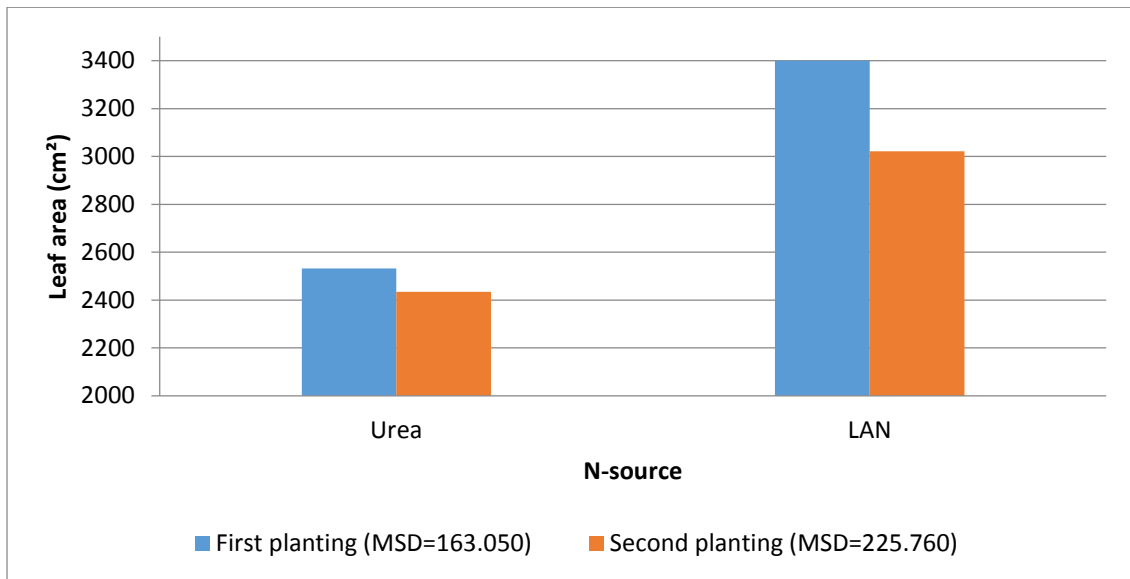
Figure 4.10   Leaf area as affected by nitrogen source at the end of week 5 for both plantings.

Phosphorus application rate influenced leaf area during both the first ($p < 0.0001$) and second ($p = 0.0151$) planting (Table 4.1). The leaf area for 0 kg P ha^{-1} was significantly smaller compared to that of 40 kg P ha^{-1}. Leaf area for the 10, 20 and 30 kg P ha^{-1} was neither significantly greater than that of the control (0 kg P ha^{-1}) nor significantly less than that of 40 kg P ha^{-1} for both plantings (Figure 4.12).
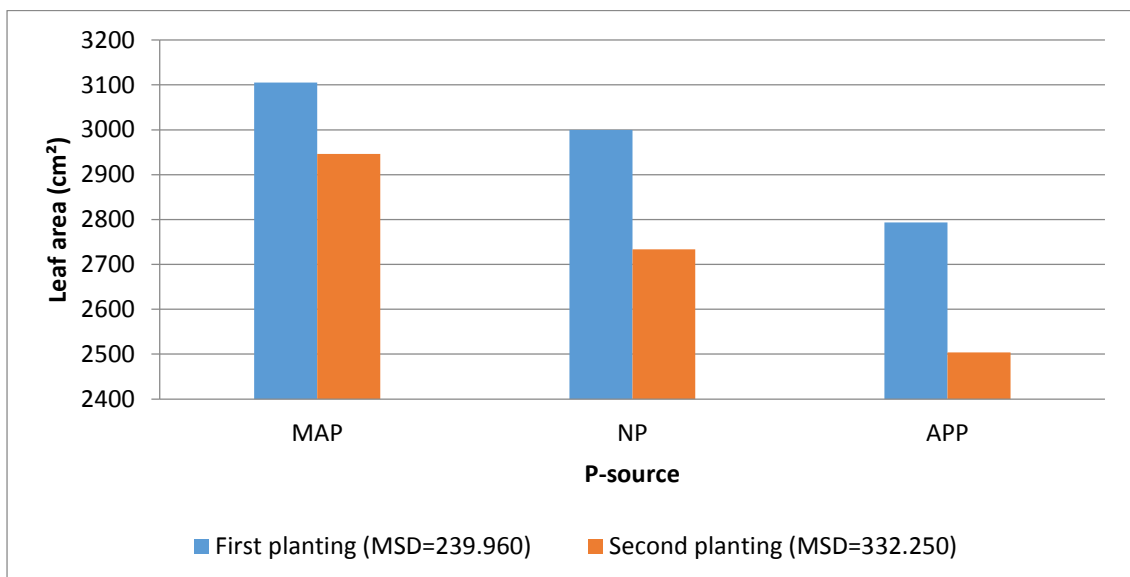

Figure 4.11   Leaf area as affected by phosphorus source at the end of week 5 for both plantings.
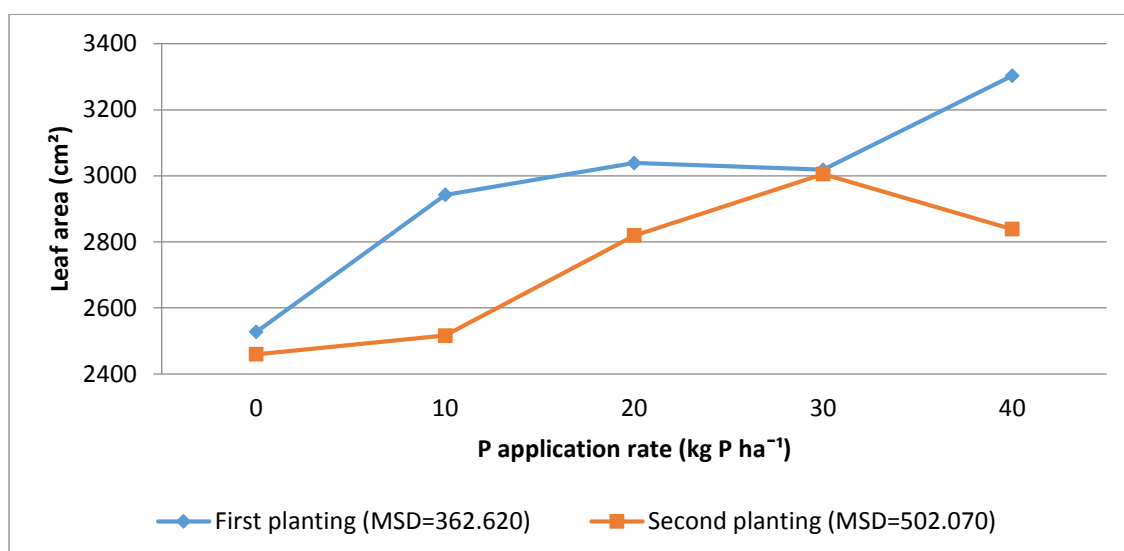
Figure 4.12 Leaf area as affected by phosphorus application rate at the end of week 5 for both plantings.

Leaf area at the end of the fifth week of the second planting was significantly ($p = 0.0274$) influenced by a nitrogen by phosphorus source interaction (Table 4.1). The LAN by MAP combination resulted in a leaf area significantly greater than the leaf area of a urea by NP as well as urea by APP combination. The leaf area of the latter treatment combination was significantly smaller than all other treatment combinations (Table 4.7).

Table 4.7: Leaf area as affected by the nitrogen by phosphorus source interaction at the end of the fifth week of plant development the second planting

			Nitrogen Source (N)		Average (P)
			Urea	LAN	
Phosphorus Source (P)		MAP	2731.830_{ab}	3160.330_a	2946.100
		NP	2573.470_b	2893.990_{ab}	2733.700
		APP	1997.510_c	3010.520_{ab}	2504.000
MSD$_{(0.05)}$ (N x P)			574.630		
Average (N)			2432.300	3021.600	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Nitrogen is necessary for a well-developed leaf area during the vegetative growth period which has been found to significantly increase with the application of N (Onasanya *et al.*,

2009). Limestone ammonium nitrate have been reported to yield a larger leaf area compared to urea (Figure 4.10) when band applied at rates higher than 90 kg N ha^{-1} (Adriaanse & Human, 1993). An explanation for the response in leaf area to N and P (Table 4.5) may be sought in the role of the plant's energy system (Mengel & Kirkby, 1987). Nitrogen, combined with different rates of P application (Figure 4.12) significantly increases maize leaf area (Agber & Ali, 2012). Phosphorus deficiencies affects the photosynthesis per unit of leaf area (Jacob & Lawlor, 1991). This suggests that both effects (reduction of leaf area and reduction of net photosynthesis per unit of leaf area) may contribute to the final reduction of biomass production (Qui & Israel, 1994; Rodriguez et al., 1998).

4.3.1.5 Dry mass

Dry mass can be defined as the plant's solid and chemical constituents excluding water, which include carbohydrates, proteins, vitamins and minerals (Hunt, 2003). Dry mass is a more reliable plant growth measure compared to fresh mass since it excludes the fluctuating water concentration of the plant (Smil, 2011). Consequently, dry mass reflects the relative efficiency at which a plant is able to transform nutrients in solution into a solid quantitative fraction (Hunt, 2003). Nutrients required for growth therefore plays a vital role in the development of dry mass (Schachtman *et al.*, 1998).

Nitrogen source significantly influenced dry mass production during the first ($p < 0.0001$) and second ($p < 0.0001$) planting (Table 4.1). Dry mass was significantly greater with LAN compared to urea-fertilized plants when measured at the end of the fifth week of the first and second planting (Figure 4.13).

Phosphorus source significantly influenced dry mass production during the first ($p = 0.0032$) and second ($p = 0.0085$) planting (Table 4.1). Mono-ammonium phosphate yielded plants with a significant greater dry mass compared to APP treated plants. Dry mass of NP treated plants NP did not significantly differ from either MAP or APP treated plants during both plantings (Figure 4.14).

Phosphorus application rate significantly influenced dry mass production during the first ($p < 0.0001$) planting only (Table 4.1). The dry mass results of the 0, 10 or 30 kg P ha^{-1} treated plants were significantly less than those treated with 20 and 40 kg P ha^{-1} (Figure 4.15).
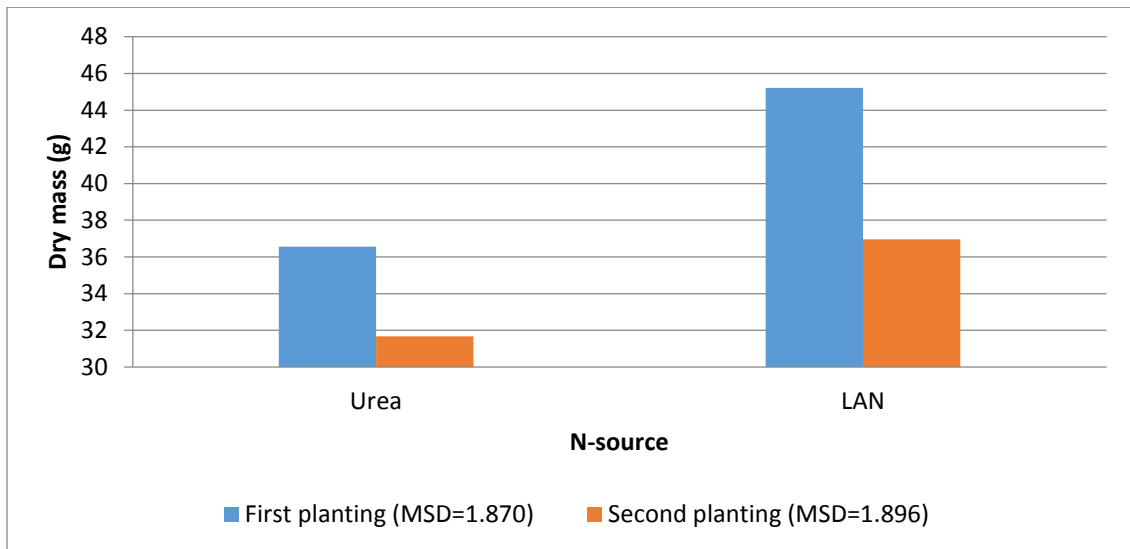
Figure 4.13 Dry mass as affected by nitrogen source at the end of week 5 for both plantings.
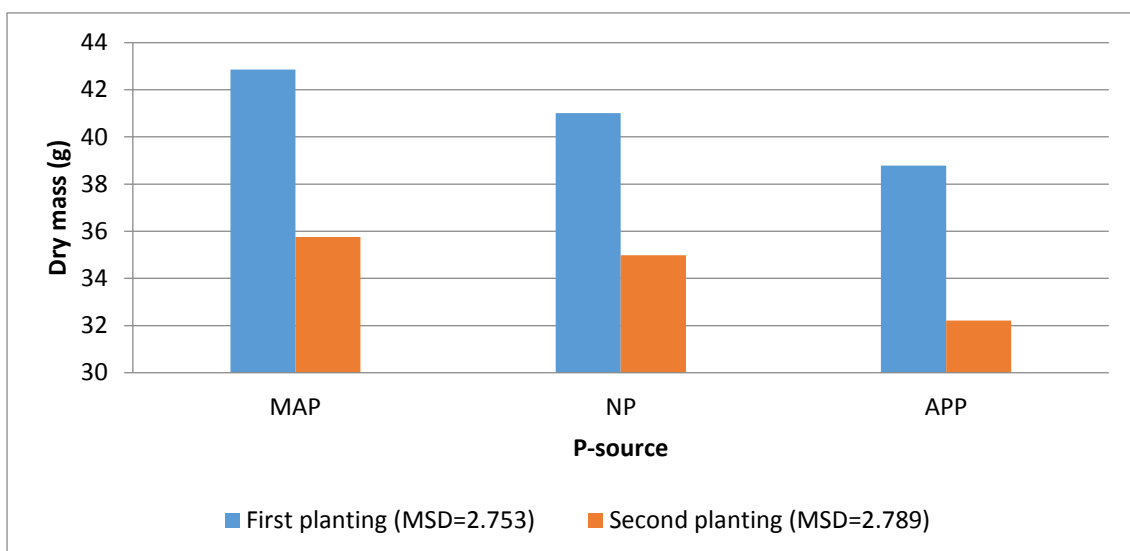

Figure 4.14 Dry mass as affected by phosphorus source at the end of week 5 of both plantings.

Nitrogen is a vital plant nutrient which makes up 1 to 4 % of a plant's dry matter (Onasanya *et al.*, 2009). Time and method of application are two important factors that significantly influence the production of dry mass during the vegetative growth period (Wasaya *et al.*, 2012). When band applied, nitrate N sources significantly increases the dry mass compared to ammonium and urea sources (Cramer & Lewis, 1993), which correlates with this study's results (Figure 4.13).

Lower dry mass production with the use of ammonium N sources during the vegetative growth period (Figure 4.13) is partly a result of altered carbon partitioning within the plant (Cramer & Lewis, 1993). Furthermore, a low-medium soil pH has been found to greatly suppress dry mass as a result of increased ammonium sensitivity and/or toxicity by the plants roots and metabolism (Britto & Kronzucker, 2002). The root system therefore plays an essential role in N uptake and dry matter partitioning (Peng *et al.*, 2012). The increase in dry mass response with increased N availability can be ascribed to increased production and translocation of photosynthates (Amanullah *et al.*, 2009).
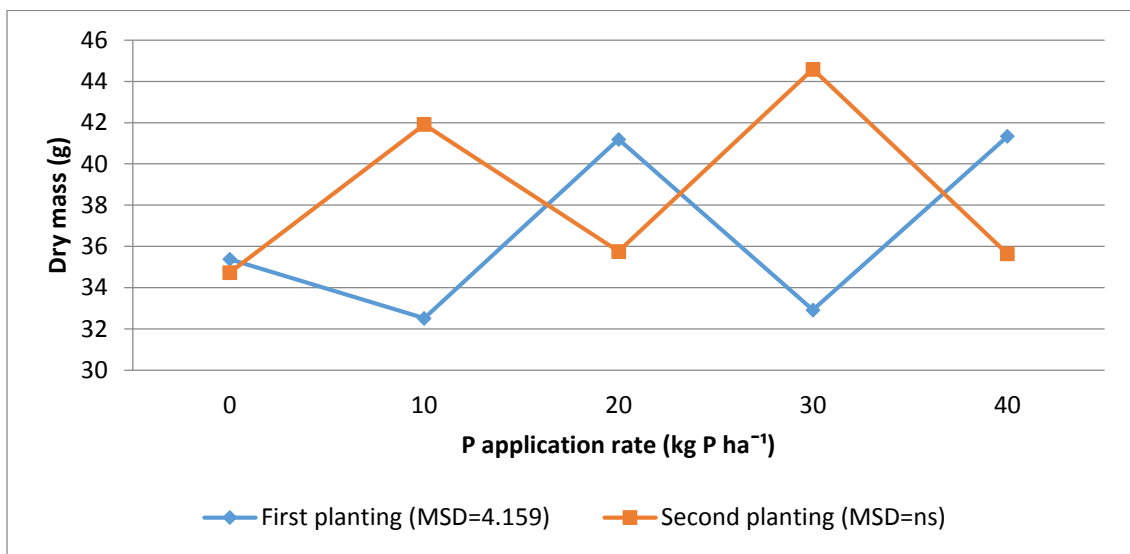

Figure 4.15   Dry mass as affected by phosphorus application rate at the end of week 5 for both the first planting and second planting.

4.3.1.6 Summary

The use of LAN as N source and MAP as P source significantly increased all aerial parameters measured throughout as well as at the end of the five week vegetative period, when compared to the other N and P sources used. A maximum P application rate of 40 kg P ha^{-1}, when compared to the control, yielded significantly greater aerial parameters throughout as well as at the end of the five week vegetative period. All other P application rates showed no significant differences when compared to both the control and optimum P application rates.

Interactions between the above mentioned main treatments (LAN, MAP and 40 kg P ha^{-1}) out performed all other treatments combinations. Both aforementioned can be ascribed to soil chemistry which favored hydrolysis and oxidation of ammonium and

phosphorus as well as the availability of nutrient ions which were easily taken up and translocated to sinks; which assist in significantly increasing the overall growth and development of maize after emergence.

4.3.2 Subsoil plant parameters

Only the subsoil plant parameters that were significantly influenced by nitrogen source, phosphorus source and phosphorus application rate will be included in the discussion. A summary on the analysis of variance evaluating the effect of treatment factors, *viz*. N source, P source and P application rate on the subsoil plant parameters is presented in Table 4.8.

The overall significant response to nitrogen source on the subsoil parameters measured is strongly reflected during both plantings (Table 4.8). The only significant reaction to P-rate was recorded by measurements of the root length of the fertilized zone. Two- and three way interactions were found, however no clear tendencies was evident between the two plantings (Table 4.8). The coefficient of variation for subsoil parameters varies between 2.1 to 21.4%.

The root system has three primary functions: the attainment of [1]water, [2]nutrients and [3]anchorage (Fitter *et al.*, 2002). The development of the root system provides a good indication of both the absorptive area and capacity of the root system to utilise soil nutrients (Van Tonder, 2008). Effective nutrient and water uptake therefore depends on the area of the root system that comes in contact with available nutrients; which is fundamentally represented by the root mass and root length (Zuo *et al.*, 2004). However, the development of the root parameters is dependent on when the nutrients are applied, on the quantity of nutrients applied, on the distance of placement from the developing roots as well as on the availability of nutrients (Emmanuel, 2000).

Table 4.8   Subsoil growth indicators as affected by nitrogen source, phosphorus source and phosphorus application rate at the end of the fifth week of plant development for both the first and second planting

Parameter	Root mass			Root length		
	Fertilized zone	Unfertilized zone	Total	Fertilized zone	Unfertilized zone	Total
First planting						
N Source (N)	*	*	*	*	*	*
P Source (P)	ns	ns	ns	ns	ns	ns
P Rate (PR)	ns	ns	ns	*	ns	ns
N×P	ns	ns	ns	*	ns	ns
N×PR	ns	ns	ns	ns	ns	ns
P×PR	ns	ns	ns	ns	*	ns
N×P×PR	*	ns	ns	ns	ns	ns
Second planting						
N Source (N)	ns	*	*	ns	*	*
P Source (P)	ns	ns	ns	ns	ns	ns
P Rate (PR)	ns	ns	ns	ns	ns	ns
N×P	*	*	ns	ns	ns	ns
N×PR	ns	ns	ns	ns	*	ns
P×PR	ns	ns	ns	ns	ns	ns
N×P×PR	*	*	ns	ns	ns	ns

*= significant (P≤0.05), ns = not significant.

4.3.2.1 Root mass

Root mass in the fertilized zone

The interaction between nitrogen source, phosphorus source and phosphorus application rate significantly influenced root mass in the fertilized zone during both the first (p = 0.0003) and second (p = 0.0402) planting (Table 4.8) as shown in Table 4.9 and Table 4.10 respectively.

Generally, the lowest root masses were recorded with urea as N source by almost all P sources and P application rates. The LAN by MAP by 10 kg P ha^{-1} interaction yielded the greatest root mass in the fertilized zone during the first planting (Table 4.9).

