Response of Cynodon dactylon (L.) Pers. to ammonium and nitrate nutrition

by

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1999

Abstract

Plants that come early in succession predominantly prefer ammonium form of nitrogen than nitrate. The hypothesis that *Cynodon dactylon* (L.) Pers., a grass in the primary succession stage would respond well when supplied with ammonium than nitrate fertiliser was tested. Seedlings of *Cynodon dactylon* were grown in culture solution and fertilised with either ammonium sulphate or potassium nitrate at a continuous concentrations of 200 ppm, 400 ppm and 600 ppm in the growth chamber. In support of my hypothesis, growth of *Cynodon dactylon* was greater from ammonium than nitrate nutrition in terms of aboveground and total plants yields at 200 ppm and 400 ppm and this was associated with efficient utilisation of this form of nitrogen by this species. Production from the nitrate treatments was restricted and showed no change with increase in external nitrogen supply. Decreased dry weights from the ammonium nutrition at 600 ppm were associated with toxicity of ammonium ions in plant tissues. High levels of nitrogen measured from the nitrate treatments were associated with the soluble nitrogen that was not assimilated for yield increase by this grass. Uptake of ammonium ion was shown to result in high of uptake phosphorus. This study shows that *Cynodon dactylon* has the capacity for increased growth under ammonium nutrition while this was limited in the nitrate nutrition. However, response is restricted when excessively fertilised with ammonium nitrogen.

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1. Introduction

Nitrogen in its various combined forms is a precious commodity in the biosphere where it forms a major component of many key compounds which are essential for the structure and functioning of plants and other biological organisms.

Nitrogen is found in inorganic forms, such as ammonia, ammonium nitrate, ammonium phosphate, ammonium sulphate, calcium nitrate, nitric phosphate, potassium nitrate, and sodium nitrate and it quickly becomes part of the soil solution or the cation exchange complex when applied to moist soils (Scarsbrook, 1965). These individually or in combination, comprise nearly all the inorganic nitrogen sources utilised in fertilisers.

Despite the great abundance of dinitrogen gas in the atmosphere, the element is commonly deficient in grassland soils from which it is available in the oxidised (nitrate) and reduced (ammonium) forms (Novoa and Loomis 1981). In aerated soils, bacteria quickly transform ammonium ions to nitrate, and the oxidised form is the principal one in plant nutrition. Nitrogen in the soil is also mildly available in the form of simple organic compounds, principally those containing free amide or amino acid groups (Scarsbrook 1965). Nitrites are a minor source of available nitrogen and are normally considered toxic. The concentration of nitrogen in the grassland soil solution is generally low, 1 mM or less, with 0.1 mM (100μM) not uncommon (Novoa and Loomis 1981). Ammonium nitrogen is generally available in much lower amounts, and tightly bound to the cation exchange complex (Scarsbrook, 1965).

Although plants can usually utilise any of these nitrogen forms, there are numerous exceptions (Bonner 1946). One form may be preferentially absorbed depending on environment and the species and age of plants (Ghosh and Burris 1950). While plants maybe similar in appearance and grow equally well on different forms of nitrogen, the plant composition may be widely different. This is because physiological response of these ions differ (Cox and Reisenauer, 1973) and plants tend differ in their ability to absorb them (Bonner,1946). For example, Arnon (1937) when comparing the growth of barley in either ammonium or nitrate nutrition obtained a more positive growth from

the latter. Similarly, Spratt (1974) also tested the effects of various combinations of ammonium and nitrate sources of nitrogen on growth and nitrogen uptake of spring wheat (*Triticum aestivum* var Manitou) at the seral stage and showed and obtained higher yields from the ammonium than the nitrate nitrogen. This mean that this species prefer ammonium nitrogen than the nitrate one. However, the general opinion is that most species tend to prefer nitrate. This is ascribed to the fact that nitrate ion is less toxic to plant tissue and can actually accumulate in large concentrations without affecting plant tissues. However, toxic effects of high levels of ammonium nitrogen on plant metabolism has been shown in a number of studies (Nittler and Kenny 1976). Toxicity of ammonium nitrogen to plants appear to be caused by two main factors (Lewis 1986):

(i) ammonium is shown to uncouple photophosphorylation at concentration as low as low as 2 mM, thus restricting ATP production in the leaves. This is probably the reason why ammonium assimilation takes place in the roots and ammonium is loaded onto the xylem supply to the leaf in small quantities.