Table 4.9 Root mass (g) of the fertilized zone as affected by a nitrogen source by phosphorus source by phosphorus application rate interaction at the end of the fifth week of plant development during the first planting

Nitrogen source (N)	Phosphorus source (P)	Phosphorus application rate (kg P ha^{-1}) (PR)					Average (N x P)
		0	10	20	30	40	
Urea	MAP	2.860$_c$	3.009$_c$	2.999$_c$	3.145$_{abc}$	3.197$_{abc}$	3.042
	NP	2.918$_c$	2.925$_c$	2.923$_c$	2.976$_c$	3.078$_{bc}$	2.964
	APP	2.912$_c$	2.905$_c$	2.937$_c$	2.864$_c$	2.929$_c$	2.909
LAN	MAP	2.974$_c$	3.604$_a$	3.074$_{bc}$	3.250$_{abc}$	3.071$_{bc}$	3.195
	NP	3.147$_{abc}$	3.059$_{bc}$	3.289$_{abc}$	2.973$_c$	3.064$_{bc}$	3.106
	APP	2.987$_c$	2.995$_c$	3.218$_{abc}$	3.539$_{ab}$	3.533$_{ab}$	3.254
MSD$_{(0.05)}$ (N x P x PR)		0.505					
Average (PR)		2.966	3.083	3.073	3.125	3.145	

*Means followed by the same letter are not significantly different (MDS = 0.05)

During the second planting, with the exception of a few treatment combinations, all other treatment combinations resulted in a significantly greater root mass compared to that of the urea by MAP by 40 kg P ha^{-1}, which was the smallest root mass recorded (Table 4.10). The only consistency between the two plantings was the LAN by MAP by 10 kg P ha^{-1} treatment which resulted in greatest root mass in the fertilized zone five weeks after emergence.

Table 4.10 Root mass (g) of the fertilized zone as affected by a nitrogen source by phosphorus source by phosphorus application rate interaction at the end of the fifth week of plant development during the second planting

Nitrogen source (N)	Phosphorus source (P)	Phosphorus application rate (kg P ha^{-1}) (PR)					Average (N x P)
		0	10	20	30	40	
Urea	MAP	2.907$_a$	2.942$_a$	2.936$_a$	2.896$_{ab}$	2.565$_b$	2.849
	NP	2.878$_a$	2.913$_a$	2.948$_a$	2.906$_a$	2.939$_a$	2.917
	APP	2.929$_a$	2.859$_{ab}$	2.918$_a$	2.884$_a$	2.816$_{ab}$	2.881
LAN	MAP	2.869$_a$	3.013$_a$	2.964$_a$	2.934$_a$	2.903$_a$	2.937
	NP	2.984$_a$	2.919$_a$	2.881$_a$	2.954$_a$	2.835$_{ab}$	2.915
	APP	2.918$_a$	2.997$_a$	2.969$_a$	2.909$_a$	2.796$_{ab}$	2.918
MSD$_{(0.05)}$ (N x P x PR)		0.304					
Average (PR)		2.914	2.941	2.936	2.914	2.809	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Root mass in the unfertilized zone

Nitrogen source significantly influenced root mass of the unfertilized zone during the first (p < 0.0001) (Table 4.8). The interaction between N source, P source and P rate was significant during the second planting (Table 4.8). Root mass of the unfertilized zone was greater with LAN compared to urea-fertilized plants at the end of the fifth week of the first planting (Figure 4.16).
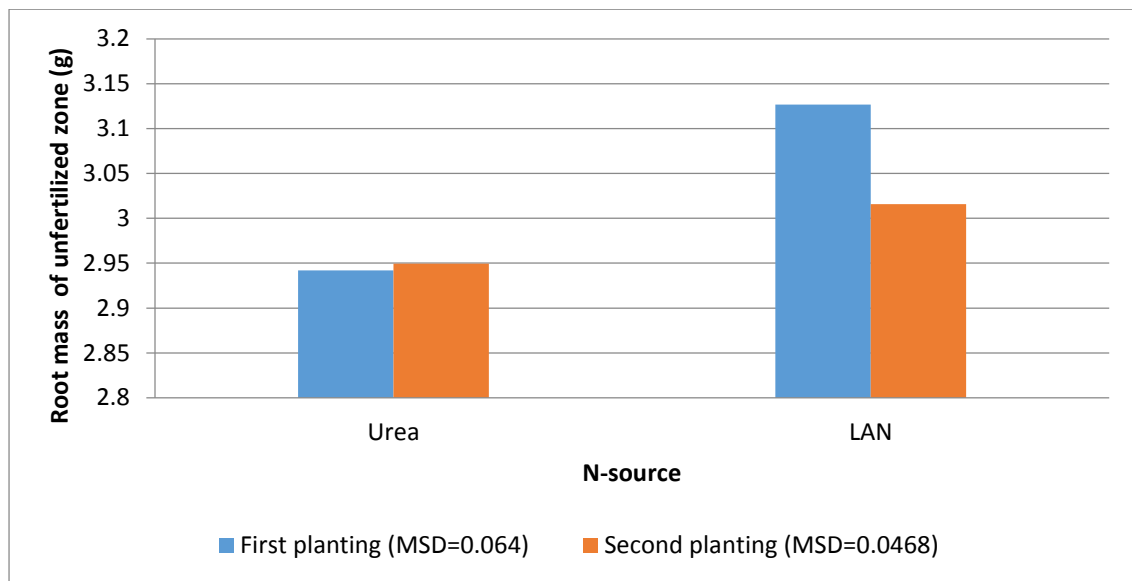

Figure 4.16   Root mass of the unfertilized zone as affected by nitrogen source at the end of week 5 for both the first and second planting.

During the second planting the greatest root mass was recorded with the LAN by APP by 10 kg P ha^{-1} interaction. Although the latter was the greatest root mass recorded, it was however not significantly greater than the three other treatment combinations (Table 4.11).

Table 4.11 Root mass (g) of the unfertilized zone as affected by a nitrogen source by phosphorus source by phosphorus application rate interaction at the end of the fifth week of plant development during the second planting

Nitrogen source (N)	Phosphorus source (P)	Phosphorus application rate (kg P ha^{-1}) (PR)					Average (N x P)
		0	10	20	30	40	
Urea	MAP	2.883$_b$	2.927$_b$	3.014$_b$	3.095$_{ab}$	3.093$_{ab}$	3.002
	NP	2.868$_b$	2.946$_b$	2.988$_b$	2.890$_b$	2.984$_b$	2.935
	APP	2.903$_b$	2.871$_b$	2.916$_b$	2.927$_b$	2.935$_b$	2.910
LAN	MAP	2.947$_b$	2.937$_b$	3.105$_{ab}$	3.057$_{ab}$	2.861$_b$	2.981
	NP	3.011$_b$	2.925$_b$	2.973$_b$	3.009$_b$	2.908$_b$	2.965
	APP	2.906$_b$	3.368$_a$	3.114$_{ab}$	3.070$_{ab}$	3.044$_{ab}$	3.100
MSD$_{(0.05)}$ (N x P x PR)		0.335					
Average (PR)		2.920	2.996	3.018	3.008	2.971	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Total root mass

Nitrogen source significantly influenced total root mass during the first ($p < 0.0001$) and second ($p = 0.0313$) planting. Total root mass was greater with LAN compared to urea-fertilized plants when measured at the end of the fifth week of both plantings (Figure 4.17).
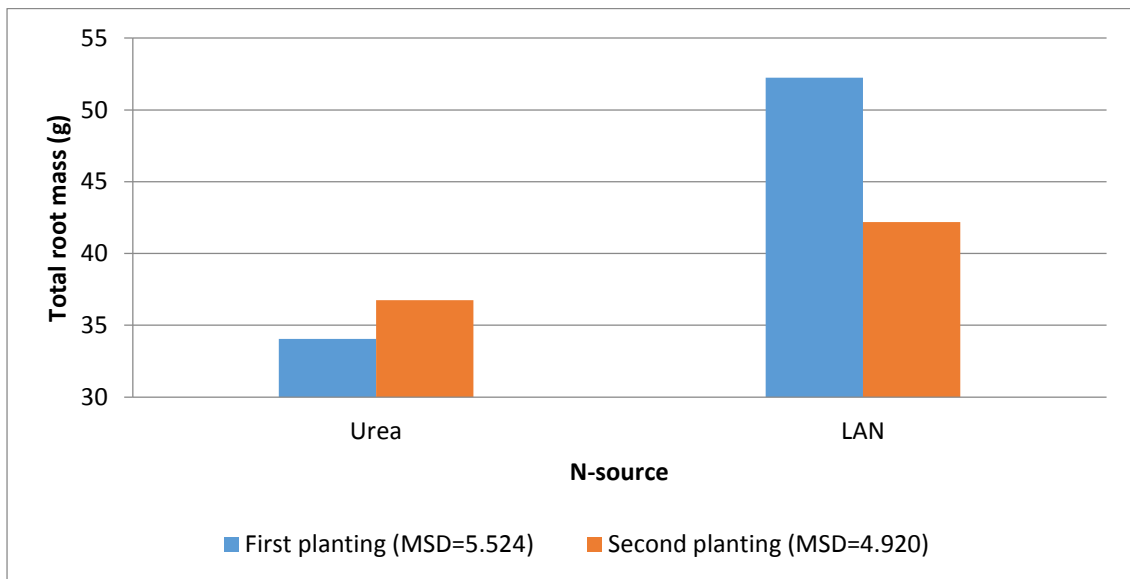

Figure 4.17 Total root mass as affected by nitrogen source at the end of week 5 for both the first and second planting.

4.3.2.2 Root length

Root length in the fertilized zone

Nitrogen source significantly influenced root length in the fertilized zone (p <0.0001) during the first planting however, only the main treatment (P-rate) will be discussed for the first planting since N source is involved in an interaction (N source by P source) during the first planting (Table 4.8).

Phosphorus application rate influenced root length of the fertilized zone during the first (p < 0.0001) planting (Figure 4.18). Root length in the fertilized zone of plants receiving 10 kg P ha^{-1} was significantly shorter compared to those plants treated with 40 kg P ha^{-1}. The root length of the fertilized zone at 0, 20 and 30 kg P ha^{-1} did not differ from either the control (0 kg P ha^{-1}) plants nor from the plants treated with 40 kg P ha^{-1} (Figure 4.18).

Root length of the fertilized zone was also significantly affected by a nitrogen by phosphorus source interaction (p = 0.0035) during the first planting (Table 4.8). The LAN by APP interaction yielded the longest root length in the fertilized zone during the first planting (Table 4.12). The shortest root length was recorded for the urea by APP interaction. This interaction effect was significantly smaller than all other treatment combinations with the exception of the urea by NP interaction (Table 4.12).
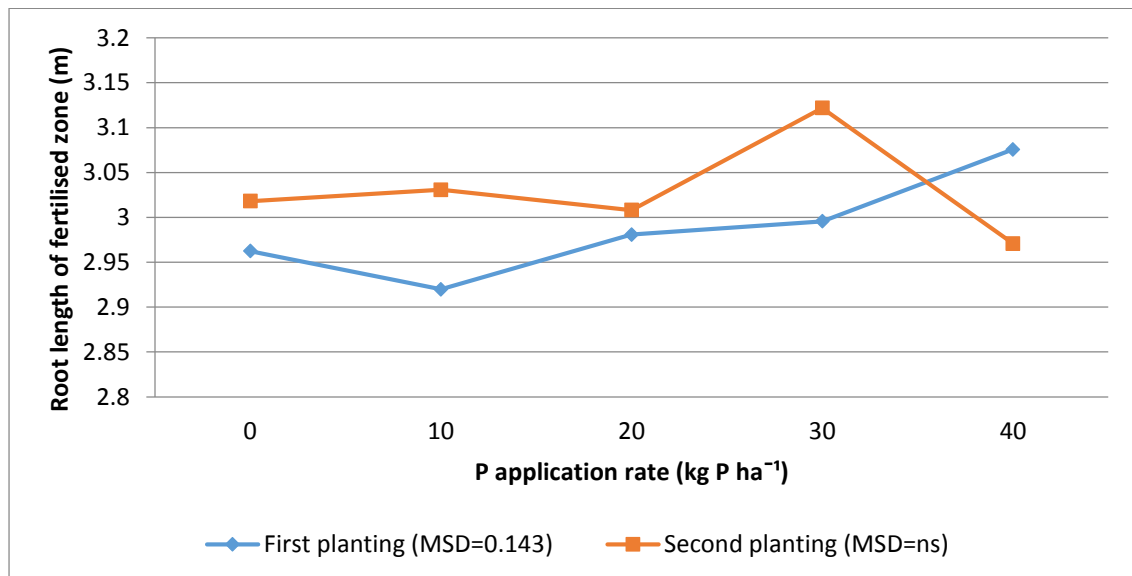

Figure 4.18   Root length of the fertilized zone as affected by phosphorus application rate at the end of week 5 for both the planting and second planting.

Table 4.12 Root length (m) in the fertilized zone as affected by a nitrogen source by phosphorus source interaction at the end of the fifth week of plant development during the first planting

		Nitrogen Source (N)		Average (P)
		Urea	LAN	
Phosphorus Source (P)	MAP	1.891_{ab}	2.179_a	2.035
	NP	1.453_{bc}	2.118_{ab}	1.786
	APP	0.814_c	2.235_a	1.525
MSD$_{(0.05)}$ (N x P)		0.679		
Average (N)		1.386	2.177	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Root length in the unfertilized zone

Nitrogen source significantly influenced root length of the unfertilized zone during the first ($p < 0.0001$) planting (Figure 4.19). Root length of the unfertilized zone was greater with LAN compared to urea-fertilized plants when measured at the end of the fifth week of both plantings (Figure 4.19). Only the first planting will be discussed since an interaction (N source by P rate) effect was found during the second planting (Table 4.8).
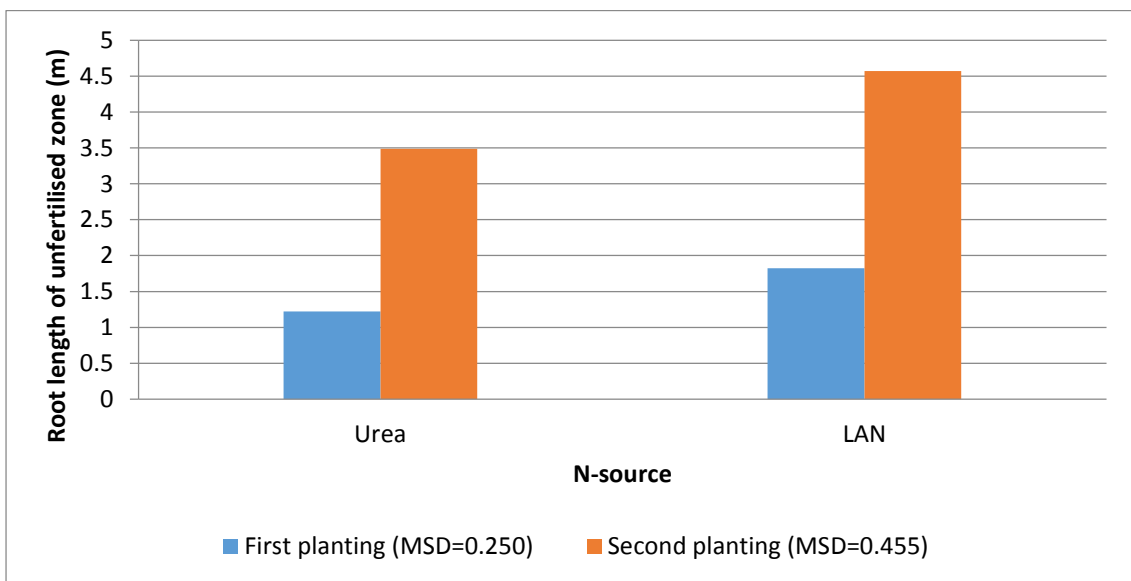

Figure 4.19 Root length of the unfertilized zone as affected by nitrogen source at the end of week 5 for both the first and second planting.

A phosphorus source by phosphorus application rate interaction significantly influenced root length of the unfertilized zone during the first (p = 0.0474) planting (Table 4.13). The nitrogen source by phosphorus application rate interaction significantly influenced root length of the unfertilized zone during the second (p = 0.0173) planting (Table 4.14).

The longest roots, during the first planting (Table 4.13), of the unfertilized zone was recorded with the APP by 40 kg P ha^{-1} combination. This root length was only significantly longer than that of the MAP by 20 kg P ha^{-1} and NP by 10 kg P ha^{-1} interactions (Table 4.13).

Table 4.13   Root length (m) of the unfertilized zone as affected by a phosphorus source by phosphorus application rate interaction at the end of the fifth week of plant development during the first planting

		Phosphorus application rate (kg P ha^{-1}) (PR)					Average (P)
		0	**10**	**20**	**30**	**40**	
Phosphorus source (P)	**MAP**	1.876$_{ab}$	1.658$_{ab}$	0.938$_{b}$	1.212$_{ab}$	1.467$_{ab}$	1.430
	NP	1.398$_{ab}$	0.925$_{b}$	1.667$_{ab}$	1.673$_{ab}$	1.705$_{ab}$	1.474
	APP	1.778$_{ab}$	1.552$_{ab}$	1.753$_{ab}$	1.205$_{ab}$	2.028$_{a}$	1.663
MSD$_{(0.05)}$ (P x PR)		1.207					
Average (PR)		1.684	1.378	1.453	1.363	1.733	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Root length of the unfertilized zone at the end of the second planting was significantly shorter for the urea by 0 and 10 kg P ha^{-1} interactions compared to the longest root length, recorded with the LAN by 20 kg P ha^{-1} combination (Table 4.14).

Table 4.14 Root length (m) of the unfertilized zone as affected by a nitrogen source by phosphorus application rate interaction at the end of the fifth week of plant development during the second planting

		Phosphorus application rate (kg P ha^{-1}) (PR)					Average (N)
		0	**10**	**20**	**30**	**40**	
Nitrogen source (N)	**Urea**	3.063$_{cd}$	2.710$_d$	4.128$_{abcd}$	3.122$_{bcd}$	4.420$_{abc}$	3.489
	LAN	4.760$_{ab}$	4.541$_{abc}$	4.998$_a$	4.549$_{abc}$	3.994$_{abcd}$	4.568
MSD$_{(0.05)}$ (N x PR)		1.669					
Average (PR)		3.912	3.626	4.563	3.836	4.207	

*Means followed by the same letter are not significantly different (MDS = 0.05)

Total root length

Nitrogen source significantly influenced the total root length during the first ($p < 0.0001$) and second ($p = 0.0081$) planting (Table 4.8). Total root length was greater with LAN compared to urea-fertilized plants when measured at the end of the fifth week of both plantings (Figure 4.20).
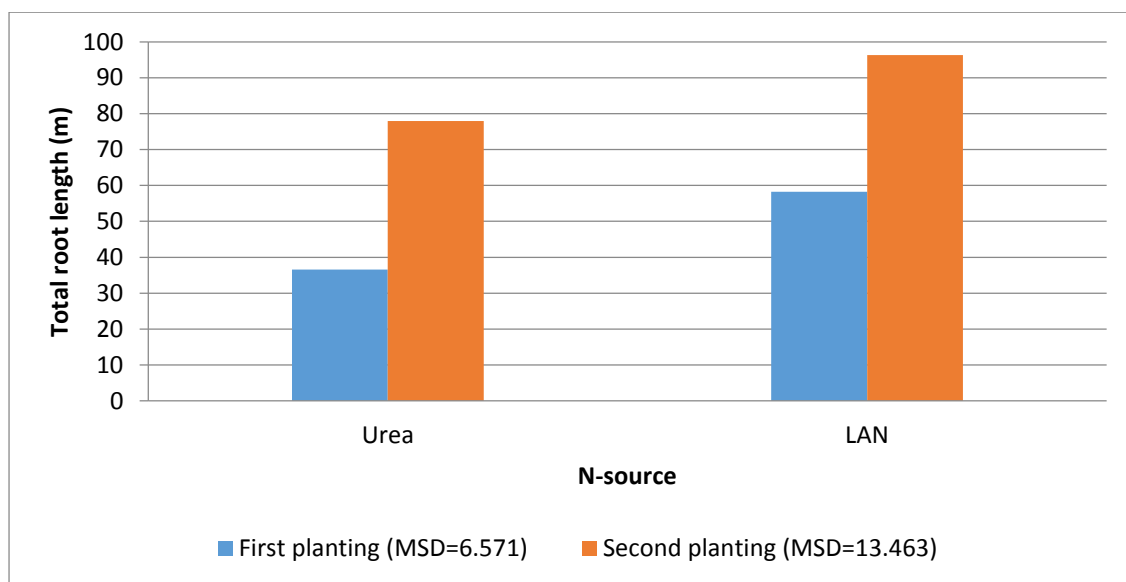

Figure 4.20 Total root length as affected by nitrogen source at the end of week 5 for both the first and second planting.

The complex root system of maize includes different root forms which develop at different stages of plant development (Zhang *et al.*, 1999). Accordingly, the root system can be divided into two categories: [1]the embryonic root system which consists of a single primary root together with various seminal roots and [2] the post-embryonic root system which consists of the shoot-borne adventitious roots (Hochholdinger *et al.*, 2004). An ordered arrangement is followed by the root system of maize; the lower the order the smaller the root diameter, the lower the dry mass and the shorter the overall root length (Merrill *et al.*, 2002).

The largest portion of a plant's root length originates from the finer and smaller root systems that primarily controls ion uptake (Eshel & Waisel, 1996). Development of maize roots is highly sensitive to the availability of nutrients in the soil solution which determines both root growth and root proliferation (Zhang *et al.*, 1999). Nitrogen and phosphorus are two of the major nutrients that have the ability to alter how the root elongates and partition carbohydrates and nutrients (Lopez-Bucio *et al.*, 2003).