(ii) ammonium absorption is electrically balanced by the excretion of H^+ by the root into the soil. Therefore acidification of the rhizosphere retards growth, resulting in stunted rooting system and greatly impaired nutrient absorption.

Limitations on growth imposed by different forms of nitrogen suggest three types of plants with respect to their nitrogen response. These include nitrate nutrition adapted species, ammonium nutrition adapted species and ammonium + nitrate adapted species (Lewis, 1986). Nitrate adapted species usually occur on neutral or alkaline soils where pH is conducive to the activity of the soil nitrifying bacteria (Alexander, 1965) which converts all or most of the available soil ammonium into nitrate. Such plants tend to be killed by high levels of soil ammonium. The ammonium adapted species generally occur where soils are acidic and the activity of the nitrifying bacteria is inhibited and the main form of available nitrogen is the ammonium ion (Alexander, 1965). Many plants growing under these conditions tend to show a better response to ammonium than nitrate nutrition. The ammonium and nitrate adapted plants have been shown to respond well to the mixture of the these two nitrogen sources. For example, Cox and Reisenauer (1973) did a study on wheat which is a crop plant. They found that the

combination of the two nitrogen sources resulted in increase in productivity of this crop by over fifty percent as opposed to supplying it with one source of nitrogen alone.

Hall, Meredith, and Altona (1949) established that fertilisation of grassland with ammonium sulphate as the nitrogen source causes a reversion of climax grassland to earlier successional stages. Roux (1954) showed a similar reversion of grasslands with ammonium sulphate fertilisation which caused disappearance of climax grasses and were replaced by earlier succession grasses such as *Eragrostis* species and *Cynodon dactylon.* Roux (1954) found that ammonium sulphate had the most stimulating effect on the growth of *Eragrostis curvula* and depressed the growth of *Trachypogon* which is a climax grassland species. Roux (1954) argued that the sensitivity of climax grass species and their replacement by earlier grasses in succession depends more on the availability of nitrogen than on acidity. Dominance of earlier succession grasses can be ascribed to their ability to respond to relatively high concentrations of the available nitrogen and perhaps their ability to withstand high salinity (Altona, 1972). However, Whiltshire (1973), has argued that it is unlikely that change in the soil pH are responsible for the relative performance of different species on nitrogen forms since soils fertilised with ammonium became more acidic. High pH levels have been noticed in climax species and this would have had some effects on the climax species such as *Themeda triandra* (Roux 1954). The results of Whiltshire (1973) indicated that nitrogen source affected yield far more than acidity. Neutral soils are shown to improve the yield of crops and does not usually improve production of the natural pastures (Brockingston 1961). Wiltshire (1973) therefore, suggested that grassland climax in highveld is dominated by calcifuge species that differ from their precursors by being less productive, more tolerant to soil acidity and tolerance of low nitrogen availability on soils containing mainly ammonium nitrogen.

Further, evidence from Warren (1966) revealed that climax species of grasslands are apparently dominated by calcifuge species as many of the grasses show a definite preference for ammonium nutrition and grow in acidic soils containing little nitrate for most part of the year. In their respective studies they showed that ruderal grasses (*Eleusine indica, Panicum laevifolium and Rhychelytrum repens*) responded to higher

levels of ammonium nitrogen than did the pioneers (*Cynodon dactylon* and *Eragrostis curvula*) or climax species (*Cymbopogon excavatus, Digitaria tricholaenoides, Elyonurus argenteus, Themeda triandra, Trachypogon spicatus* and *Trystachya hispida*).