Nitrogen is highly mobile within plants after passive uptake by the roots (Marschner, 1995). However, the adsorption of ammonium at medium soil acidity (pH 5.5) renders a N source such as urea, which hydrolyzes to ammonium, less available compared to a source like LAN, since the nitrate ion is not adsorbed by the soil clay colloids (Robinson & Robinson, 1983; Hodge, 2004). Nitrate also requires less energy for root uptake compared to ammonium (Havlin *et al.*, 1999). The latter is supported by the higher root dry mass (Figures 4.16 and 4.17) and longer root lengths (Figures 4.19 and 4.20) with the use of LAN compared to urea.

Root dry matter provides only limited information about the efficiency at which the root system absorbs water and nutrients from a fertilized zone (Mekonnen *et al.*, 1997). For this root length may be a more appropriate parameter (Schortemeyer *et al.*, 1993). Root length and dry mass in the fertilized root zone of plants fertilized with LAN (Tables 4.9 and 4.10) outperformed plants fertilized with urea (Hodge *et al.*, 1999). The same is true for the unfertilized root zones (Figures 4.16, 4.18 to 4.20).

Phosphorus is one of the most vital nutrients required for both root growth and the increase in root mass, since P is required in important processes of cell expansion, cell elongation as well as carbon partitioning between the roots and the leaves (Raghothama, 1999), as discussed in Section 4.3.1.4 and 4.3.1.5. The availability of P is affected by various factors such as soil acidity, P form applied, soil moisture and temperature (Abel *et al.*, 2002). Root length and mass are therefore two important indicators that reflected on the quality and quantity of P availability (Liu *et al.*, 2004).

Acidification (pH≤5.5) significantly reduces the concentration of polyphosphate (Tables 4.9 and 4.10) in solution after fertilization, by reducing the amount of P supplied from APP as a result of the decreased rate of hydrolysis (McBeath *at al*., 2007a). However, P availability in the orthophosphate form is substantially higher (Tables 4.9 and 4.10) since the orthophosphate forms, MAP and NP, are not subjected to hydrolysis after fertilization (Lynch & Brown, 2001). The application of a P source that is readily available (MAP) directly after application significantly increases the root length (Tables 4.12 to 4.14) and root dry weight (Tables 4.9 to 4.11) of the fertilized zone (Tables 4.12 to 4.14), the unfertilized zone (Tables 4.9 to 4.11) as well as that of the total root mass and length (Lynch & Brown, 2001).

The concentration at which P is available is another important characteristic that influences both root mass and root length development. A beneficial characteristic of increased P availability is the increase of lateral root growth (Figures 4.17 and 4.19) which is a result of the increase in the absorption capacity of the root (Qu *et al*., 2003; Zhang *et al*., 2012). Moderate increases in P concentration can have profound effects on root morphology and growth stimulation (Singh & Sale, 2000; Williamson *et al*., 2001).

The root biomass of both the fertilized (Tables 4.9 and 4.10) and unfertilized (Table 4.11) zones have shown that a P application as low as 10 kg P ha^{-1} perform similarly to an application level of 40 kg P ha^{-1}; which is in accordance with the findings of Williamson *et al*. (2001). The increase in root dry mass with the addition of P leads to increased lateral root length development due to a significant positive relationship found between the root dry weight and root length (Murphy & Smucker, 1995; Costa *et al*., 2000).

Root length (Table 4.12), for plants fertilized with 40 kg P ha^{-1} was significantly longer compared to those of lower P applications levels. This might be because the lengths of mature cortical cells were found to be significantly longer with sufficient P supply compared with those grown under lower phosphate availabilities (Williamson *et al*., 2001). Root branching (which increases length, mass and P acquisition) is consequently regulated by the availability of phosphorus (Lopez-Bucio *et al*., 2003).

4.3.2.3 Summary

Roots of the fertilized zone yielded a significantly heavier mass when fertilized with 10 kg P ha^{-1}, compared to a significantly lighter mass when fertilized with 40 kg P ha^{-1}. In contrast, roots of the fertilized zone were significantly shorter when fertilized with 10 kg P ha^{-1} compared to that of the 40 kg P ha^{-1}. Both the aforementioned shows that the root mass and root length of this study was inversely correlated with regards to P application

rate. In general, LAN by MAP treatment combinations yielded significantly heavier and longer root measurements compared to all urea treatment combinations.

All urea treatments, for roots of the fertilized zone yielded lighter and shorter root measurements compared to LAN. Roots of the unfertilized zone yielded significantly heavier and longer root measurements with LAN independently, as well as LAN by APP treatment combinations. The P application rate of 10 kg P ha^{-1} yielded a significantly heavier root mass while the roots proved to be a longer with 10 kg P ha^{-1}.

Both the total root mass and root length was only affected by N source only; where LAN yielded both significantly heavier and longer root measurements compared to urea.

4.4 Conclusion

Nitrogen source proved to be of greater significance importance for vegetative growth and development of maize during both plantings compared to that of P source and P application rate. The use of LAN, which provides immediate availability of N after application as well as ease of uptake, yielded the greatest aerial and subsoil parameter measurements throughout the trial period. Comparing phosphorus sources, the response of the aerial and subsoil parameter groups were inconsistent. Both orthophosphate (MAP and NP) sources yielded significantly greater aerial parameter measurements compared to that of the polyphosphate source (APP), while subsoil parameters results between ortho- and polyphosphate sources were inconsistent. Roots mass and length of the fertilized zone were significantly greater with the use of an orthophosphate (MAP) source, while the polyphosphate (APP) source yielded a significant greater root mass and length within the unfertilized zone. With the exception of root mass of the fertilized zone and root length of the unfertilized zone; which yielded significantly greater measurements with the use of 10 kg P ha^{-1}, the overall response to 40 kg P ha^{-1} yielded significantly greater parameter measurement throughout the vegetative growth period.

CHAPTER 5

INFLUENCE OF PHOSPHORUS AND NITROGEN SOURCES ON NUTRIENT CONCENTRATION AND UPTAKE DURING THE EARLY GROWTH AND DEVELOPMENT OF MAIZE (*Zea mays* L.)

5.1 Introduction

The cultivation of a high-yielding quality maize (*Zea mays* L.) crop is reliant on an adequate and balanced nutrient fertilization program (Sahrawat, 2008). Grains are dependent on an adequate supply of at least sixteen nutrients that stimulate optimum growth and development (White & Zasoski, 1999). Each of these nutrients are equally important to the plant, however are required in vastly different quantities, with roles that are now reasonably well established and have been reported in detail in the texts of Mengel and Kirkby (1987), Bergmann (1992) and Marschner (1995) have grouped the nutrients into three main categories namely, primary macronutrients, secondary macronutrients and micronutrients.

Nitrogen (N), phosphorus (P) and potassium (K) are the three primary macronutrients and most commonly form part of the fertilization program. These nutrients are also needed in the highest quantity by plants (Havlin *et al.*, 1999). Sulphur (S), Calcium (Ca) and magnesium (Mg) are the three secondary macronutrients, which are required in lesser amounts compared to the primary macronutrients (Brady & Weil, 2008). Boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn) are known as the micronutrients (Morris *et al.*, 2007). Micronutrients are required in trace quantities, however are just as important to plant development and commercial crop production compared to the macronutrients (Havlin *et al.*, 1999). Micronutrients act as activators of many plant functions, such as plant metabolism, enzyme systems and physiological processes, and are especially important for the overall performance and health of the maize crop (Marschner, 1995).

A nutrient interaction occurs when the supply of one nutrient affects the absorption and/or utilization of another nutrient (Morris *et al.*, 2007). Therefore, nutrient interactions may occur either in the soil, at the root surface or within the plant and can accordingly be categorized into two major categories. The first category describes interactions that occur as a result of the chemical bond that develop between ions (Fageria, 2001). The second category describes the interaction between those ions with similar chemical

properties. These ions will consequently compete for site of adsorption, absorption, transport and function on plant root surfaces and/or within plant tissue (Robson & Pitman, 1983; Marschner, 1995; Fageria, 2001). Nutrient interactions may be positive (synergistic), negative (antagonistic) or may even have no effect on each other at all (Fageria, 2001). When nutrients in combination yield a growth response greater than the sum of their individual effects, the interaction is positive. The opposite will yield a negative interaction response (Fageria, 2001).

These nutrient interactions are influenced by various factors such as the concentration of the nutrient, temperature, light intensity, soil moisture, soil pH, root architecture, soil aeration, rate of plant transpiration and respiration, plant age and growth rate, plant species and internal nutrient concentration of plants (Mengel & Kirkby, 1987). An acidic soil with a pH of 5.5 or less, as with this study and most South African soils, is one of the greatest factors affecting the uptake of macro- and micronutrients (Brady & Weil, 2008). Acidic soils will generally result in decreased uptake of macronutrients, while possibly increasing the uptake of toxic amounts of metal nutrients such as Mn (Kochian *et al.*, 2004).

Taking all factors ascribed into consideration an investigation into the response of maize to various P and N forms on a medium soil pH of 5.5 is justified. The objective of this study is to evaluate the quantitative nutrient concentration and nutrient uptake response to different nitrogen and phosphorus sources as well as different P application rates during the early growth (5 weeks) of maize.

5.2 Materials and methods

Refer to Chapter 3, Section 3.1, 3.2 and 3.3.

5.3 Results

Only nutrient concentration (Table 5.1) and uptake (Table 5.2) parameters that were significantly influenced in the biomass by nitrogen source, phosphorus source and phosphorus application rate will be included in the discussion. A summary on the analysis of variance evaluating the effect of treatment factors, *viz.* N source, P source and P application rate on the nutrient concentration and uptake parameters is presented in Table 5.1 and Table 5.2 respectively. Molybdenum however, will not be included in the discussion below since it was not significantly affected by any of the abovementioned treatment factors. The overall significant response to nitrogen source and phosphorus

application rate on the nutrient concentration in the biomass is strongly reflected during both plantings (Table 5.1). The response of nutrient concentration in the biomass to phosphorus source however, appears to only have had a significant influence during the first planting (Table 5.1).

Table 5.1   Nutrient concentration in biomass as affected by nitrogen source, phosphorus source and phosphorus application rate at the end of the fifth week of plant development for both the first and second planting

Element	Nutrients												
	N	P	K	S	Ca	Mg	Na	Cu	Mn	Zn	Fe	B	Mo
First planting													
N Source (N)	*	*	*	*	ns	*	*	*	ns	*	ns	ns	ns
P Source (P)	ns	*	ns	*	ns	*	ns	*	*	*	ns	ns	ns
P Rate (PR)	*	ns	ns	*	ns	*	ns	*	ns	*	ns	ns	ns
N×P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Second planting													
N Source (N)	*	*	*	ns	*	*	*	ns	ns	*	*	*	ns
P Source (P)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P Rate (PR)	ns	*	ns	ns	*	*	ns	*	ns	*	ns	ns	ns
N×P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*= significant (P≤0.05), ns = not significant.

The overall significant response to nitrogen source on nutrient uptake in the biomass is strongly reflected during both plantings (Table 5.2). The response of nutrient uptake in the biomass to phosphorus source and phosphorus application rate, although apparent during both plantings, showed that phosphorus source was of greater influence during the first planting while phosphorus application rate was of greater significant influence during the second planting (Table 5.2). The coefficient of variation for nutrient concentration and nutrient uptake varies between 1.13 to 14.86% and 1.1 to 7.41%, respectively.

Table 5.2 Nutrient uptake in biomass as affected by nitrogen source, phosphorus source and phosphorus application rate at the end of the fifth week of plant development for both the first and second planting

Element	N	P	K	S	Ca	Mg	Na	Cu	Mn	Zn	Fe	B	Mo
Nutrients													
First planting													
N Source (N)	*	*	*	*	*	*	ns	*	*	*	ns	*	ns
P Source (P)	*	ns	ns	*	*	ns	ns	ns	*	ns	ns	ns	ns
P Rate (PR)	*	*	*	*	*	ns	*	*	*	*	ns	*	ns
N×P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P×PR	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
N×P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Second planting													
N Source (N)	*	*	*	*	*	*	*	*	*	ns	*	ns	ns
P Source (P)	*	ns	ns	ns	*	*	ns	ns	ns	*	ns	*	ns
P Rate (PR)	ns	*	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
N×P	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N×P×PR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*= significant (P≤0.05), ns = not significant.

5.3.1 Macronutrients

5.3.1.1 Nitrogen

Concentration

Nitrogen source significantly influenced nitrogen concentration in the biomass (Table 5.1) during the first ($p < 0.0001$) and second ($p = 0.0289$) planting. Nitrogen concentration during the both plantings was significantly greater with the use of urea compared to LAN (Figure 5.1). Phosphorus application rate influenced nitrogen concentration in the biomass during the first ($p = 0.0161$) planting only (Table 5.1). Nitrogen concentration for the 10 and 30 kg P ha^{-1} application rates were neither significantly smaller than the control (0 kg P ha^{-1}), nor significantly greater than that of 20 and 40 kg P ha^{-1} application rates (Figure 5.2). However, the control did result in a significantly greater nitrogen concentration compared to that of the 20 and 40 kg P ha^{-1}.
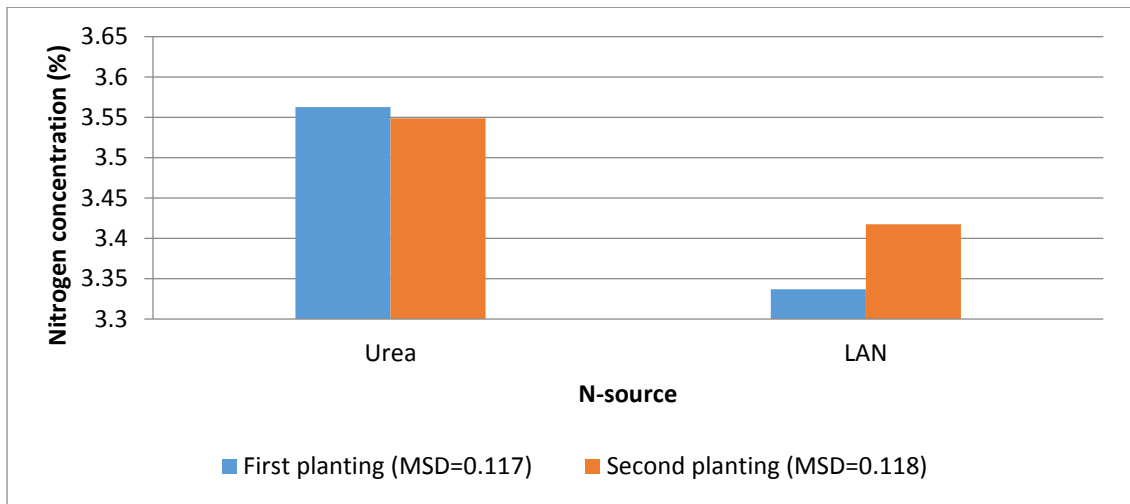
Figure 5.1　　Nitrogen concentration in biomass as affected by the nitrogen source at the end of week 5 for both the first and second planting.
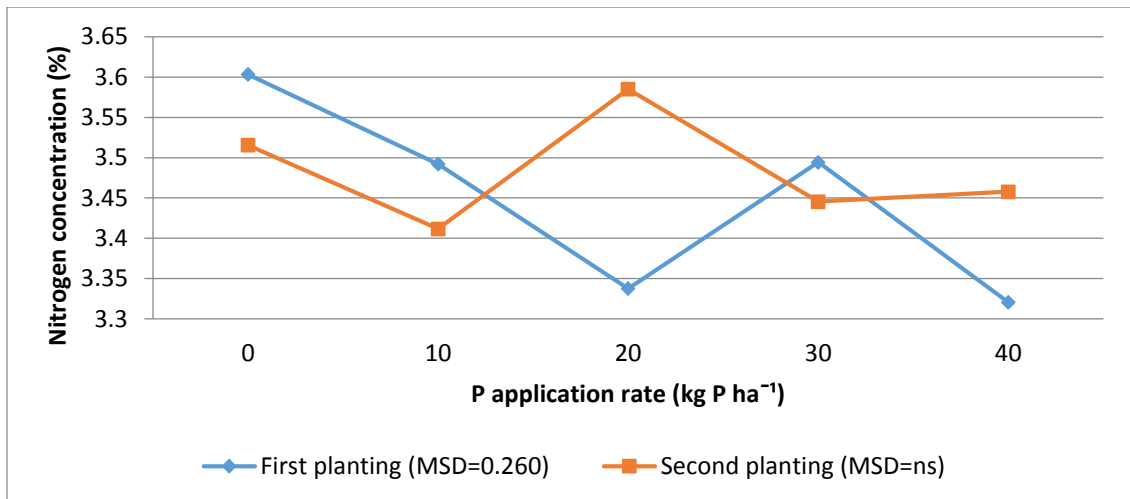

Figure 5.2　　Nitrogen concentration in biomass as affected by the phosphorus application rate at the end of week 5 for the first planting.

Uptake

Nitrogen source significantly influenced nitrogen uptake in the biomass (Table 5.2) during the first ($p < 0.0001$) and second ($p = 0.0010$) planting. Nitrogen uptake during both plantings was significantly greater with LAN compared to urea (Figure 5.3 a).

Phosphorus source significantly influenced nitrogen uptake in the biomass during the first ($p = 0.0459$) and second ($p = 0.0457$) planting (Table 5.2). Monoammonium phosphate resulted in significantly greater nitrogen uptake compared to APP, while NP did not significantly differ from either MAP or APP during both plantings (Figure 5.3 b).
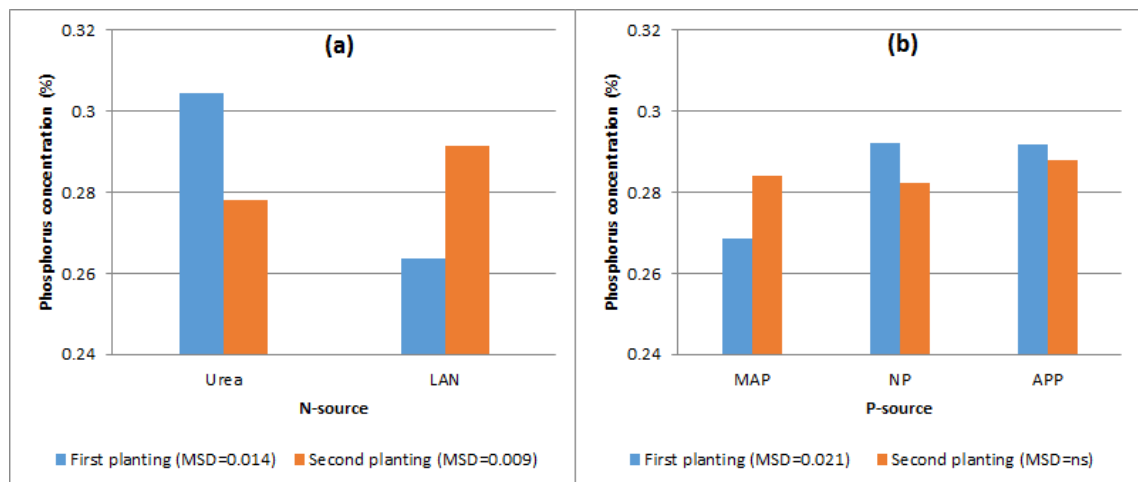
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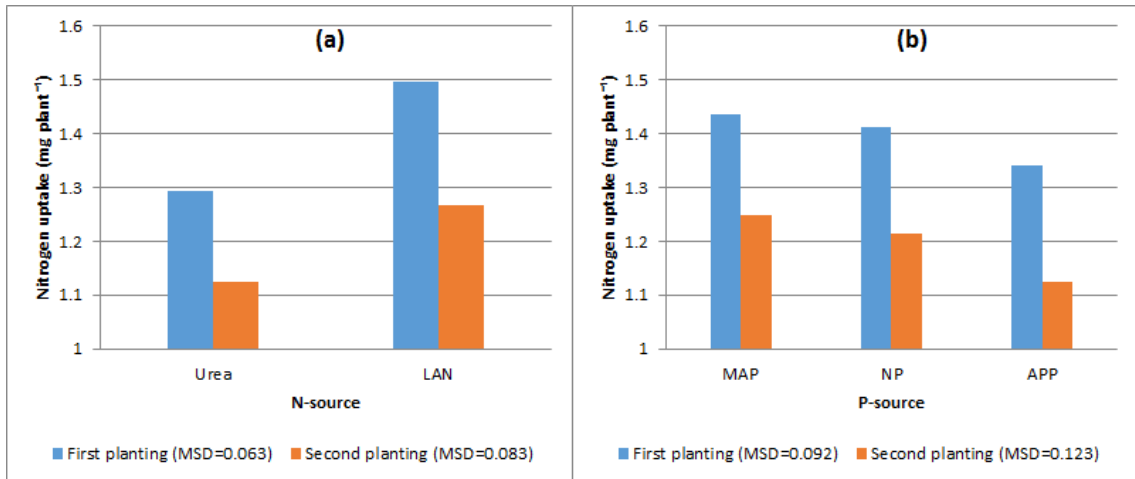
Figure 5.3   Nitrogen uptake in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate influenced nitrogen uptake in the biomass during the first (p = 0.0161) planting only (Table 5.2). Nitrogen uptake with an application of 20 kg P ha^{-1} was neither significantly greater than the control (0 kg P ha^{-1}), nor significantly lower than application rates ranging from 10 to 40 kg P ha^{-1} (Figure 5.4). However, the control did result in a significantly lower nitrogen uptake compared to applications of 10, 30 and 40 kg P ha^{-1} (Figure 5.4).
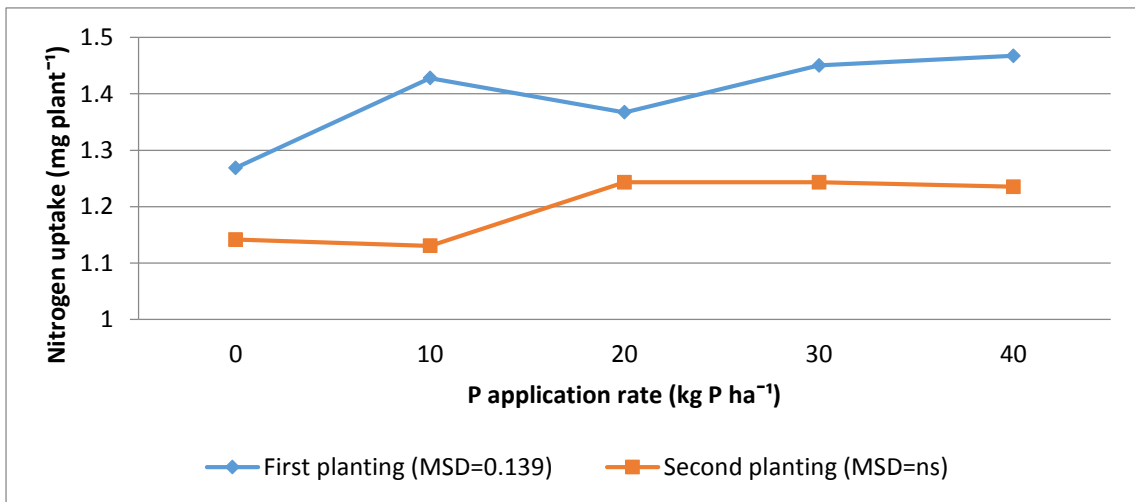
Figure 5.4   Nitrogen uptake in biomass as affected by phosphorus application rate at the end of week 5 for the first planting.

5.3.1.2 Phosphorus

Concentration

Nitrogen source significantly influenced phosphorus concentration in the biomass (Table 5.1) during the first (p < 0.0001) and second (p = 0.0050) planting. Phosphorus concentration during the first planting was significantly greater with the use of urea compared to LAN while the opposite is true for the second planting (Figure 5.5 a).

Phosphorus source significantly influenced phosphorus concentration in the biomass (Table 5.1) during the first (p = 0.0129) planting only. Nitrophosphate and APP yielded no significant phosphorus concentration differences, however both aforementioned P sources yielded significantly greater phosphorus concentrations compared to MAP (Figure 5.5 b).

Figure 5.5   Phosphorus concentration in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate influenced phosphorus concentration in the biomass during the second (p = 0.0131) planting only (Table 5.1). Phosphorus concentration for the 0, 20 and 30 kg P ha^{-1} application rates were neither significantly greater than the 10 kg P ha^{-1} application rate, nor significantly lower than the 40 kg P ha^{-1} application rate (Figure 5.6). The application of 40 kg P ha^{-1} did result in a significantly greater phosphorus concentration compared to that of the 10 kg P ha^{-1} (Figure 5.6).
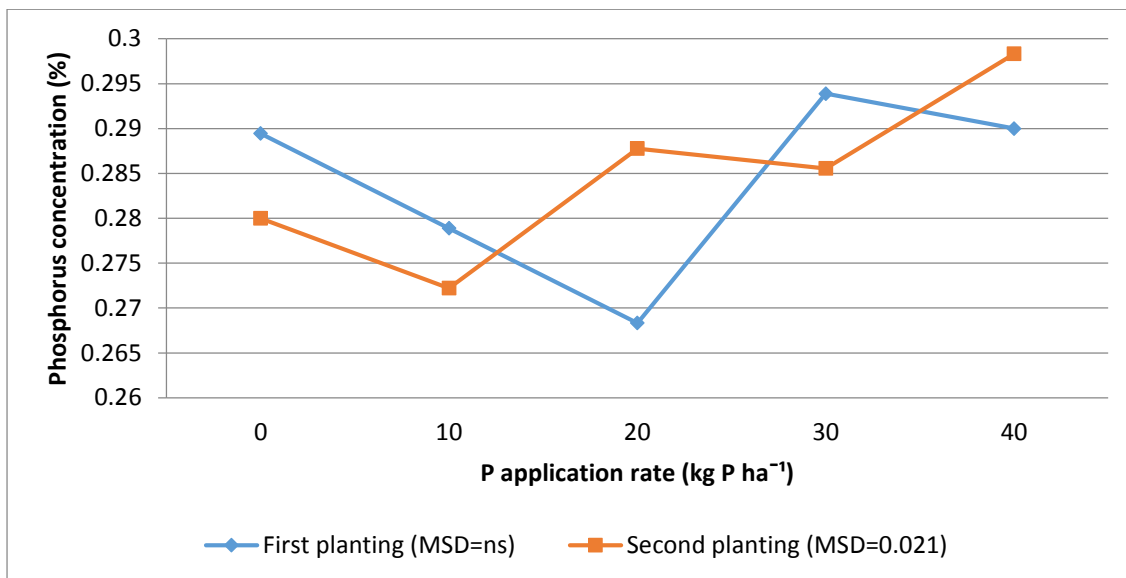
Figure 5.6   Phosphorus concentration in biomass as affected by the phosphorus application rate at the end of week 5 for the second planting.

Uptake

Nitrogen source significantly influenced phosphorus uptake in the biomass (Table 5.2) during the first ($p = 0.0100$) and second ($p < 0.0001$) planting. Phosphorus uptake during both plantings was significantly greater with the use of LAN compared to urea (Figure 5.7).
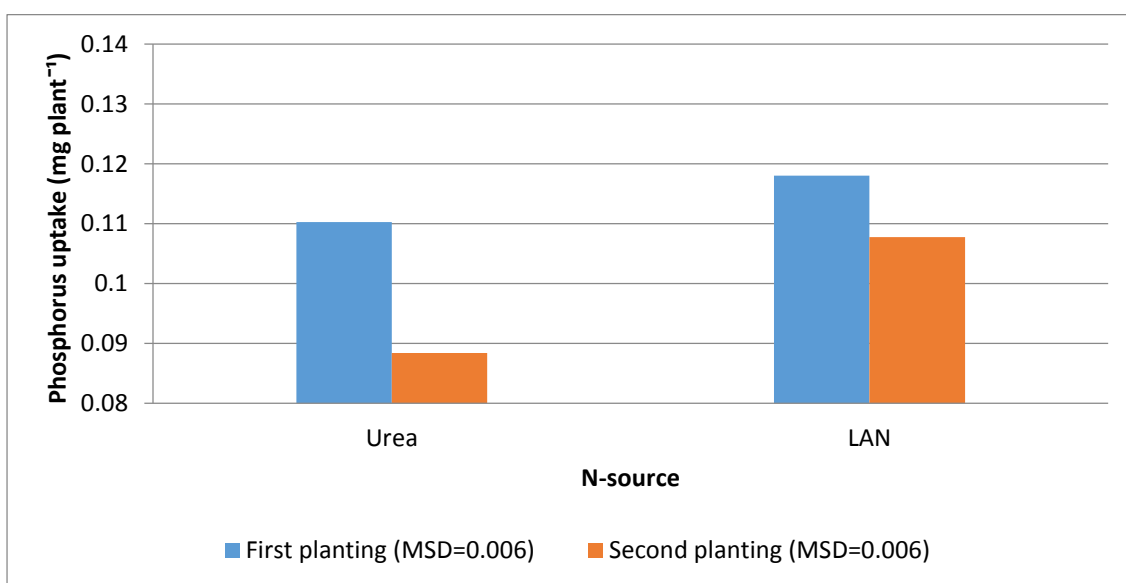

Figure 5.7   Phosphorus uptake in biomass as affected by the nitrogen source at the end of week 5 for both the first and second planting.

Phosphorus application rate influenced phosphorus uptake in the biomass during the first (p = 0.0001) and second (p = 0.0033) planting (Table 5.2). During the first planting, phosphorus uptake was similar for applied rates 0, 10 and 20 kg P ha^{-1} however, these were significantly lower than the phosphorus uptake obtained at 30 and 40 kg P ha^{-1} (Figure 5.8). The control yielded a significantly lower phosphorus uptake compared to that of 40 kg P ha^{-1} (Figure 5.8).

During the second planting phosphorus uptake in the biomass with application rates of 20 and 30 kg P ha^{-1} was neither significantly greater than the control and 10 kg P ha^{-1} application rates, nor significantly lower than the 40 kg P ha^{-1} application rate (Figure 5.8). An application rate of 40 kg P ha^{-1} resulted in significantly greater phosphorus uptake compared to that of the 0 and 10 kg P ha^{-1} (Figure 5.8).
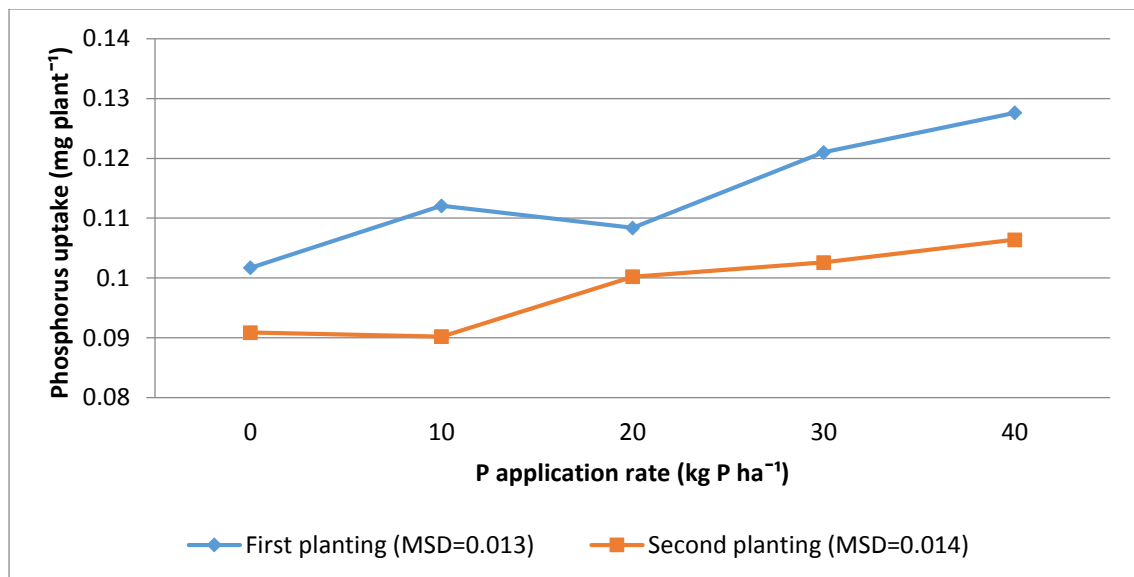
Figure 5.8   Phosphorus uptake in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

5.3.1.3 Potassium

Concentration

Nitrogen source significantly influenced potassium concentration in the biomass (Table 5.1) during the first (p = 0.0002) and second (p = 0.0006) planting. Potassium concentration during the first planting was significantly greater with the use of LAN compared to urea while the opposite is true for the second planting (Figure 5.9).
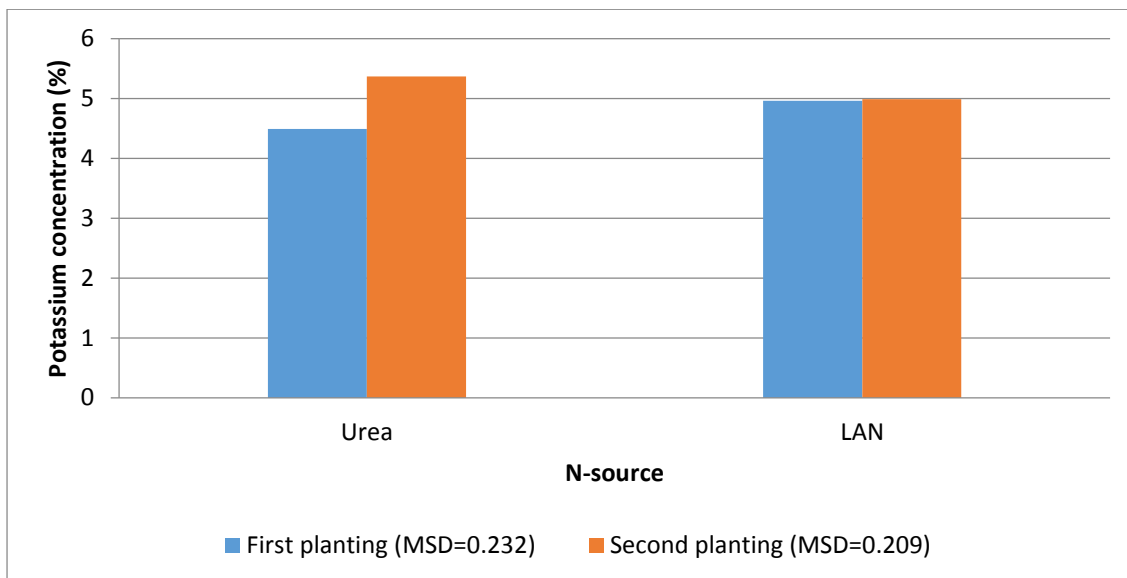
Figure 5.9   Potassium concentration in biomass as affected by the nitrogen source at the end of week 5 for both the first and second planting.

Uptake

Nitrogen source significantly influenced potassium uptake in the biomass (Table 5.2) during the first ($p < 0.0001$) and second ($p = 0.0091$) planting. Potassium uptake during both plantings was significantly greater with the use of LAN compared to urea (Figure 5.10).
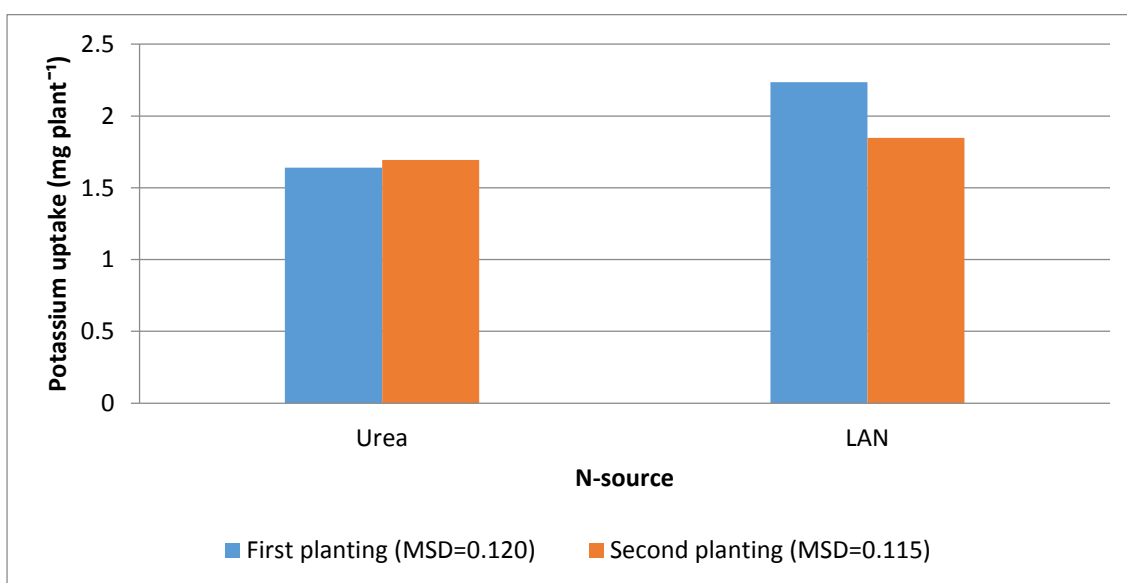

Figure 5.10   Potassium uptake in biomass as affected by the nitrogen source at the end of week 5 for both the first and second planting.

Potassium uptake in the biomass was also significantly affected by the interaction between phosphorus source and phosphorus application rate (Table 5.3). A significant greater potassium uptake was obtained with MAP applied at 10, 20 and 40 kg P ha^{-1} as well as NP applied at 30 and 40 kg P ha^{-1}. With the exception of NP applied at a rate of 10 kg P ha^{-1}, the remaining treatment combinations yielded a significantly lower total potassium uptake compared to the above mentioned treatment combinations.

Table 5.3: Potassium uptake in biomass (mg plant^{-1}) at week 5 of the first planting as affected by the interaction of phosphorus source and phosphorus application rate

		Phosphorus application rate (kg P ha^{-1}) (PR)					Average (P)
		0	10	20	30	40	
Phosphorus source (P)	MAP	1.662$_b$	2.238$_a$	2.205$_a$	1.871$_b$	2.107$_a$	2.017
	NP	1.673$_b$	1.901$_{ab}$	1.887$_b$	2.178$_a$	2.130$_a$	1.954
	APP	1.826$_b$	1.771$_b$	1.723$_b$	1.890$_b$	1.995$_b$	1.841
MSD$_{(0.05)}$ (P x PR)		0.305					
Average (PR)		1.720	1.970	1.938	1.980	2.077	

*Means followed by the same letter are not significantly different (MDS = 0.05)

5.3.1.4 Sulphur

Concentration

Nitrogen source significantly influenced sulphur concentration in the biomass (Table 5.1) during the first (p < 0.0001) planting only. Sulphur concentration during the first planting was significantly greater with the use of urea compared to LAN (Figure 5.11 a).

Phosphorus source significantly influenced sulphur concentration in the biomass (Table 5.1) during the first (p = 0.014) planting only. Nitrophosphate yielded a significant greater sulphur concentration compared to MAP, while sulphur concentration with the use of APP did not significantly differ from either NP or MAP sources (Figure 5.11 b).
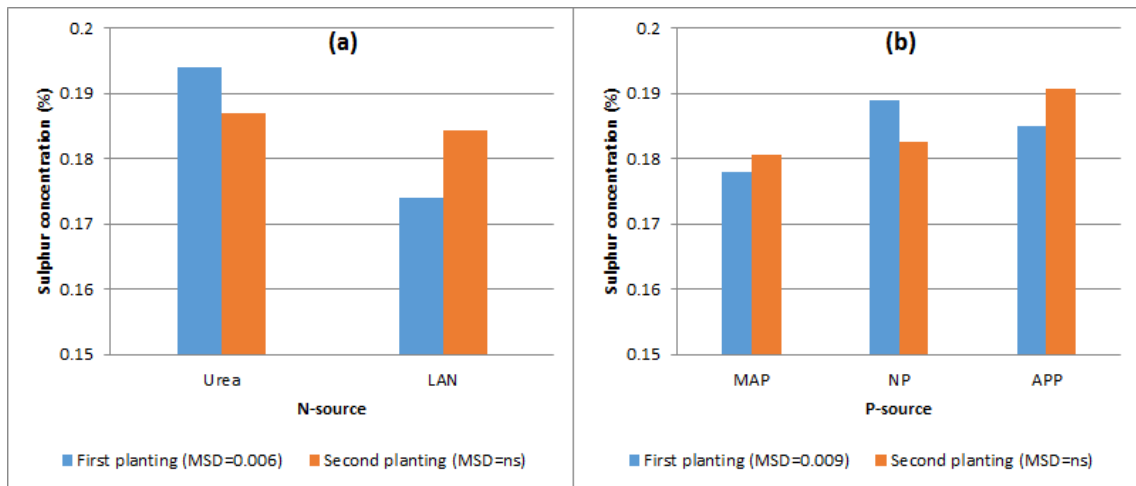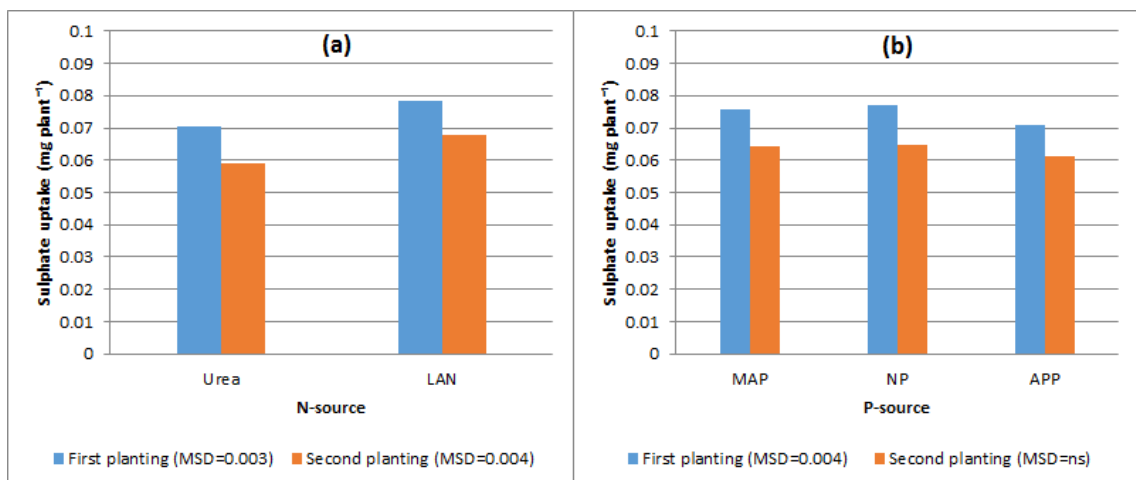
Figure 5.11   Sulphate concentration in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for the first planting.