Most of Chloridoid grass species tend to occupy early stages of succession while most of the Andropogonoides tend to occupy secondary and climax stages. In the present study three grass species *Eragrostis curvula, Cynodon dactylon* and *Bothriochloa insculpta* belonging tribe Chlorideae and three from Angropogoneae which includes *Cymbopogon plurinoides, Themeda triandra* and *Enteropogon macristachyus* were grown under three different concentrations of ammonium or nitrate nitrogen. The objective was to investigate whether successional patterns in terms of response to nitrogen source, reflect phylogenetic differences between these tribes. The hypothesis was that application of ammonium nitrogen would increase yield from the Chloridoid species while nitrate nutrition would increase yield from the Adropogonoid species. However, during experimentation all the species chosen for this study died except *Cynodon dactylon.* As a result of this experimental problem and time limitation, this experiment was continued with *Cynodon dactylon* alone and the hypothesis was modified slightly. The modified hypothesis was that *Cynodon dactylon* would respond well when supplied with ammonium nitrogen while it would show a more restricted response to nitrate nutrition. Response to nitrogen source has been measured in terms of biomass production, nitrogen uptake, and the influence of nitrogen source on the uptake of phosphorus.

2.Materials and Methods

SECTION A

2.1 Growth of plants

2.1 1 Selection of plants and germination of seeds.

Three species of the tribe Andropogonae and three of tribe Chloridae were chosen to represent different stages of plant succession. Seeds of these species were obtained from Kruger National Park (Table 1) and sown in acid washed river sand in 2. 5 litre polythene containers covered with black plastic bags to aid in germination. These were provided with constant environmental conditions as described below.

Table . Initial list of species used in the experiment. N/S = information not supplied

Species	Tribe	Prime number	Germination percentage	Seed collection locality
Cymbopogon plurinoides	Andropogoneae	01P9	NS	Kruger National Park
Themeda triandra	Andropogoneae	018F	N/S	Kruger National Park
Enteropogon macristychyus	Andropogoneae	016K	N/S	Kruger National Park
Cynodon dactylon	Chlorideae	02JC	77.75%	Ex. Gunsen seed Kliprivier
Eragrostis curvula	Chlorideae	03F8	67%	Ex. Tanganyika via Plant and Quality control
Bothriochloa insculpta	Chlorideae	Q1E2	16%	Kruger National Park

2.1.2. Environmental conditions

A plant growth room was used to provide a 15 hour day with light intensity at mean plant height 315+/-30μmol^{-1} m^{-2} s^{-1} (photosynthetically active radiation). The day temperature was at 28^{0}C and night temperature at 15^{0}C. Relative humidity was kept at 65%.

2.1.3 Planting of seedlings

Five days after planting the seeds, all five species except *Eragrostis curvula* (which failed to germinate) started to germinate. When the plants had grown to about 30 mm long which was fourteen days after germination, the seedlings were transferred to 25 L polystyrene pots supplied with a cover containing 12 holes. Seedlings from each species were washed with distilled water to remove sand on the roots. Three replicate seedlings of each species were each rolled with a small piece of foam rubber (c.15 cm x 2 cm) and were stuck in the holes of the lid to aid in suspending them in the culture media. These were then suspended in the 25 litre polystyrene pot containing nutrient solution, each supplied with different concentrations of nitrate or ammonium nitrogen as described below. Even more than aiding in the suspension of seedlings in the culture media, covers are also useful in providing shade and reduction of water loss, the exclusion of much of the dust in the air and are the most practical support in water cultures (Hewitt 1952).

2.1.4. Culture solution

Each 25 litre polythene container received a Long Ashton nutrient solution prepared from the following macro nutrient stock solution: 0.15 M $MgSO_4.7H_20$, 0.20 M K_2SO_4, 0.40 M $CaCl_2.H_20$, 0.133 M $NaH_2PO_4.2H_2O$, 0.133 M Na_2HPO_4. To make up N-free nutrient solution in 25 litres, 250 ml of $MgSO_4.7H_2O$ and K_2SO_4 and 125ml of NaH_2PO_4 and $Na_2HPO_4.12H_2O$ were placed in each container and the solution was made up to three quarters full before the $CaCl_2.2H_2O$ was added. $CaCl_2.2H_2O$ could not be added with other macro nutrients because it precipitates out, thereby making the solution useless. The micro-nutrients were supplied in all the three treatments as: Fe-EDTA (2.8 μg Fe ml^{-1}), $MnCl_2.4H_2O$ (0.550 μg Mn ml^{-1}), H_3BO_3 (0.330 μg B ml^{-1}),

$Zn\ Cl_2$ (0.065 μg Zn ml^{-1}), $CuCl_2.2H_2O$ (0.064 μg Cu ml^{-1}), Na_2MoO_4 (0.046 μg Mo ml^{-1}).