Uptake

Nitrogen source significantly influenced sulphur uptake in the biomass (Table 5.2) during the first ($p < 0.0001$) and second ($p < 0.0001$) planting. Both the first and second planting's sulphur uptake was significantly greater with the use of LAN compared to urea (Figure 5.12 a).

Phosphorus source significantly influenced sulphur uptake in the biomass (Table 5.2) during the first ($p < 0.0001$) planting only. Sulphur uptake yielded no significant difference with the use of MAP compared to NP (Figure 5.12 b). However, these P sources did yield a greater sulphur uptake compared to that of APP fertilized plants.

Figure 5.12   Sulphate uptake in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate influenced uptake of sulphur in the biomass during the first (p < 0.0001) planting only (Table 5.2). Sulphur uptake with 10, 20 and 30 kg P ha^{-1} was neither significantly lower compared to that of the 40 kg P ha^{-1}, nor significantly greater than that of the 0 kg P ha^{-1} (Figure 5.13). Sulphur uptake for the 40 kg P ha^{-1} was significantly greater than that of the 0 kg P ha^{-1}.
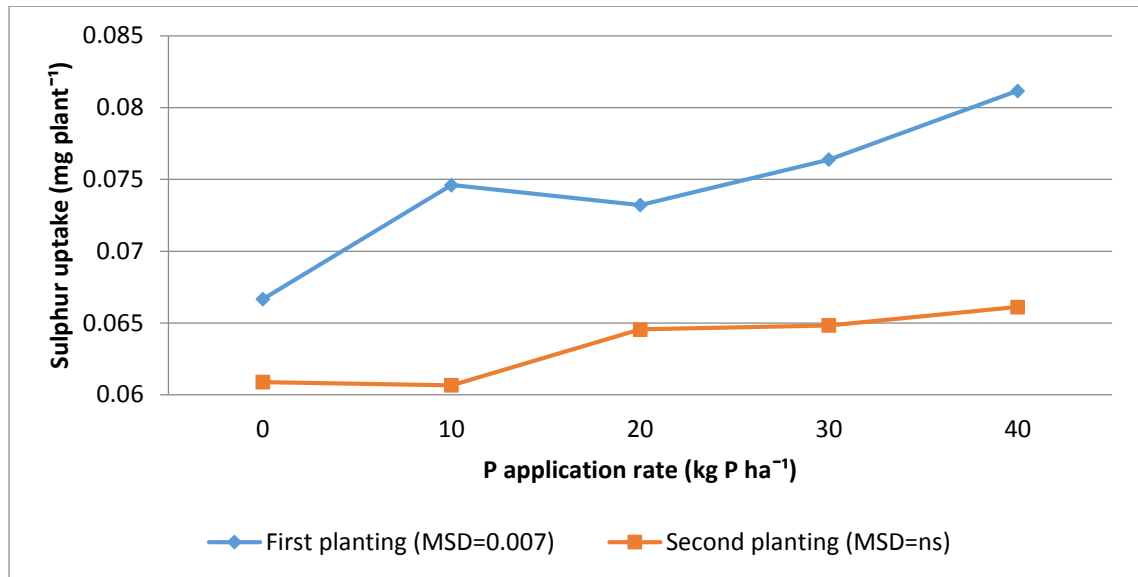

Figure 5.13   Sulphate uptake in biomass as affected by phosphorus application rate at the end of week 5 for the first planting.

5.3.1.5 Calcium

Concentration

Nitrogen source significantly influenced calcium concentration in the biomass (Table 5.1) during the second (p = 0.02226) planting only. Calcium concentration during the second planting was significantly greater with the use of LAN compared to urea (Figure 5.14).

Phosphorus application rate significantly influenced calcium concentration in the biomass during the second (p = 0.0131) planting only (Table 5.1). Calcium concentration for the 0, 10 and 40 kg P ha^{-1} application rates were neither significantly greater than the 30 kg P ha^{-1} application rate, nor significantly lower than the 20 kg P ha^{-1} application rate (Figure 5.15). However, the 20 kg P ha^{-1} did result in a significantly greater calcium concentration compared to that of the 30 kg P ha^{-1}.
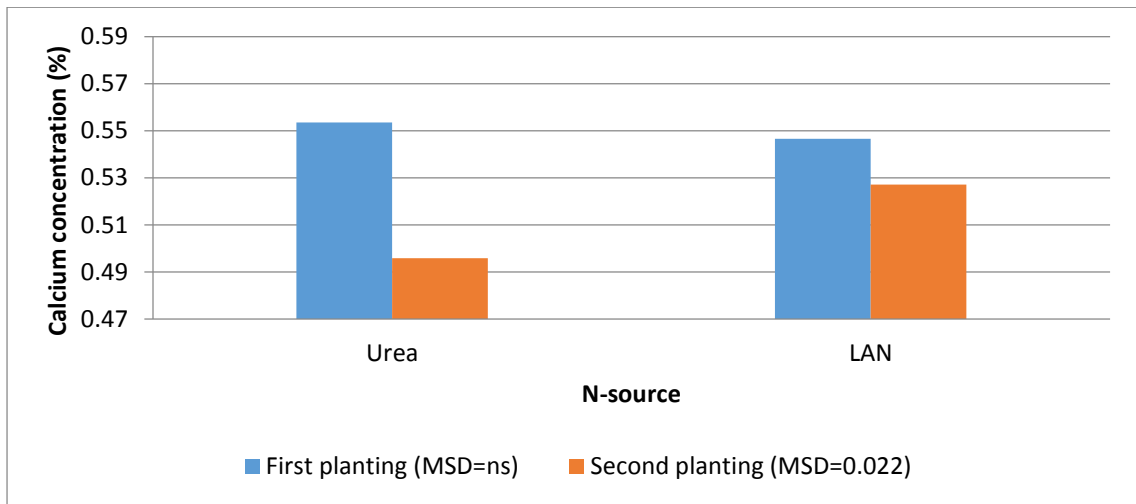
Figure 5.14   Calcium concentration in biomass as affected by the nitrogen source at the end of week 5 for the second planting.
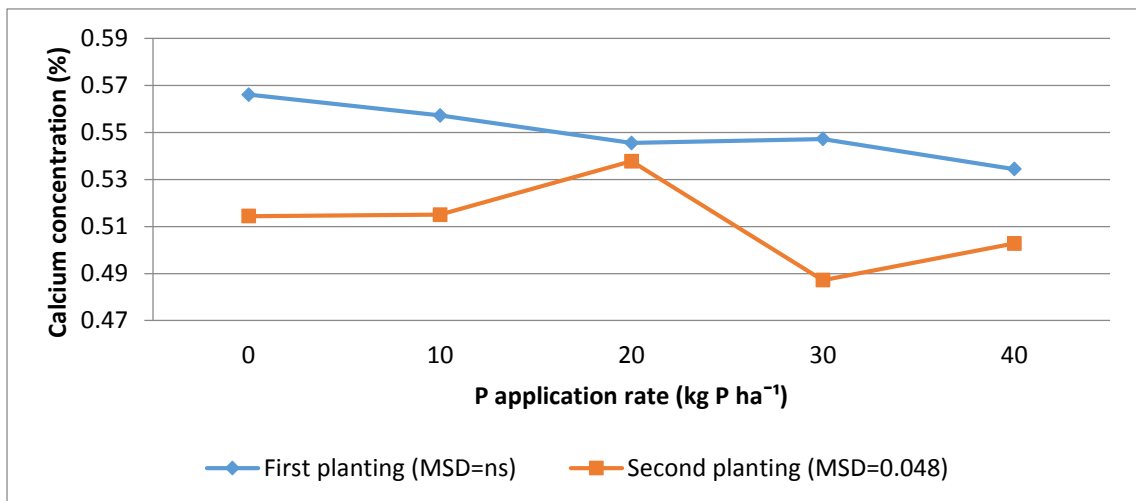

Figure 5.15   Calcium concentration in biomass as affected by phosphorus application rate at the end of week 5 for the second planting.

Uptake

Nitrogen source significantly influenced calcium uptake in the biomass (Table 5.2) during the second ($p < 0.0001$) planting only. Calcium concentration was significantly greater with the use of LAN compared to urea (Figure 5.16 a).

Phosphorus source significantly influenced the total calcium uptake in the biomass (Table 5.2) during the second ($p = 0.0057$) planting only. Calcium concentration (Figure 5.16) with the use of MAP and NP, although not significantly different when compared

with each other, yielded a greater calcium concentration compared to that of APP fertilized plants (Figure 5.16 b).
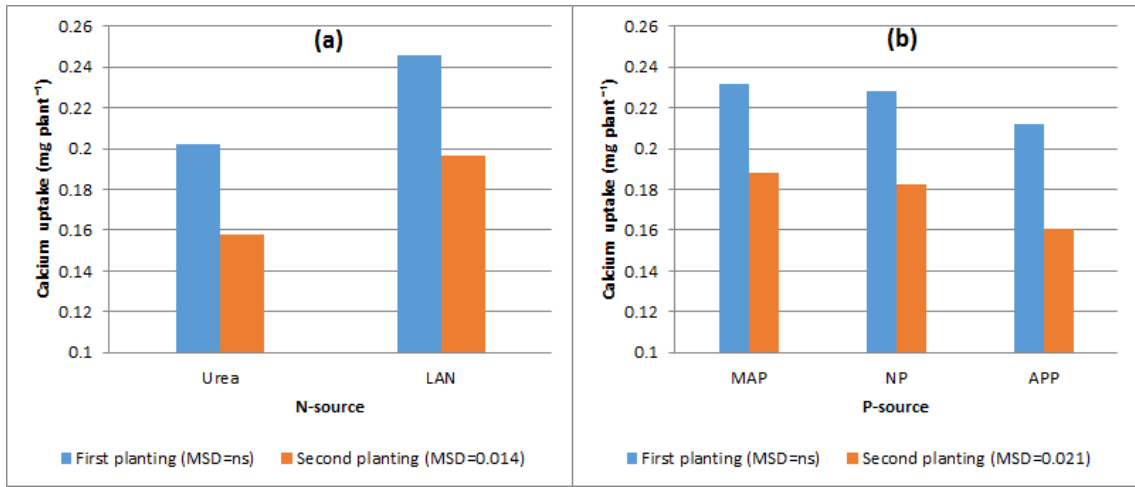

Figure 5.16   Calcium uptake in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate significantly influenced calcium uptake in the biomass during the first ($p = 0.0161$) planting only (Table 5.2). All rates of P application, with the exception of the control (0 kg P ha^{-1}) showed no significant difference in calcium uptake (Figure 5.17). The control resulted in the lowest significant uptake of calcium when compared to all other rates of P application (Figure 5.17).
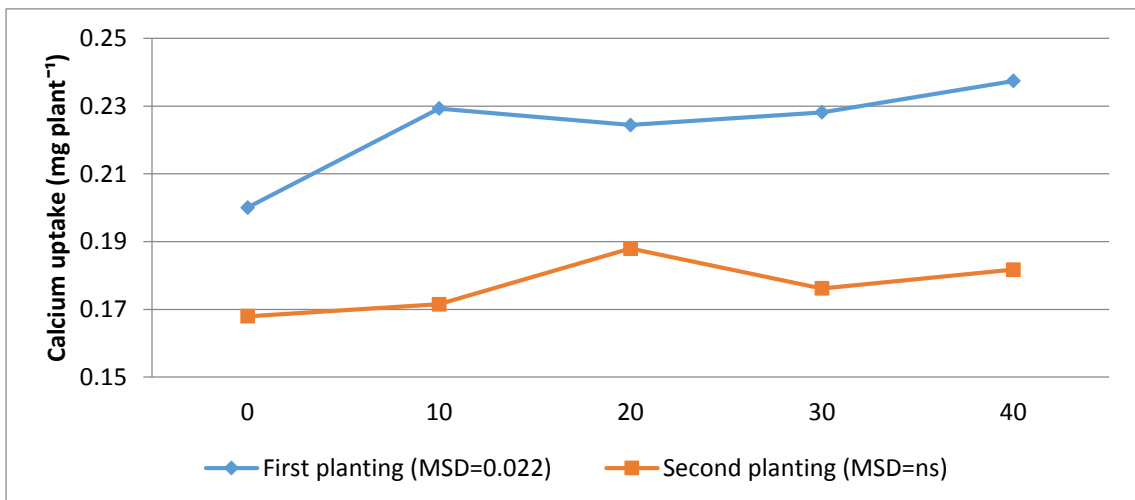

Figure 5.17   Calcium uptake in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

5.3.1.6 Magnesium

Concentration

Nitrogen source significantly influenced magnesium concentration in the biomass (Table 5.1) during the first ($p < 0.0001$) and second ($p = 0.0226$) planting. Magnesium concentration during both plantings was significantly greater with the use of urea compared to LAN (Figure 5.18 a).

Phosphorus source significantly influenced magnesium concentration in the biomass (Table 5.1) during the first ($p = 0.0173$) planting only. Ammonium polyphosphate yielded a significant greater magnesium concentration compared to MAP, while magnesium concentration with the use of NP did not significantly differ from either NP or MAP sources (Figure 5.18 b).
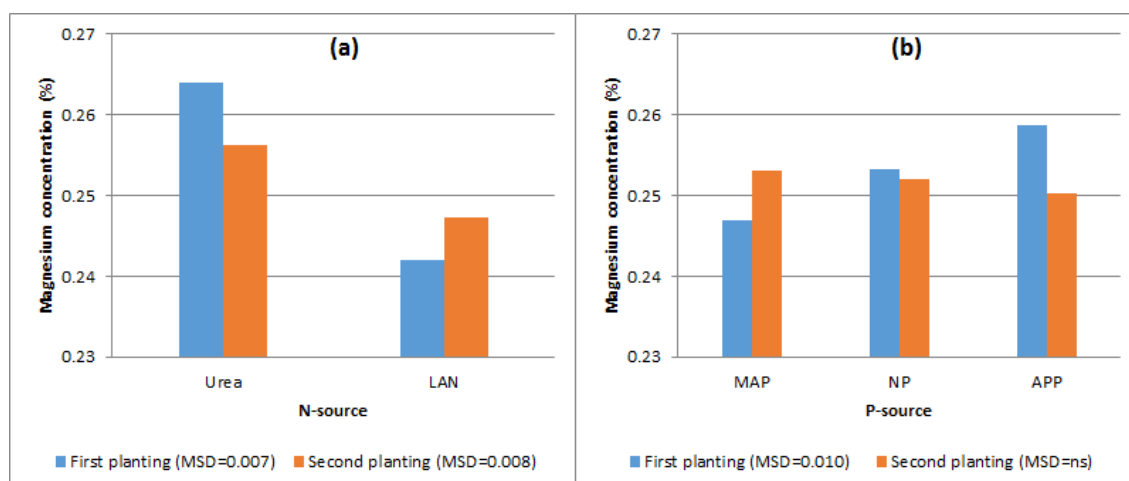

Figure 5.18   Magnesium concentration in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate significantly influenced magnesium concentration in the biomass during the first ($p < 0.0001$) and second ($p = 0.0038$) planting (Table 5.1). During the first planting, magnesium concentration for the 20 and 30 kg P ha^{-1} application rates were neither significantly greater than the 40 kg P ha^{-1} application rate, nor significantly lower than that of the 10 kg P ha^{-1} application rate (Figure 5.19). The 0 and 10 kg P ha^{-1} resulted in a significantly greater magnesium concentration compared to that of the 40 kg P ha^{-1}.

During the second planting, magnesium concentration in the biomass with the 10 and 20 kg P ha^{-1} application rates was neither significantly greater than the 30 and 40 kg P ha^{-1} application rates, nor significantly lower than the 0 kg P ha^{-1} application rate (Figure

5.19). The 0 kg P ha^{-1} resulted in a significantly greater magnesium concentration compared to that of the 30 and 40 kg P ha^{-1} application rates.
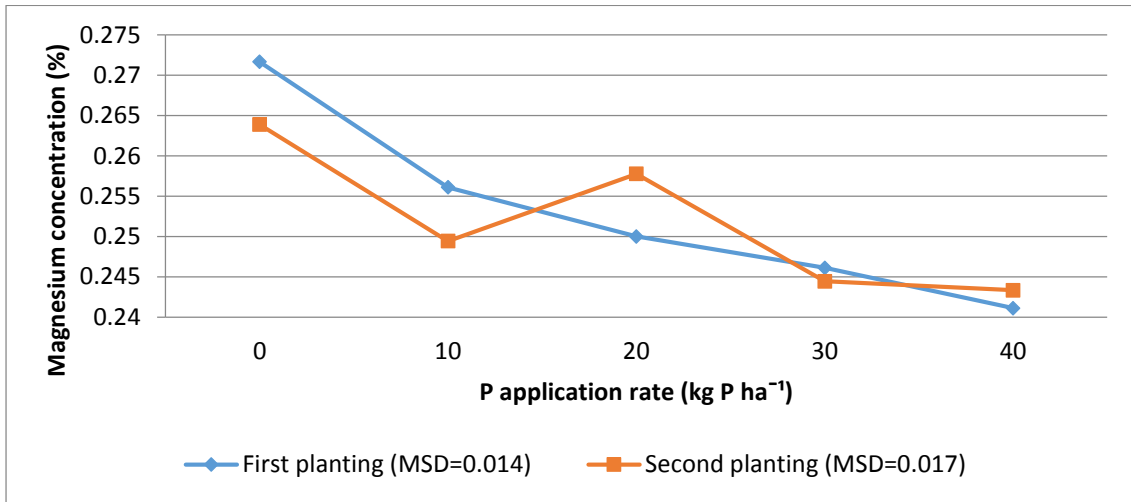

Figure 5.19   Magnesium concentration in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

Uptake

Nitrogen source significantly influenced magnesium uptake in the biomass during the first ($p < 0.0001$) and second ($p = 0.0009$) planting (Table 5.2). Magnesium uptake during both plantings was significantly greater with the use of LAN compared to urea (Figure 5.20 a).
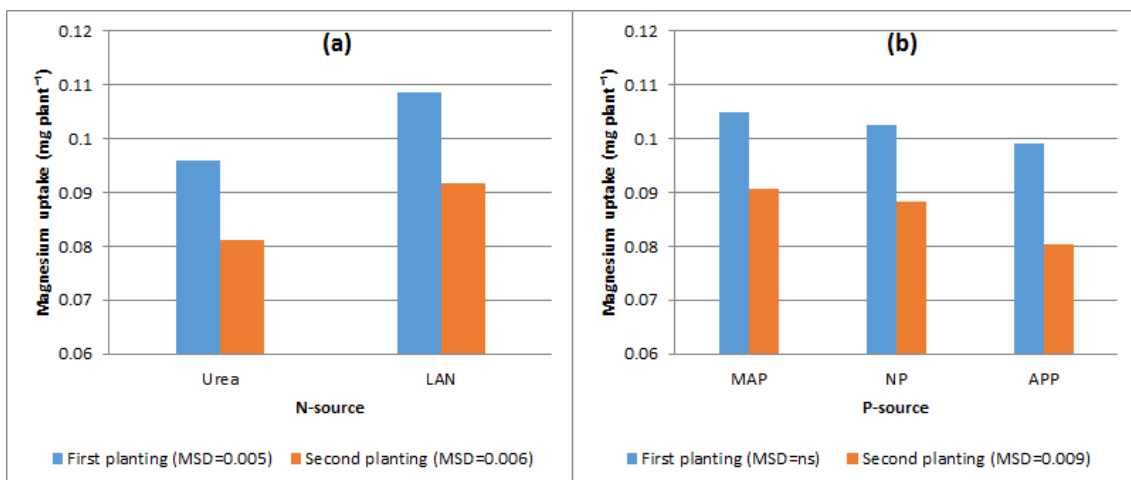

Figure 5.20   Magnesium uptake in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus source significantly influenced magnesium uptake in the biomass (Table 5.2) during the second (p = 0.0195) planting only. Monoammonium phosphate resulted in significantly greater magnesium uptake compared to APP, while NP did not significantly differ from either MAP or APP sources (Figure 5.20 b).

5.3.2 Micronutrients

5.3.2.1 Copper

Concentration

Nitrogen source significantly influenced copper concentration in the biomass during the first (p < 0.0001) planting only (Table 5.1). The copper concentration during the first plantings was significantly greater with the use of urea compared to LAN (Figure 5.21 a).

Phosphorus source significantly influenced copper concentration in the biomass during the first (p = 0.0253) planting only (Table 5.1). Ammonium polyphosphate yielded a significant greater copper concentration compared to MAP, while copper concentration with the use of NP did not significantly differ from either NP or MAP sources (Figure 5.21 b).
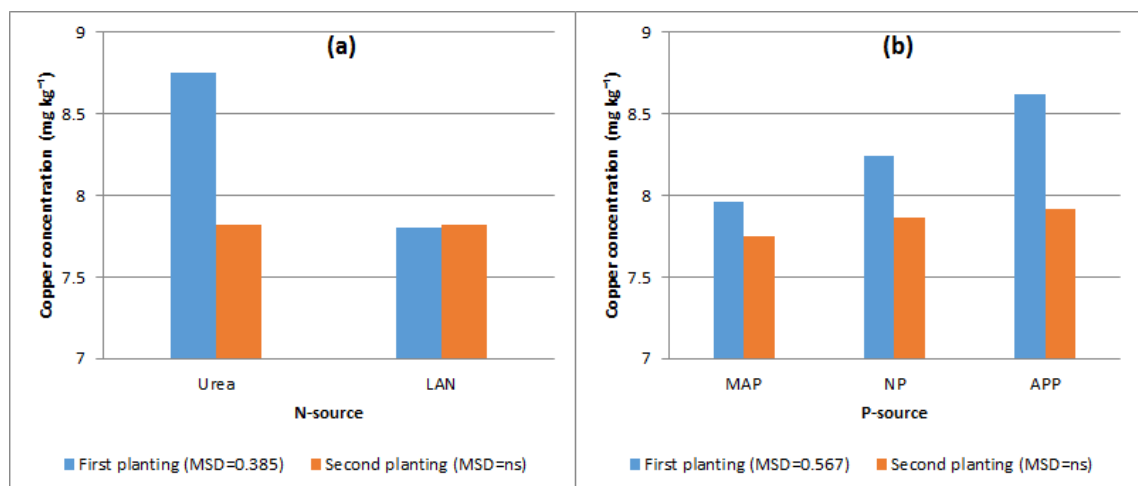

Figure 5.21   Copper concentration in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate significantly influenced copper concentration in the biomass during the first (p < 0.0001) and second (p = 0.0059) planting (Table 5.1). During the first planting the 20, 30 and 40 kg P ha^{-1} applications yielded similar copper concentrations (Figure 5.22). The control and 10 kg P ha^{-1}, although not significantly different from each

other, yielded a significantly greater copper concentration when compared to all other P application rates.

During the second planting, the copper concentration in the biomass for 10, 20 and 40 kg P ha^{-1} was neither significantly greater than the 30 kg P ha^{-1} application rate, nor significantly lower than the control (Figure 5.22). The control yielded a significantly greater copper concentration compared to that of the 30 kg P ha^{-1} application.
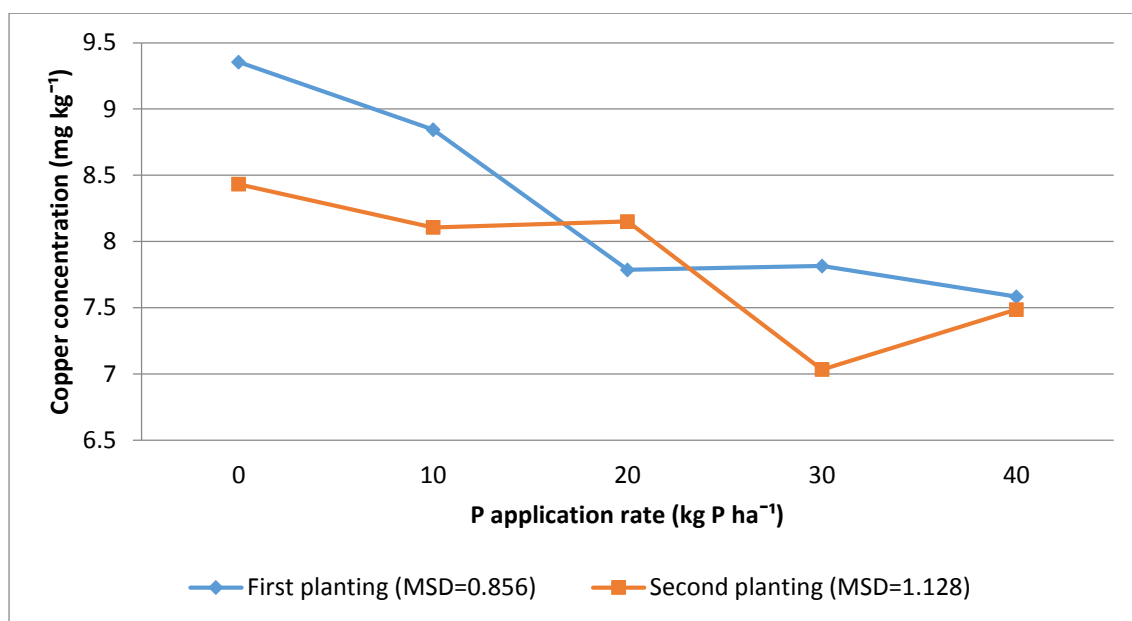

Figure 5.22   Copper concentration in biomass as affected by phosphorus application rate at the end of week 5 for the first planting.

Uptake

Nitrogen source significantly influenced copper uptake in the biomass during the first (p = 0.0005) and second (p = 0.0012) planting (Table 5.2). Copper uptake during both plantings was significantly greater with LAN compared to urea (Figure 5.23).

Phosphorus application rate significantly influenced copper uptake in the biomass during the first (p = 0.0249) planting only (Table 5.2). Copper uptake with 30 and 40 kg P ha^{-1} was neither significantly greater than the control and 20 kg P ha^{-1}, nor significantly lower than the 10 kg P ha^{-1} application rate (Figure 5.24). The application of 10 kg P ha^{-1} resulted in a significantly greater copper uptake compared to that of 0 and 20 kg P ha^{-1}.
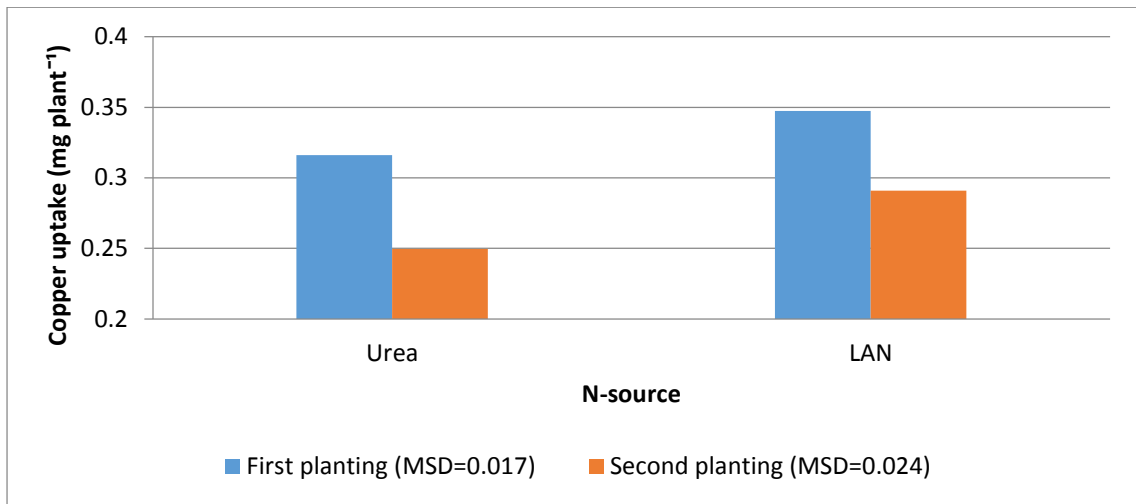
Figure 5.23 Copper uptake in biomass as affected by nitrogen source at the end of week 5 for both the first and second planting.
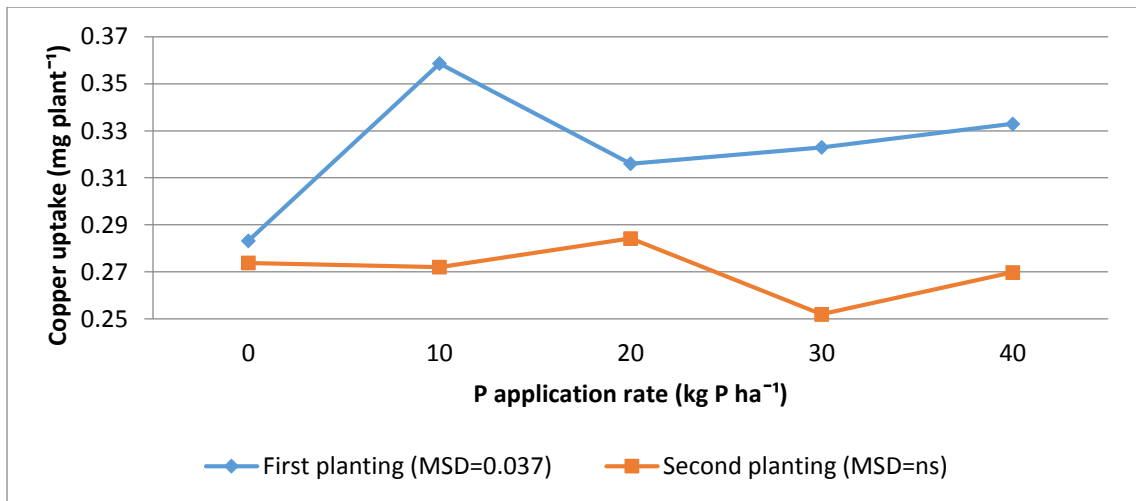

Figure 5.24 Copper uptake in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

5.3.2.2 Manganese

Concentration

Phosphorus source significantly influenced manganese concentration in the biomass during the first ($p = 0.0138$) planting only (Table 5.1). Monoammonium phosphate and APP yielded no significant difference in manganese concentration compared with each other, however both the former yielded a significantly lower manganese concentration compared to NP (Figure 5.25).
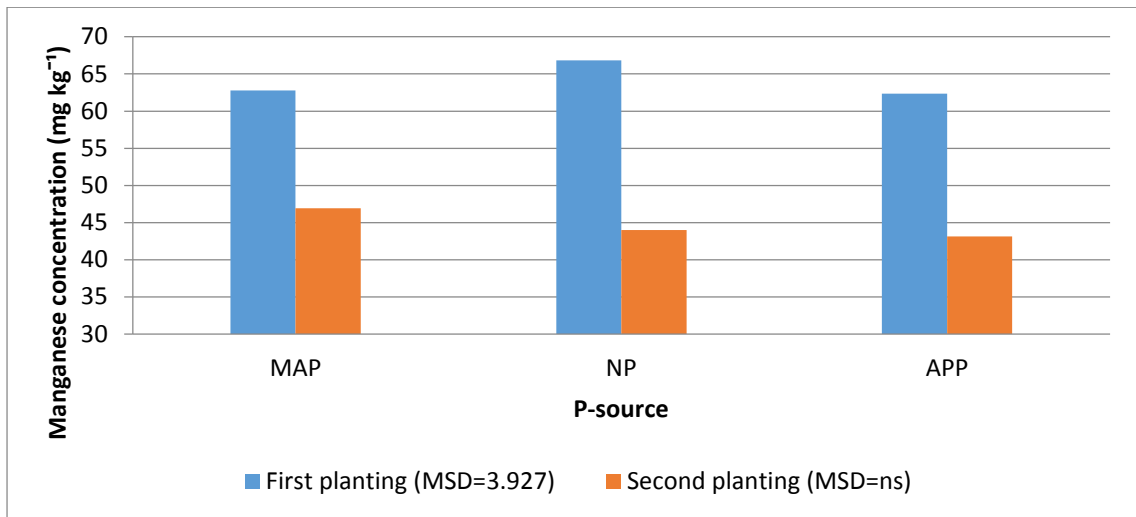
Figure 5.25   Manganese concentration in biomass as affected by phosphorus source at the end of week 5 for both the first and second planting.

Uptake

Nitrogen source significantly influenced manganese uptake in the biomass during the first ($p < 0.0001$) and second ($p = 0.0011$) planting (Table 5.2). Manganese uptake during both plantings was significantly greater with the use of LAN compared to urea (Figure 5.26).

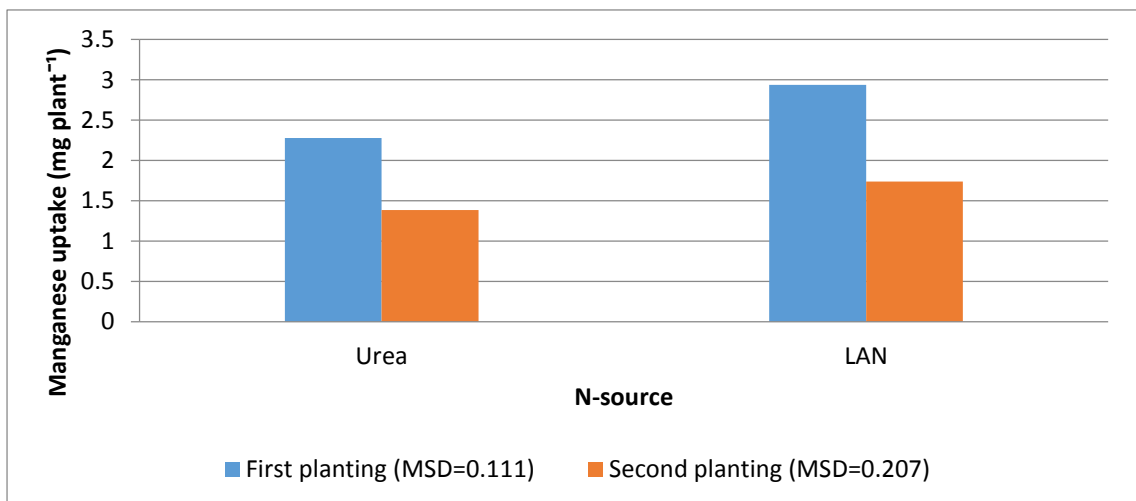**Figure 5.26**   Manganese uptake in biomass as affected by nitrogen source at the end of week 5 for both the first and second planting.

The significant greatest manganese uptake in the biomass was obtained with MAP applied at 40 kg P ha^{-1} as well as NP applied at 30 and 40 kg P ha^{-1} (Table 5.4). The lowest manganese uptake was recorded with 0 kg P ha^{-1} for all P sources used. All other

treatment combinations yielded similar manganese uptake but was significantly lower than that of the first mentioned.

Table 5.4: Manganese uptake in biomass (mg plant^{-1}) at week 5 of the first planting as affected by the interaction of phosphorus source and phosphorus application rate

		Phosphorus application rate (kg P ha^{-1}) (PR)					Average
		0	**10**	**20**	**30**	**40**	**(P)**
Phosphorus source (P)	**MAP**	2.197$_d$	2.604$_{bc}$	2.736$_b$	2.626$_{bc}$	3.276$_a$	2.688
	NP	2.162$_d$	2.648$_{bc}$	2.650$_{bc}$	3.074$_a$	3.159$_a$	2.739
	APP	2.174$_d$	2.427$_{cd}$	2.320$_{cd}$	2.569$_{bc}$	2.525$_{bc}$	2.403
MSD$_{(0.05)}$ (P x PR)		0.282					
Average (PR)		2.178	2.560	2.569	2.756	2.987	

*Means followed by the same letter are not significantly different (MDS = 0.05)

5.3.2.3 Zinc

Concentration

Nitrogen source significantly influenced zinc concentration in the biomass during the first (p < 0.0001) and second (p = 0.0385) planting (Table 5.1). Zinc concentration during the both plantings was significantly greater with the use of urea compared to LAN (Figure 5.27 a).

Phosphorus source significantly influenced zinc concentration in the biomass during the first (p = 0.0005) planting only (Table 5.1). Monoammonium phosphate and NP yielded a similar zinc concentration, which was significantly lower compared to the zinc concentration of APP (Figure 5.27 b).

Phosphorus application rate significantly influenced zinc concentration in the biomass during the first (p < 0.0001) and second (p < 0.0001) planting (Table 5.1). During both plantings, zinc concentration for the 20 kg P ha^{-1} application rate was not significantly different from the 10, 30 and 40 kg P ha^{-1} application rates (Figure 5.28). The control resulted in significantly greater zinc concentration compared to that of 30 and 40 kg P ha^{-1} applications.
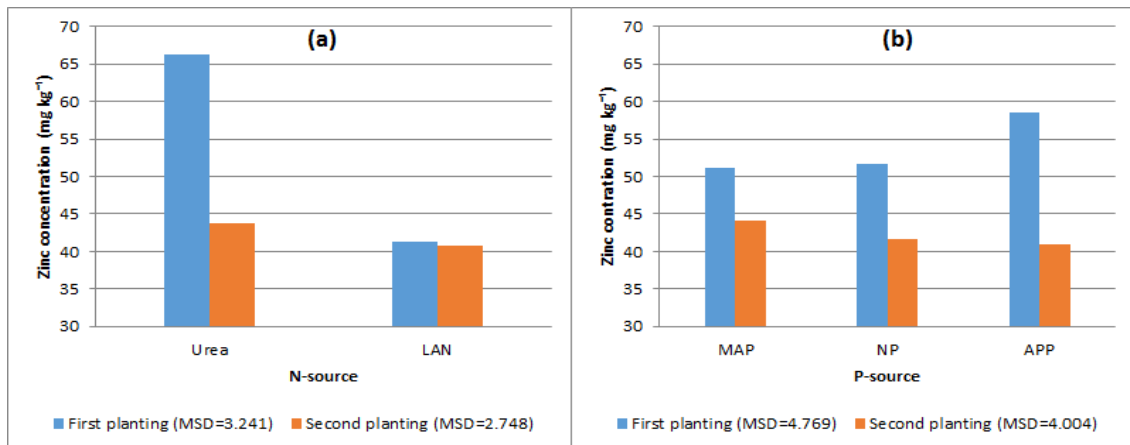
103

Figure 5.27 Zinc concentration in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Figure 5.28 Zinc concentration in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

Uptake

Nitrogen source significantly influenced zinc uptake in the biomass during the first (p < 0.0001) planting only (Table 5.2). For this planting zinc uptake was significantly greater with the use of urea compared to LAN (Figure 5.29 a).

Phosphorus source significantly influenced zinc uptake in the biomass during the second (p = 0.0067) planting only (Table 5.2). Monoammonium phosphate resulted in significantly greater zinc uptake in comparison to APP, while NP did not significantly differ from either MAP or APP (Figure 5.29 b).

Phosphorus application rate significantly influenced the uptake of zinc in the biomass during the first (p = 0.0369) and second (p = 0.0111) planting (Table 5.2). During the first planting total zinc uptake with 0, 20 and 40 kg P ha^{-1} applications was neither significantly greater than the 30 kg P ha^{-1} nor significantly lower than the 10 kg P ha^{-1} applications. The 10 kg P ha^{-1} application did however, yield a significant greater zinc uptake compared to that of 20 kg P ha^{-1} applications (Figure 5.30).

During the second planting zinc uptake in the biomass with 10 and 30 kg P ha^{-1} was not significantly different than the 0, 20 or 40 kg P ha^{-1} applications (Figure 5.30).

Figure 5.29   Zinc uptake in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Figure 5.30   Zinc uptake in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

5.3.2.4 Iron

Concentration

Nitrogen source significantly influenced iron concentration in the biomass during the second ($p < 0.0001$) planting (Table 5.1). Iron concentration during the second planting was significantly greater with the use of LAN compared to urea (Figure 5.31).
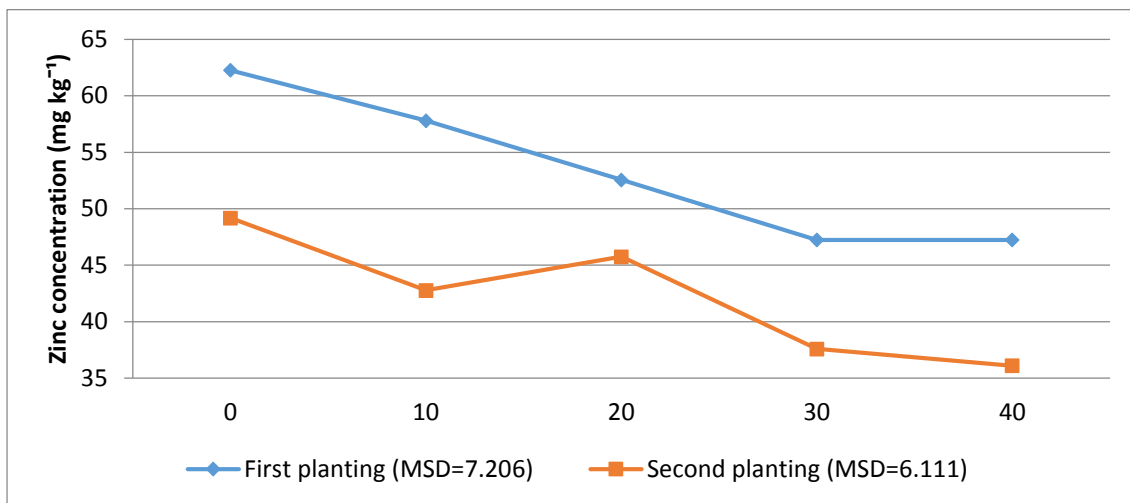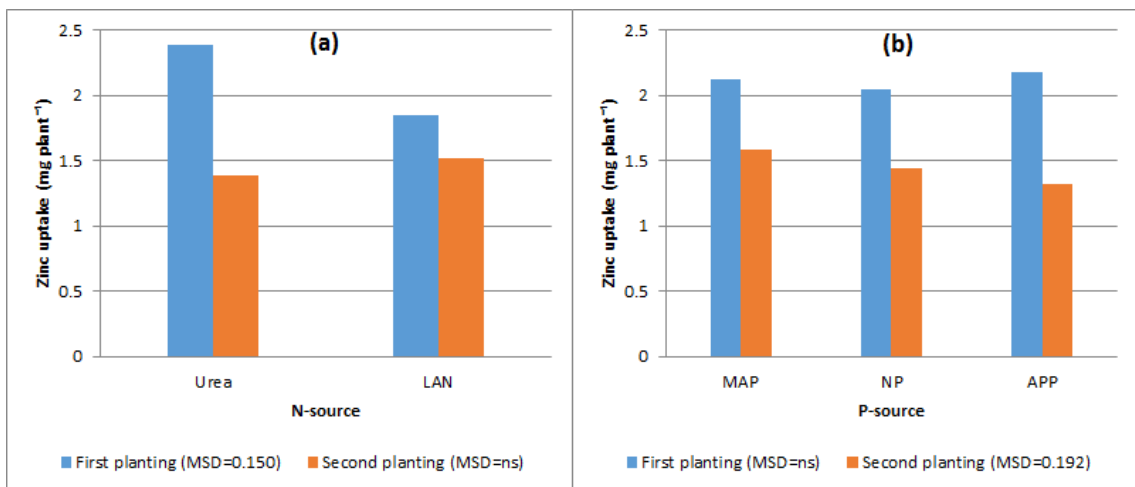

Figure 5.31 Iron concentration in biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

Uptake

Nitrogen source significantly influenced iron uptake in the biomass during the second ($p < 0.0001$) planting only (Table 5.2). Iron uptake during the second planting was significantly greater with the use of LAN compared to urea (Figure 5.32).