Ammonium sulphate and potassium nitrate were used as the sources of ammonium and nitrate nitrogen respectively. Three concentration levels of nitrogen were chosen for each nitrogen source. Ammonium or nitrate nitrogen at 200 parts per million (ppm) was chosen as the lower limit because it was considered to be above the deficiency level for almost any culture solution and moreover one below this level is sometimes difficult to keep ammonium and nitrate in solution and not adsorbed onto the containers; 600 ppm was used as the upper limit because it has been found to be optimal or even supra-optimal for the growth of many species in solution culture (Hewitt 1952) and 400 ppm represented the medium concentration for the two sources of nitrogen used in this experiment. Each pot was constantly aerated to allow uniform distribution of the solution and proper oxygenation of nutrient medium.

For the first two weeks of growth of seedlings, the Long Ashton culture solution was kept at 200 ppm to allow seedlings to establish in the nutrient media. This was done in order to minimise the shock that might be experienced by young seedlings when they are subjected into high nutrient medium. To prevent nutrient depletion, the solution was renewed each week

2.1.5. Establishment of plants

After two weeks of growth of the plants in the phytotron chamber all the species started to die out except *Cynodon dactylon* which established well. Therefore, only *Cynodon dactylon* was used to carry in the study and the rest of the species initially considered were discarded due to time limitations.

2.1.6. Harvest and determination of yield.

After eight weeks of growth in the nutrient media, the *Cynodon dactylon* plants were harvested and divided into shoot and roots. From each plant, the outline of five leaves chosen at random from the second node of the tiller were drawn on the graph paper with 1 mm^2 grids and the average area was determined by counting the number of

squares. Shoots (aboveground plant parts), roots (belowground plant parts) and the measured leaves were dried in a forced-draught oven at 80 ^{0}C for 48 hours. The aboveground and belowground weights were determined using model TS4KD Ohaus balance and leaf weights were determined using Mettler AE 200 balance.

2.1.7. Dry matter partitioning

All the morphological parameters were weighted by dividing the integral of the polynomial over the total plant production (total plant mass). The parameters that were included are leaf weights, leaf areas, root weights and shoot weights. These values give an insight on how much of the dry matter has been allocated for a particular growth parameter in relation to the total mass of the plant.

SECTION B

2.2. Chemical Analysis

2.2.1. Preparation of sample chemical analysis

Samples of dried leaf material were obtained from each plant. The material was finely ground using a micro hammer mill. This material was used for the estimation of leaf nitrogen and phosphorus concentrations.

2.2.2. Kejdahl digestion

Two analytical procedures are available for determination of total nitrogen, the Kejdhal methods and the Dumas method. The Dumas method is rather complicated and their low analytical capacity (less than 10 samples per hour) limits their use for total nitrogen determination (Bremmner, 1965). Thus, for this study nitrogen concentration in the leaves was measured by the modified salicylic acid-thiosulphate modification of the Kejdahl method to include nitrate-nitrogen. An amount of 0.1 gram (g) of ground leave material of each of the 26 samples were placed in thick-walled digestion tubes. To each sample was added 1 ml of distilled water. Eight standards with different nitrogen concentration (0.25 mg N ml^{-1} to 3.5 mg N ml^{-1}) were prepared from stock solution. Three distilled water blanks were included for the correction of the results. 3 ml of concentrated sulphuric acid containing salicylic acid was added to both the samples and the standards in order to convert nitrogen in the samples to ammonium when heated in the digestion block. This conversion was promoted by adding a spatula tip of sodium thiosulphate and selenium-catalyst tablet in each tube. The tubes were then heated gently in the digestion block at 150 ^{0}C for 4 hours to drive the water off. Digestion was carried further by increasing temperature at one hour intervals from 220 ^{0}C to 250 ^{0}C to 280 ^{0}C to300 ^{0}C and finally 350 ^{0}C for four hours until the digest was clear.

The digests were cooled to 150 ^{0}C and the liquid inside was rolled on the walls of the tubes to wash the residue back into the acid. The digestion was repeated again by heating following the above temperature sequence at intervals of 30 minutes until the digest was clear again. The digests were then cooled. To the still warm digest (150

^{0}C), about 5-10 ml of water was added to drop by drop down the side of each tube until the violent reaction subsided, then an additional 25 ml of water was added and the solution was thoroughly mixed. The digest was transferred to 50 ml volumetric flasks and the volume of each was made up to the mark. This was allowed to settle until the solution cleared. The ammonium content was determined by the phenol-hypochlorite method (Smith 1980) as outlined below.

2.2.3. Colorimetric determination of Kjedahl digestion

From each standard and sample digest, a volume of 0.5 ml was placed into 50 ml volumetric flasks. To each flask 25 ml of EDTA-Na_2 was added followed by 2 ml of Reagent A. Reagent A is made up of equal parts of 0.5% mass per volume of sodium nitroprusside and 10% phenol in 95% ethanol. Reagent B (3.5 ml) made up of four parts of alkaline phosphate buffer and one part of 1.5% sodium hypochlorite were then added. The volume was made up to mark with distilled water and left for about 1 hour for the blue colour to develop.

Absorbence of the acid digest was determinedat 635 nm with a model 4001/4 B and L Spectronic spectrophotometer. The standard curve was drawn up and the unknown leaf nitrogen concentration values from each sample were determined from their absorbency values on the standard curve.

2.2.4. Total phosphorus determination.

The tri-acid digestion method for total phosphorus (P) determination was used in this study. A 0.1 g sample of finely ground leaf material from each plant was placed into thick walled digestion tubes and pre-digested by adding 1 ml of concentrated nitric acid and heated in the digestion block at 180 ^{0}C for 1 hour until the samples were almost dry. Three blanks were included and placed randomly with the samples. The samples were then cooled and 1 ml of a triacid (HNO_3:$HCLO_4$:H_2SO_4) mix was added to each. The samples were digested further for 1 hour at 180 ^{0}C until the white fumes had dissipated and the solution was clear. The samples were then cooled and diluted to 25 ml with distilled water and mixed thoroughly. Murphy and Riley solution was prepared as outlined below for the colorimetric determination of phosphate.

Solution:

(a) 140 ml of 2.5 M H_2SO_4 was dissolved in 860 ml of distilled water

(b) 2.64 g of ascorbic acid dissolved in 150 ml of distilled water.

(C) 20 g of ammonium molybdate dissolved in 500 ml distilled water and 75 ml of this solution was used.

(d) 0.5486 g of antimony potassium tartrate dissolved in 200 ml of distilled water and 25 ml of this was used.

All the reagents were mixed together in the sequence as outlined above.

A test range was run on a few representative samples to determine the optimum aliquot size for photometric measurements that will give absorbence readings of about 0.400. This is because the relationship between the amount of phosphorus and absorbence is not linear above a certain amount of P.

An aliquot of 0.8 ml was found to be the optimum size for photometric measurements and this amount of each digest was placed in a 50 ml volumetric flask, and 8 ml of this Murphy and Riley reagent was added to each flask and the solution made up to 50 ml and the blue colour allowed to develop for 1 hour. Absorbence was read at 882 nm with a Model 4001/4 Spectronic spectrophotometer. The concentration of phosphorus

was found by means of a calibration curve prepared by the above procedure of known amounts of KH_2PO_4. All the measurements were done under the same temperature.

3. Statistical analysis

Two way analysis of variance (ANOVA) in Statistica for windows version 5.1 (StatSoft, Inc. 1996) was found to be the appropriate statistical test for this kind of data. Where ANOVA identified significant differences, an LSD multiple range comparison test was performed to locate the significant differences.

4. Results

4.1. Biomass Productivity

4.1.1. Total dry mass

Results for the total dry mass production are presented in Figure 1. Total plant yields were significantly higher from the ammonium than the nitrate treatments as shown by two way ANOVA ($F_{(1,19)} = 140.53$, $p < 0.05$). Levels of nitrogen on total dry mass production was found to be significantly different ($F_{(219)} = 126.17$ $p < 0.05$). The interaction of the nitrogen source and level were also found to be significantly different ($F_{(2,19)} = 68.45$ $p<0.05$). The trend in Figure 1 shows that whole plant mass was enhanced in the ammonium treatments receiving 200 ppm and 400 ppm ammonium nitrogen than in the nitrate nitrogen receiving plants. Nitrate nutrition appear to have a restricted effect at these nitrogen levels for the total plant yield. Yield was substantially reduced in plants receiving 600 ppm nitrogen.