Figure 5.32   Iron uptake in biomass as affected by phosphorus application rate at the end of week 5 for the second planting.

5.3.2.5   Boron

Concentration

Nitrogen source significantly influenced boron concentration in the biomass during the second ($p < 0.0001$) planting (Table 5.1). Boron concentration during the second planting was significantly greater with the use of urea compared to LAN (Figure 5.33).
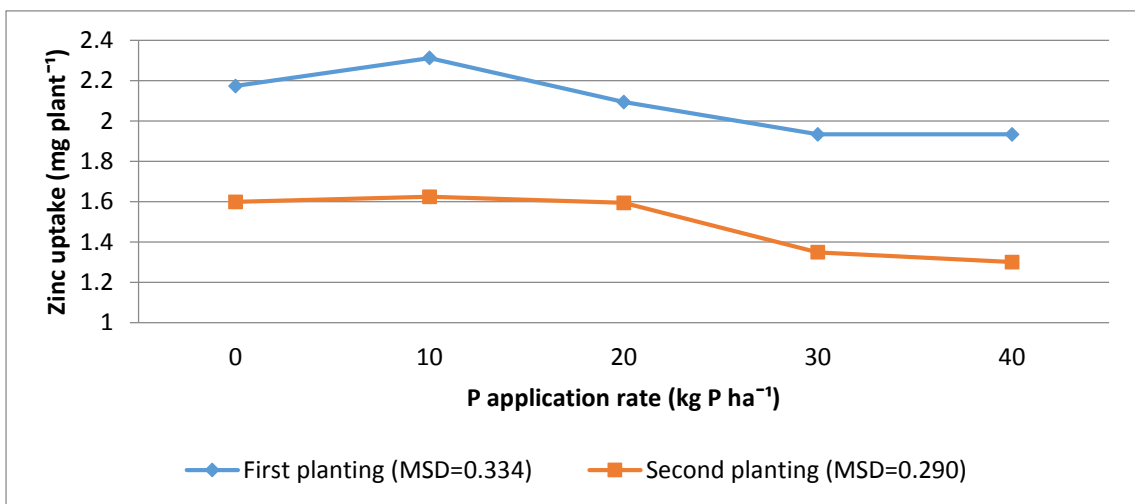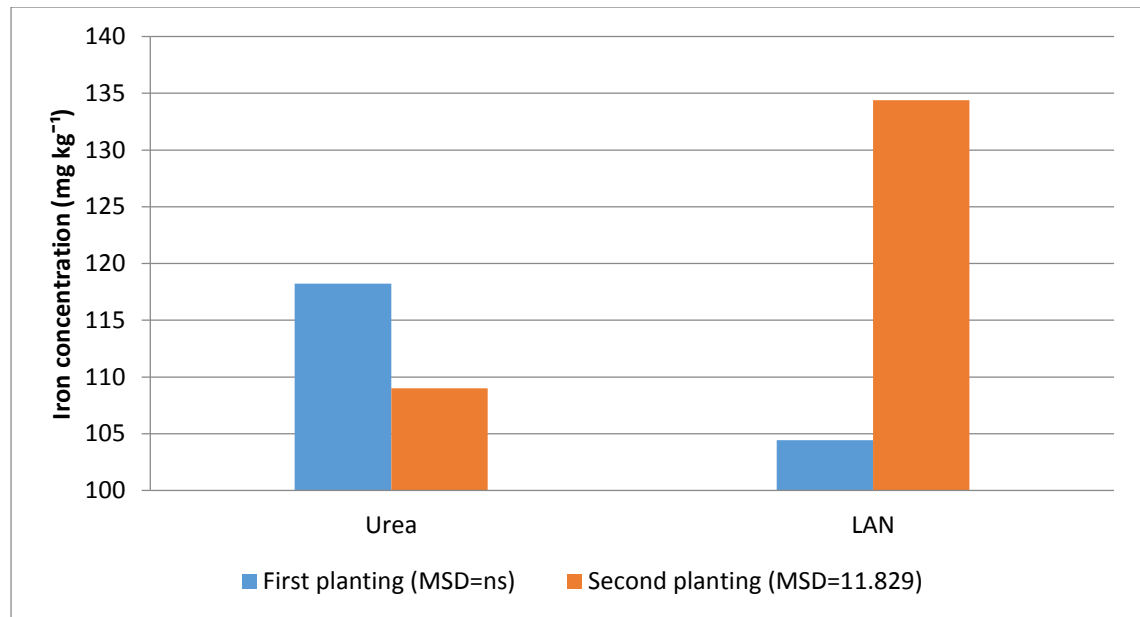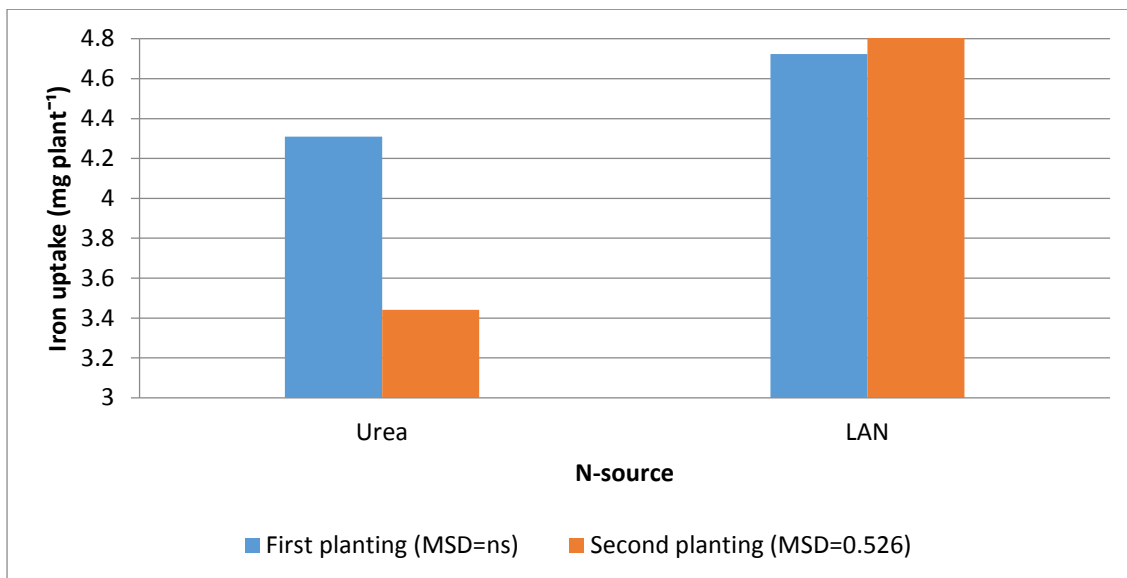

Figure 5.33   Boron concentration in the biomass as affected by phosphorus application rate at the end of week 5 for both the first and second planting.

Uptake

Nitrogen source significantly influenced boron uptake in the biomass during the first ($p < 0.0001$) planting only (Table 5.2), where boron uptake was significantly greater with LAN compared to urea (Figure 5.34 a).

Phosphorus source significantly influenced boron uptake in the biomass during the second ($p = 0.0426$) planting only (Table 5.2). Monoammonium phosphate resulted in a significantly greater boron uptake in comparison to APP, while NP did not significantly differ from either MAP or APP sources (Figure 5.34 b).
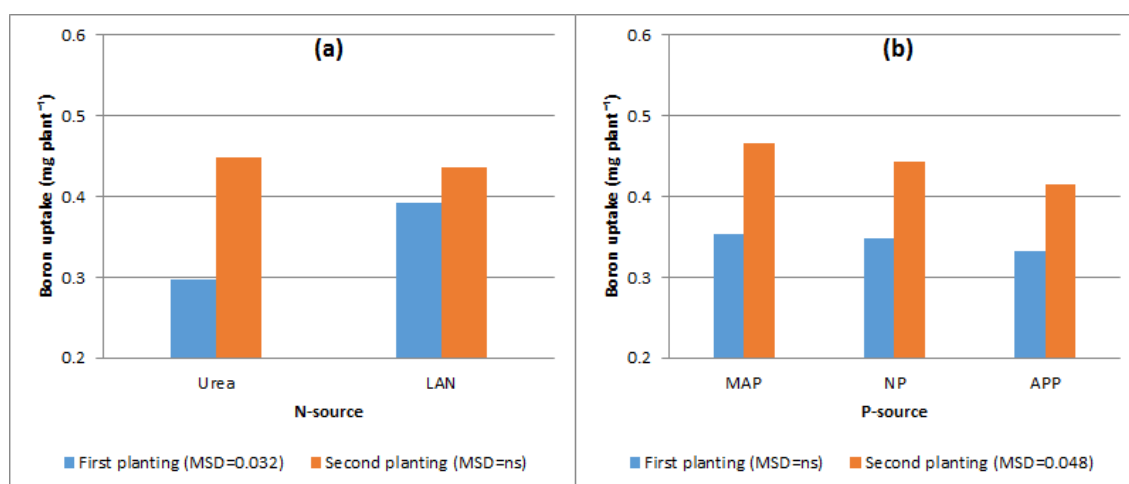
Figure 5.34 Boron uptake in biomass as affected by a) the nitrogen source and b) phosphorus source at the end of week 5 for both the first and second planting.

Phosphorus application rate also significantly influenced boron uptake in the biomass during the first planting ($p = 0.0028$) only (Table 5.2). Boron uptake with 20 and 30 kg P ha^{-1}, was neither significantly greater than that of 0 kg P ha^{-1}, nor significantly lower than the boron uptake of 10 and 40 kg P ha^{-1}. Both 10 and 40 kg P ha^{-1} yielded a significantly greater boron uptake compared to that of 0 kg P ha^{-1} (Figure 5.35).
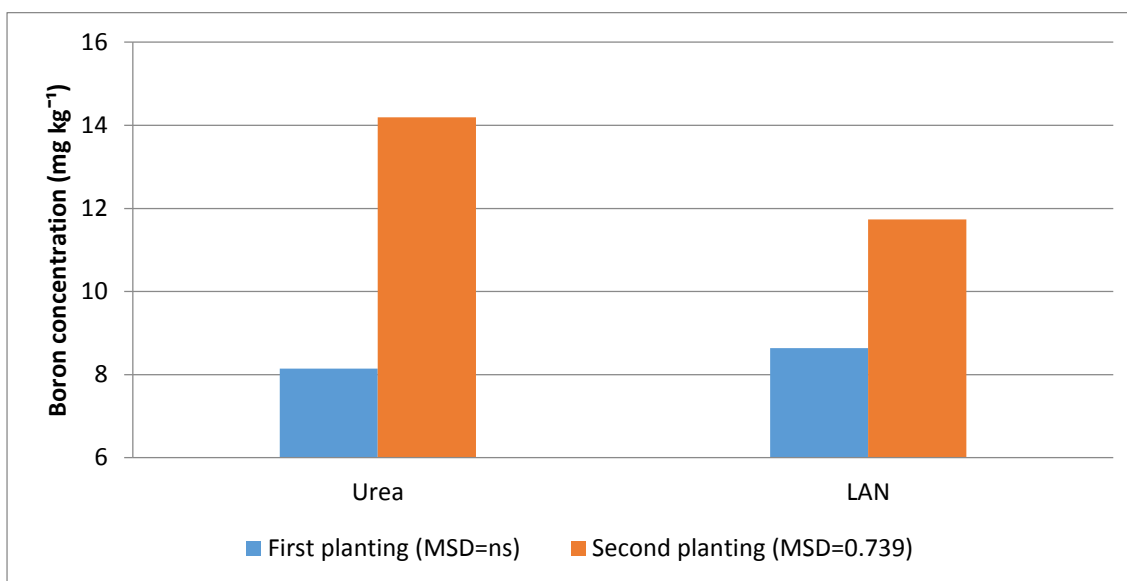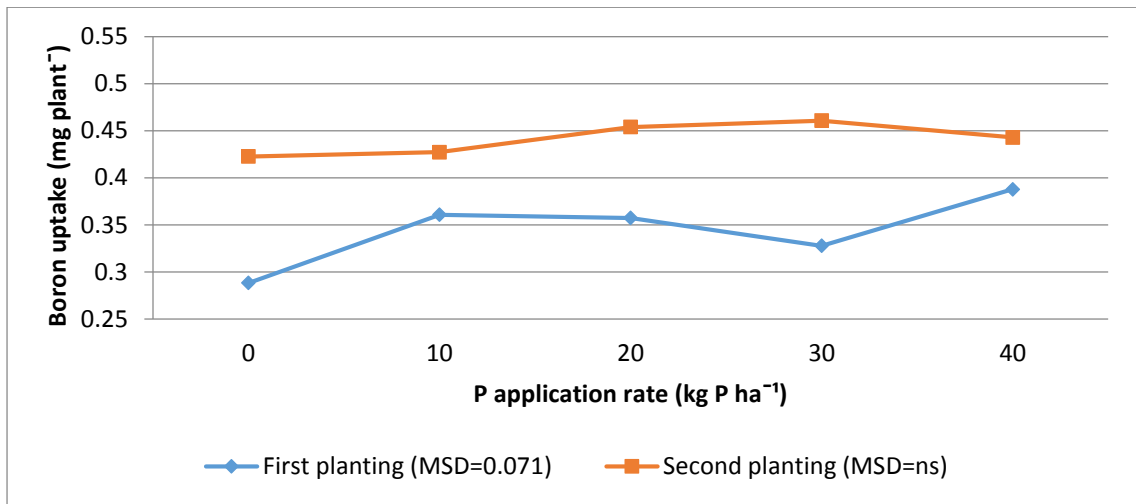
Figure 5.35   Boron uptake in biomass as affected by phosphorus application rate at the end of week 5 for the first planting.

5.4 Discussion

5.4.1 Nutrient reactions to N source

5.4.1.1 Macronutrients

The concentration and uptake of certain macro and micronutrients was affected by either a synergistic (positive) or an antagonistic (negative) response (Sumner & Farina, 1986; FSSA, 2007) to the treatment factors, *viz.* N source, P source and P application rate according to Mulder's chart (Figure 5.36).
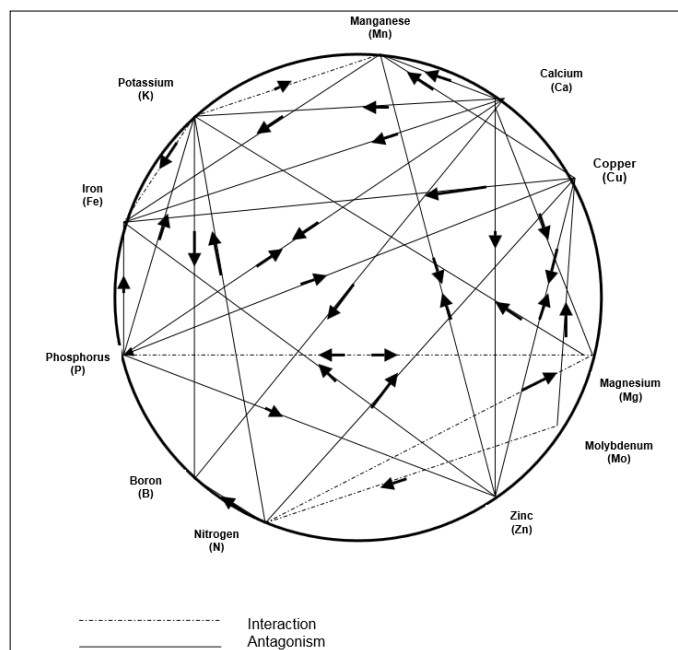
Figure 5.36   Mulder's chart (FSSA, 2007).

Thresholds (Table 5.5) will be used as reference to compare the concentration of nutrients in this study to standards delineated by Campbell & Plank (2000). The significant response to main treatments will be looked at individually, with the exception of K and Mg which reacted to an interaction between P source and P application rate (Table 5.2).

Nitrogen supply increases the growth and development of maize, consequently, increasing the demand for all nutrients (Mengel & Kirkby, 1987). This demand can result in the uptake of nutrients by the plant at concentrations less or greater than that needed for sufficiency (Table 5.5), which is a function of the nutrient supply within the root zone (Wilkinson *et al.*, 1998).

Table 5.5   Sufficiency reference ranges of nutrients for maize (for the whole plant) during early growth and development until before tasseling (Campbell & Plank, 2000), as well as measured nutrient concentration ranges for main treatments

| Nutrient		Sufficiency reference ranges				Experiment ranges		
		Deficient	Low	Sufficient	Toxic	N	P	P rate
N	Percent (%)	< 3	-	3 – 4	> 4	3.33 – 3.56	3.38 – 4.49	3.34 – 3.60
P		< 0.25	0.25 – 0.3	0.3 – 0.5	> 0.5	0.28 – 0.30	0.27 – 0.29	0.27 – 0.30
K		< 2	-	2 – 3	> 3	4.50 – 5.37	4.70 – 5.23	4.65 – 5.23
Ca		< 0.4	-	0.25 – 0.8	> 0.8	0.50 – 0.55	0.52 – 0.56	0.49 – 0.57
Mg		<0.25	-	0.15 – 0.6	> 0.6	0.24 – 0.26	0.25 – 0.26	0.24 – 0.27
S		< 0.12	-	0.15 – 0.4	> 0.4	0.17 – 0.19	0.18 – 0.19	0.18 – 0.19
Fe	(mg kg^{-1})	< 15	16 – 30	30 – 250	> 250	104.4 – 134.4	106.3 – 123.9	106.6 – 129.5
Mn		< 15	16 – 20	20 – 150	> 150	42.73 – 65.32	43.15 – 66.84	40.97 – 67.11
Zn		< 15	16 – 20	20 – 70	> 70	40.82 – 66.21	41.03 – 58.51	37.58 – 62.27
Cu		< 5	-	5 – 25	> 25	7.81 – 8.75	7.75 – 8.62	7.03 – 9.36
B		< 10	-	5 – 25	> 25	8.15 – 14.19	4.46 – 13.12	7.83 – 13.19
Mo		< 0.1	-	0.1 – 2	> 25	1.54 – 2.15	1.62 – 1.92	1.44 – 2.04

Nitrogen and S are both vital for protein synthesis (Mengel & Kirkby, 1987). The ratio of N to S concentration in plant tissue is a reflection of the ability of both N and S to partake in the protein synthesis process (Brunold & Suter, 1984). A plant's N:S ratio should generally be between15:1 and 20:1 (Cram, 1990). The N:S ratio for this study was 18:1. A change in the ratio below or above the optimum N:S range suggests that protein metabolism, growth and development have significantly been altered (Friedrich & Schrader, 1978).

Fertilization with either urea or LAN, at an application rate of 120 kg N ha^{-1}, yielded a N concentration within the sufficiency range of 3 to 4% (Table 5.5). Nitrogen concentration with urea (≈3.56%) was on average slightly greater than with LAN (≈3.38%) (Figure 5.1). Nitrogen uptake on the other hand proved to be greater when the maize plant was fertilized with LAN (Figure 5.3 a) compared to when N is supplied in the urea form; which concurs with the results of Fageria (2001). This is because LAN is superiorly used by the maize plant compared to urea, especially when band applied at rates higher than 90 kg P ha^{-1} (Adriaanse & Human, 1993). In addition, immediate availability and ease of nitrate uptake via LAN after placement, compared to its urea counterpart, cannot be dismissed (Havlin *et al.*, 1999).

Nitrogen has a strong regulatory effect on S concentration and uptake (Havlin *et al.*, 1999) however, the use of both urea (≈0.19%) and LAN (≈0.18%), yielded an S concentration (Figure 5.11 a) within the sufficiency range of 0.15 to 0.4% (Table 5.5). An explanation for the lower S concentration with LAN may reside in the low initial soil Ca concentration of 302 mg kg^{-1} (Table 3.1). The addition of Ca via LAN exerts an initial inhibitory response (5.29%) on S availability for plant uptake (Fageria, 2001). A lack of S availability will consequently limit the efficiency of LAN, since adequate S is extremely important to achieve maximum usage of the applied N source (Fazili *et al.*, 2008). Limestone ammonium nitrate yielded a greater S uptake (Figure 5.12 a) compared to urea which concur with the findings of Clarkson *et al.* (1989).

The synergism between N fertilization and P uptake is ascribed to the fact that N firstly increases P assimilation and concentration within the maize plant as a result of increased root growth, and secondly as a result of the increased ability of roots to absorb P from the soil which is then translocated to the necessary sinks (Marschner, 1995). However, this synergy between N and P becomes slightly inhibited by the initial medium soil pH (5.5) which reduces P availability (Terman *et al.*, 1977; Wilkinson *et al.*, 1998). The use of either urea or LAN, at an application rate of 120 kg N ha^{-1}, yielded a P concentration within the low range of 0.25 to 0.3% (Table 5.5). The phosphorus concentration (Figure 5.5 a) with urea (≈0.29%) was on average similar to that of LAN (≈0.28%), which is in

accordance to the findings of Barber (1984). The uptake of P in contrast was slightly greater when fertilized with LAN compared to that of urea (Figure 5.7). The aforementioned is related to decreased concentration in larger plants as a result of increased dilution (Ali *et al.*, 1998).

Fertilization with either urea or LAN, at an application rate of 120 kg N ha^{-1}, yielded a K concentration in excess of 3% which is considered as toxic according to Campbell & Plank (2000) (Figure 5.9 and Table 5.5). The aforementioned contradicts the antagonistic reaction of N on K as delineated by Mulder's chart. Potassium concentration with urea (≈4.93%) was on average similar to that of LAN (≈4.98%) (Figure 5.9 a).

The use of either urea (≈0.53%) or LAN (≈0.54%), yielded Ca concentrations within the maize plant, within the sufficiency range of 0.25 to 0.8% (Figure 5.14 and Table 5.5). The increase in Ca uptake when fertilized with LAN (Figure 5.16 a) is soundly in line with the findings of Kawasaki (1995).

Fertilization with either urea or LAN, at an application rate of 120 kg N ha^{-1}, yielded a Mg concentration within the sufficiency range of 0.15 to 0.6% (Figure 3.18 and Table 5.5). This is in accordance with the synergistic reaction of N on Mg (FSSA, 2007). Magnesium concentration as a result of N fertilization with urea (≈0.26%) and LAN (≈0.25%) was similar.

Nitrogen positively influenced the uptake of both K and Mg when applied as LAN (Table 5.2). Greater uptake of K (Figure 5.10) and Mg (Figure 5.20 a) with LAN is a result of the increase in the relative growth of both aerial and subsoil parameters, availability, ease of uptake and reduction of N losses via volatilization compared to plants supplied with urea (Claasen & Wilcox, 1974; Fageria, 2001). In addition, the initial medium soil pH (5.5) also favour an increase in K concentration as a result of its exchangeability with NH$_4^+$, due to the close relation between relative size and charge (Havlin *et al.*, 1999).

5.4.1.2 Micronutrients

Micronutrient interactions with N occur frequently as a result of the change in the soil and/or the rhizosphere pH (Fageria, 2001). According to Mulder's chart N fertilization exerts an antagonistic reaction on Cu and B. However, fertilization with either urea or LAN, at an application rate of 120 kg N ha^{-1}, yielded a Cu (Figure 21 a) and B (Figure 5.33) concentration within the sufficiency range of 5 to 25 mg kg^{-1} (Table 5.5) at the end of the vegetative growing period. Copper concentration was greater with urea (≈8.28 mg kg^{-1}) compared to that of LAN (≈7.81 mg kg^{-1}). Boron concentration was also greater with

urea ($\approx$11.17 mg kg^{-1}) compared to that of LAN ($\approx$10.17 mg kg^{-1}). The results of this study therefore, leans more towards a synergistic, instead of an antagonistic response between N fertilization and Cu (Figure 21 a) as well as B (Figure 5.33) concentration.

The Zn concentration for both N sources was well within the sufficiency range of 20 to 70 mg kg^{-1} at the end of the vegetative growing period (Figure 27 a and Table 5.5). Nitrogen generally have no effect on Zn concentration and or uptake (Mengel & Kirkby, 1987). Knowing this it is still interesting to note that urea ($\approx$54.97 mg kg^{-1}) was found to yield a greater concentration compared to LAN ($\approx$41.08 mg kg^{-1}) (Figure 5.27 a). Urea is more acidifying than LAN and this could partly explain the greater Zn availability with a decrease in soil pH (Wilkinson *et al.*, 1998; Fageria, 2001).

Urea ($\approx$113.61 mg kg^{-1}) yielded a lower Fe concentration compared to LAN ($\approx$119.42 mg kg^{-1}); even though Fe concentration for both N sources was within the sufficiency range of 30 to 250 mg kg^{-1} at the end of the vegetative growing period (Figure 5.31 and Table 5.5). This may be related to a slight increase in soil pH with the addition of Ca contained in LAN (Wilkinson *et al.*, 1998; Fageria, 2001).

The total uptake of Cu (Figure 5.23), Mn (Figure 5.26) and Fe (Figure 5.32) was greater with the addition of LAN compared to the uptake of Zn (Figure 5.27 a) and B (Figure 5.34 a) which was greater with the addition of urea.

5.4.2 Nutrient reactions to P source and P application rate

5.4.2.1 Macronutrients

Phosphorus is a major growth limiting factor for commercial maize production in both acid and alkaline soils of temperate regions (Brady & Weil, 2008). Evaluating the reaction between P and other nutrients is critical in maintaining a balanced nutrient supply in order to promote crop growth and development (Fageria & Baligar, 1997). Not all nutrients tested in this study yielded a positive interaction with P (Table 5.1 and Table 5.2).

Even though N concentration stayed within the sufficiency range of 3 to 4% (Table 5.5) across all rates of P application (Figure 5.2), an antagonistic reaction was found where an increase in P application rate resulted in a decrease in N concentration. Nitrogen uptake (Figure 5.4) as a function of P application rate, in contrast to the aforementioned, increased as P application rate increased and was the greatest at the highest P application rate (40 kg P ha^{-1}). Nitrogen uptake was superior with MAP and the lowest with APP (Figure 5.3 b). Increased P application rate yielded a synergistic response to N uptake during the growth and development of maize, where increasing P rates

increased the N concentration within the maize plant (Figure 5.4) which is in accordance with Terman *et al.* (1977) and Sumner & Farina (1986).

This study yielded no significant reaction between either P source or P application rate on the K concentration within the maize plant, which is in accordance with the findings of (Claasen & Wilcox, 1974). A positive reaction was found where an increase in P application rate resulted in an increase in K concentration. Potassium concentration was in the toxic range (Table 5.5) across all rates of P application (Figure 5.25 and Table 5.5. Potassium uptake (Table 5.3) as a function of P application rate is in agreement to the aforementioned, where K uptake increased as P application rate increased and was the greatest at the highest P application rate (40 kg P ha^{-1}).

Both NP and APP increased plant P and S concentration greatly compared to that of MAP (Table 5.1). Fertilization with either MAP, NP or APP yielded a P and S concentration within the low range of 0.25 to 0.5% and 0.15 to 0.4%, respectively (Table 5.5). Phosphorus concentration (Figure 5.5 b) as a result of P fertilization with MAP (≈0.28%) was on average slightly lower compared to that of both NP (≈0.29%) and APP (≈0.29%). Sulphur concentration (Figure 5.11 b) with MAP (≈1.80%) was on average also slightly lower compared to that of both NP (≈1.86%) and APP (≈1.86%).

According to Mulder's chart P exerts an antagonistic reaction on Ca concentration however, this study yielded no reaction between P source and Ca concentration. An antagonistic reaction, on the other hand, between increasing P application rate and decreasing Ca concentration within the maize plant was observed (Figure 5.15), even though Ca concentration stayed within the sufficiency range of 0.25 to 0.8% (Table 5.5).

Fertilization with MAP, NP or APP yielded the same Mg concentration (≈0.253%) which within the sufficiency range of 0.15 to 0.6% (Figure 18 and Table 5.5). Even though Mg concentration stayed within the sufficiency range across all rates of P application, an antagonistic reaction was observed, where increased P rates decreased the Mg concentration within the maize plant (Figure 5.18 b); which contradicts the first mentioned.

Phosphorus uptake was not significantly affected by P source however, the total uptake of S (Figure 5.12 b) and Ca (Figure 5.16 b) proved to be greater with NP, while the uptake of N (Figure 5.3 b) and Mg (Figure 5.20 b) was greater with MAP (Table 5.2).

The effect of NP on the concentration and uptake of S, P and Ca can be explained from NP's certificate of analysis (Table 3.6). Nitrophosphate was the only P source that contained appreciable amounts of S and Ca in addition to N and P when tested against

MAP (Table 3.5) and APP (Table 3.7). It can therefore be stated that the addition of NP consequently increased the availability as well as the concentration and/or uptake of both S and Ca. The increased concentration (Figure 5.18 b) and uptake (Figure 5.20 b) response between P and Mg was expected since Mg is an activator of kinase enzymes and activates most reactions involving phosphate transfer (Tagliavini *et al.*, 1992).

An increase in the P (Figure 5.8), S (Figure 5.13) and Ca (Figure 5.17) uptake was found to be positively correlated with an increase in the application rate of P. In addition to the aforementioned, P and S uptake was the greatest at the highest P application rate (40 kg P ha^{-1}).

5.4.2.2 Micronutrients

This study yielded no significant reaction between either P source or P application rate on Fe concentration within the maize plant, which is in accordance with the findings of (Claasen & Wilcox, 1974) however, a positive reaction was found where an increase in P application rate resulted in an increase in Fe concentration.

Fertilization with either MAP (≈7.86 mg kg^{-1}), NP (≈8.05 mg kg^{-1}) or APP (≈8.27 mg kg^{-1}) yielded a Cu concentration within the sufficiency range of 5 to 25 mg kg^{-1} (Figure 5.21 b and Table 5.5). Even though Cu concentration stayed within the sufficiency range across all rates of P application, an antagonistic reaction was noted, where increased P application rates reduced the Cu concentration within the maize plant (Figure 5.22); which affirms the findings delineated in Mulder's chart and by Smilde (1973). The aforementioned may be attributed to the soil acidifying effect of the ammonium contained in APP, which increases the Cu concentration due to increased availability (Jackson & Carter, 1976).

Monoammonium phosphate, NP and APP all yielded a Mn and Zn concentration within the sufficiency range of 20 to 150 mg kg^{-1} and 20 to 70 mg kg^{-1}, respectively (Table 5.5). However, NP increased the Mn concentration (Figure 5.25) while APP increased the Zn concentration (Figure 5.27 a, and Table 5.1).

Manganese concentration with NP (≈55.42 mg kg^{-1}) was on average greater than that of MAP (≈54.54 mg kg^{-1}) while APP (≈52.75 mg kg^{-1}) yielded the lowest Mn concentration as a result of P source (Figure 5.21 b). Zinc concentration with MAP (≈47.62 mg kg^{-1}) was on average greater than that of NP (≈46.69 mg kg^{-1}) while APP (≈49.77 mg kg^{-1}) yielded the greatest Zn concentration as a result of P source (Figure 5.21 b). The Mn-P reactions are also supported by Smilde (1973).

Zinc (Figure 5.29 b) and B (Figure 5.32 b) uptake proved to be greater when fertilized with MAP compared to NP and APP (Table 5.2). The increased Zn concentration and uptake is a result of the positive response to ammonium when applied at a low initial soil pH (Wilkinson *et al.*, 1998; Fageria, 2001). Phosphorus and B are both involved in adsorption as well as in precipitation reactions with the sesquioxide minerals within the soil solution (May & Pritts, 1993). Both P and B are involved in root tip elongation which in turn affect the total uptake capability of B (Pollard *et at.*, 1977). Reactions of B with P are generally found at a soil pH of 5.5 and lower (May & Pritts, 1993).

Even though both the Cu, Zn and B concentration stayed within the sufficiency range across all rates of P application, an inverse reaction was found when the P application rate increased. Copper (Figure 5.24) and B (Figure 5.35) uptake as a function of P application rate increased as P application rate increased and was the greatest at the maximum P application rate (40 kg P ha^{-1}), while Zn uptake (Figure 5.30) decreased with increasing P application rate.

5.5 Conclusion

All treatment factors *viz.* N source, P source and P application rate affected the concentration and uptake of nutrients. Nutrient concentration within the plant varied with the application of both urea and LAN, however the efficiency of LAN is significantly greater in the total uptake of nutrients. Both orthophosphate sources, namely MAP and NP outperformed their APP counterpart. The uptake of S, N, P, Ca, Na and B increased with P application rate and was greatest at 40 kg P ha^{-1} however, the contrary was found for Cu and Zn uptake. Nutrient concentration of N, Mg, Cu and Zn was greater with the control. The concentration of nutrients generally tested greater in smaller plants, while the uptake however, seem to be stimulated by an increasing P application rate as a consequence of increased vegetative growth.

CHAPTER 6

SUMMARY, SYNTHESIS AND RECOMMENDATIONS

Understanding maize's (*Zea mays* L.) nitrogen (N) and phosphorus (P) requirements during the vegetative stage is extremely important, since maize is ranked chief cereal crop in South Africa. Understanding the chemical reactions of N and P both within the soil as well as in the plant is undoubtedly the most important prerequisite of an effective nutrient management program, with N and P occurring in different forms with varying soil and crop reactions. The objectives of this study was to evaluate the quantitative growth, nutrient concentration and nutrient uptake parameters of maize response to different phosphorus sources monoammonium phosphate - MAP, nitrophosphate - NP and ammonium polyphosphate - APP) as well as P application rates (0, 10, 20, 30 and 40 kg P ha^{-1}) during the early growth (5 weeks) of maize with both urea and limestone ammonium nitrate - LAN) as N basis.

A glasshouse experiment was conducted with 40.5 L pots each filled with a dark brown sandy-loam topsoil pertaining a medium soil pH of 5.5. The aerial and subsoil parameters were tested through integrated treatments consisting of three main factor treatments *viz.* N source (urea and LAN), P source (MAP, NP and APP) and P application rate (0, 10, 20, 30 and 40 kg P ha^{-1}), replicated three times and independently subjected to a randomized complete block design with a factorial combination. Treatments were band applied in a single 0.34 m row, 50 mm below and 50 mm away from the maize seeds; which were planted with a between row spacing of 0.91 m, 50 mm below the soil surface. After planting the soil was watered and maintained at field capacity for the duration of the experiment. Plants were then allowed to grow for five weeks. The aerial parameters of three plants per pot were measured on a weekly basis following emergence while the subsoil parameters were taken at the end of the five week vegetative growing period. The experiment was repeated at two planting dates.

Plant growth and development in a sandy soil depend on the manner and degree in which roots exploit the soil to take up nutrients; especially with P, since P movement in the soil is limited compared to that of N. Aerial and subsoil parameters showed a significant greater response to nitrogen source; which was strongly reflected during both plantings, compared to P source and P application rates. Plants treated with LAN yielded

both greater aerial and subsoil measurements compared to urea, which is primarily ascribed to immediate availability of N.

Aerial and subsoil plant parameter response to phosphorus source and P application rate was greater during the first planting. The overall root length and root mass of plants fertilized with MAP was significantly greater compared to that of both NP and APP. In addition to the aforementioned the amount of leaves, plant height, stem thickness, leaf area and dry mass of the maize plants were significantly greater when fertilized with MAP compared to APP, however did not significantly differ from NP. Both aerial and subsoil parameter measurements were significantly greater when P was applied at 40 kg P ha^{-1}. Subsoil parameter response in and away from the fertilized band however, was inconsistent.

After the morphological parameters were measured the dry material was subjected to chemical analysis (Omnia Nutriology®) in order to evaluate the quantitative nutrient concentration and nutrient uptake response as a result of the three main treatment factors. Nutrient concentration and uptake was used to determine whether plant and soil nutrient interactions were synergistic or antagonistic.

Inorganic P when applied to the soil, exist predominantly in the orthophosphate form and may either be adsorbed or precipitated onto the soil mineral surfaces depending on the soil's pH. Water-soluble P, in addition to the aforementioned may also be incorporated into the plant's biomass. A soil with a low pH is positively charge and increases in positive charge as the pH decreases. Therefore, the application of P to a dark brown sandy loam soil with a medium pH of 5.5 and a very high iron content of 18 mg kg^{-1} (Table 3.1) gives way to sorption reactions where the negatively charged P ions are fixed to the positively charged Fe^{3+} and Al^{3+} sesquioxide mineral surfaces of the soil. The adsorption of positively charged metal ions such as zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu), which had respective soil analysis values of 0.7, 32.5, 18 and 1 mg kg^{-1} in the high to very high ranges (Table 3.1), onto the surfaces of synthetic iron oxides further increases the positive charge of the sesquioxide mineral surfaces, thus increasing fixation of negatively charged ions such as P. As a result of the latter, the P within the soil solution, available for passive uptake and reactions with other nutrients, will be low even though it is in equilibrium with P held onto the particle surfaces of the soil.

Phosphorus and sulphur (S) uptake was significantly greater with the use of NP and LAN compared to that of both MAP and APP as well as that of urea. This is most probably because NP was the only P source to have had additional S (Table 3.6). Furthermore, the synergistic response between N and P (where the addition of N, especially in the

nitrate form, increases the total P uptake) seem to be one reasonable explanation for the increased P uptake with the addition of NP and LAN. Another reason for increased P uptake with NP and LAN rests in the additional calcium (Ca) and or magnesium (Mg) within the NP and LAN granules (Table 3.6). This meant that both NP and LAN had the added benefit of increasing the soil pH ultimately counteracting the sorption reaction, making greater quantities of the residual P available for plant uptake. Though not measured, it seemed that a great quantity of P was adsorbed by the sesquioxide mineral surfaces since P was only taken up in the low ranges (0.25 to 0.3 %).

Even though P and S uptake was greater with NP and LAN, the P and S concentration however, was greater when the plant was fertilized with APP and urea. This clearly shows the relationship between growth, concentration and uptake. The greater the vegetative growth, the greater the uptake of nutrients, however the lower the nutrient concentration within the biomass. This is because larger plants contain a greater water fraction per unit area compared to smaller plants, and as a result yield lower nutrient concentration analysis due to dilution, even though the total uptake is greater.

Superior nitrogen (N) and potassium (K) uptake is strongly related to the stimulation of vegetative growth and development with the use of LAN and MAP. The NH_4^+ contained within both aforementioned N and P sources is highly similar with regards to K^+ charge, size and hydration energy which means NH_4^+ and K^+ competes for sorption. The efficiency of N and K use by field crops depends not only on the cation exchange behaviour of NH_4^+ and K^+ dissociated from the applied inorganic fertilizer salts but may also be strongly affected by Ca^{2+} depending on soil pH. The addition of NH_4^+ (within both LAN and MAP) to a soil that was highly saturated with K^+ (150 mg kg^{-1}), yielded an antagonistic reaction between NH_4^+ and K^+. The use of LAN and MAP released large quantities of NH_4^+ into the soil solution that favoured the adsorption/substitution of NH_4^+ in the place of K^+. As a result, greater quantities of K^+ is available for plant uptake. Fertilization with a N source containing Ca^{2+} (LAN) allows for rapid K^+ uptake. This is because Ca increases root respiration, membrane integrity, plant metabolism and consequently K absorption. Calcium is more strongly held to the soil particles than K^+, allowing for greater K uptake with the application of LAN. Also, as the maize plant increases in biomass so does the requirement for N and K, two essential nutrients respectively required for energy production as well as the structural integrity of the plant.

Superior uptake of Ca and Mg by the maize plant with the application of LAN and MAP is mainly attributed to the greater growth and development of the aerial and subsoil parameters with LAN and MAP. Greater growth consequently increases the nutrient requirement and therefore increased uptake will persist. This is in line with the synergistic

response between NH_4^+ and Ca^{2+} and Mg^{2+}, where NH_4^+ has been reported to enhance Ca^{2+} and Mg^{2+} uptake. The NO_3^- contained within LAN also assist in increased Ca^{2+} uptake. Also, since Ca^{2+} is held very strongly by the soil particles within an acid soil, the addition of LAN not only improves soil pH but also assist in increased availability and uptake of Ca^{2+} by the roots. Stimulation of Ca concentration within the maize plant's biomass is firstly related to the increase of both soil pH and Ca availability with the application of NP and LAN, and secondly related to greater growth.

Various micronutrients and other ions commonly found in the soil *viz.* Al, play an important role in the uptake of P by plants. Nitrogen and P on the other hand, are involved in either energy and/or transfer processes which assist with the translocation of micronutrients to the necessary sinks once assimilated by the root system. Micronutrients, with the exception of Zn, are highly immobile once in the plant. Zinc is assimilated throughout the season and is mainly stored in the stems during the vegetative stage, while Fe is assigned to the leaves. Copper and Mn are distributed to both leaves and stems and are assimilated mostly before the flowering period. In order to achieve an optimum yield, farmers have come to rely on a fertilization program where greater quantities of N and P are applied to maize hybrids. As a consequence, greater quantities of micronutrients are translocated through the plant as a response to N and P fertilization.

Biomass concentration of B, Zn and Fe was greater with urea compared to LAN, while B and Fe uptake was superior with the use of LAN. Urea allowed for greater Zn uptake. The uptake of all micronutrients was greater with MAP and was superior to the use of APP. The nutrient concentration of all micronutrients were greater with the use of APP compared to MAP and NP. The aforementioned clearly shows the relationship between micronutrient concentration and uptake as a response to N and P fertilization. The greater the vegetative sink, the greater the requirement and translocation of the aforementioned micronutrients. The availability of micronutrients as a consequence of a slight decrease in the soil pH with the use of ammonium N and P sources, may also have resulted in the increased uptake of micronutrients.

It is recommended that:

- Further research be done to establish the beneficial effects attributed to orthophosphates when in combination with LAN (band applied at planting).
- The trial be extended till the end of the maize flowering period as to determine whether APP availability increases if the hydrolysis period is extended.

- The trial be executed on a soil with a low soil pH (KCl) of ≤4.5 in order to determine the economic viability of treatment factors in the general South African environment.
- The experiment be performed in the field to determine how orthophosphate and polyphosphate sources compare when environmental conditions are not controlled.

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