4.1.2. Aboveground mass

Figure 2 shows the trend in the aboveground dry mass produced by *Cynodon dactylon* treated with different concentrations of either ammonium or nitrate nutrition. The effects source of nitrogen on the aboveground mass production at the end of the experiment were significantly different as indicated by two way ANOVA ($F_{(1,19)} = 158.86$, $p<0.05$). Two way ANOVA also showed that levels of nitrogen supplied to *Cynodon dactylon* had a significant effect ($F_{(2,19)} = 109.35$, $p<0.05$) for the aboveground dry mass production. Interaction between the nitrogen source and the level of nitrogen were shown to be significantly different ($F_{(2,19)} = 78.83$, $p< 0.05$). The trend in Figure 2 shows that aboveground production was more from plants receiving 200 ppm and 400 ppm of ammonium nitrogen as compared to the nitrate treated plants. There was a marked reduction in the aboveground productivity at 600 ppm on the ammonium series. There was little effect on aboveground productivity from nitrate nutrition of plants with change in nitrogen concentration

4.1.3. Belowground mass

Figure 3 shows the belowground productivity of *Cynodon dactylon* at the end of the experiment. The sources of nitrogen on the beowground productivity showed a significant effect the belowground productivity as shown by two way ANOVA ($F_{(1,16)} = 8.95$, $p < 0.05$). Furthermore, two way ANOVA also revealed a significant difference between the means of different nitrogen concentrations ($F_{(2,16)} = 10.24$, $p < 0.05$) on this component. Interaction effects between the nitrogen treatments and the nitrogen concentrations for the belowground mass produced were not significantly different. The trend in Figure 3 shows that belowground mass production was highest in the 200 ppm ammonium nitrogen and was negatively affected as the nitrogen increased to advance to 600 ppm. Although the nitrate treated plants had produced significantly less than ammonium treatment, similar negative impact was observed at 400 ppm as compared to the 200 ppm nitrate concentration and no significant effect was shown as with increase of external nitrogen to 600 ppm. At this level, both treatments had produced equally on the belowground.

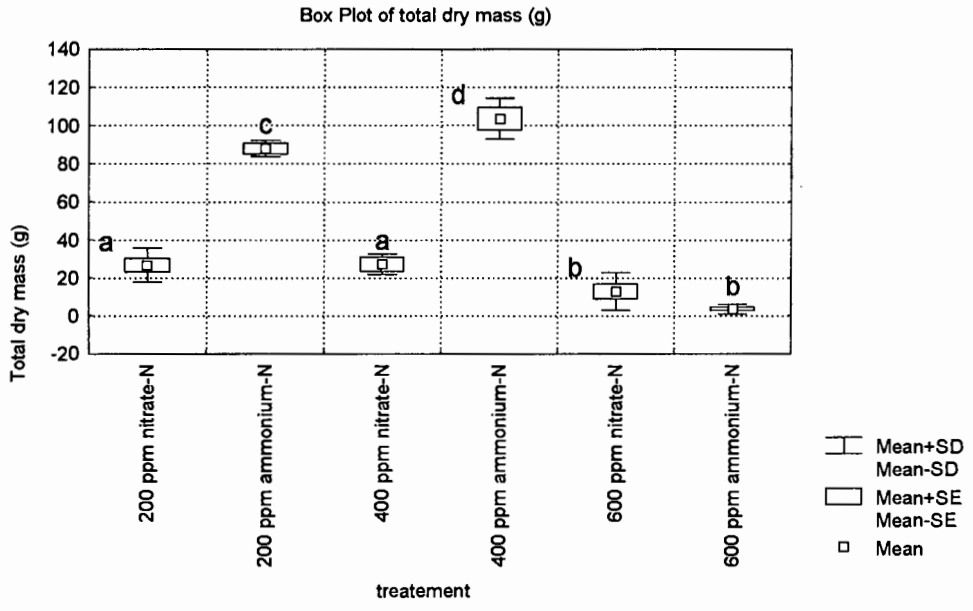

Fig. 1. Box and whiskers plots showing total mass produced by *Cynodon dactylon* treated with different concentrations of either ammonium or nitrate nitrogen. LSD multiple range comparisons are indicated by letters to show where the difference lies.

Two way ANOVA

a) Nitrogen source :$F_{(1,19)} = 140.53$, $p<0.05$

b) Concentration :$F_{(2,19)} = 126.17$, $p<0.05$

c) Interaction :$F_{(2,19)} = 68.45$, $p<0.05$

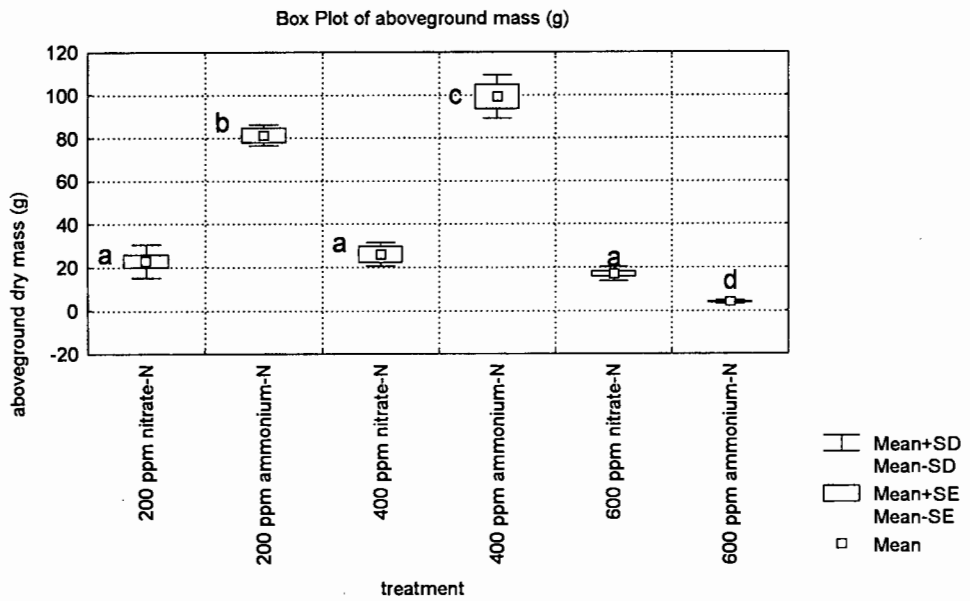

Fig. 2. Box and whiskers plot showing the effect of nitrogen source and level on aboveground dry mass production of *Cynodon dactylon.* LSD multiple range comparisons are indicated by letters to showe significant differences.

Two way ANOVA

a) Nitrogen sources : $F_{(1,19)} = 158.86$, $p<0.05$

b) Concentrations : $F_{(2,19)} = 109.35$, $p<0.05$

c) Interaction effects: $F_{(2,19)} = 78.83$, $p<0.05$

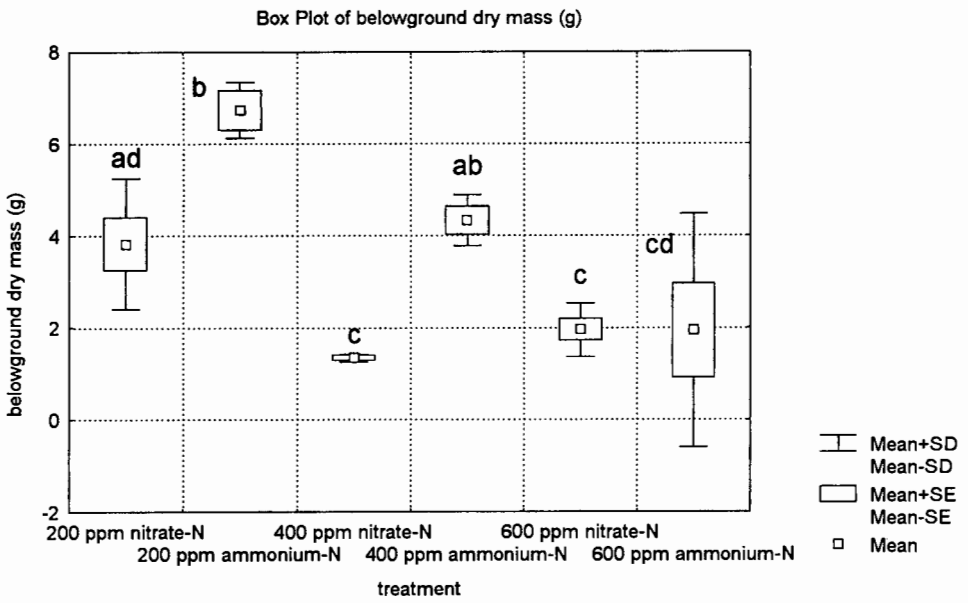

Fig. 3. Box and whiskers plots showing the effect of ammonium and nitrate as nitrogen sources on belowground productivity of *Cynodon dactylon*. LSD multiple range comparisons are indicated by letters to show the significant difference.

Two way ANOVA

a) Nitrogen sources : $F_{(1,16)} = 8.95$, $p < 0.05$

b) Concentrations : $F_{(2,16)} = 10.24$, $p < 0.05$

c) Interaction effects: Not significant

4.2.Dry matter partitioning

4.2.1. Shoot to root ratio (S:R)

The effect of nitrogen source and level on dry matter partitioning between shoot and root in *Cynodon dactylon* is presented in Figure 4. Two-way ANOVA showed no significant difference on the shoot to root ratio between the ammonium and nitrate nitrogen treatments. Levels of nitrogen showed a significant effect on shoot to root ratio ($F_{(2,17)} = 51.68$, $p < 0.05$). Interaction between the treatments and the nitrogen concentration levels was also found to be significantly different ($F_{(2, 17)} = 4.94$, $p < 0.05$).

The trend in Figure 4 shows that plants receiving 200 ppm and 400 ppm ammonium nitrogen allocating more of the dry mass to the shoots than plants under nitrate nutrition. At the highest nitrogen level (600 ppm), there was a marked reduction in the allocation of dry mass to the shoots in both nitrogen treatments.

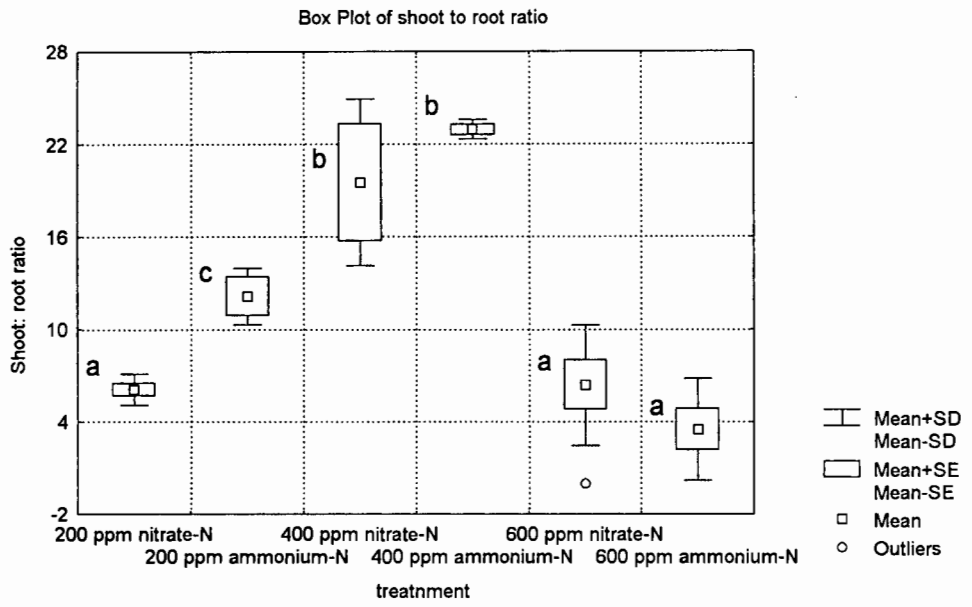

Fig. 4. Box and whiskers plots showing the effects source and level of external nitrogen on the shoot to root ratios in *Cynodon dactylon* treated with different concentrations of either ammonium or nitrate nitrogen. LSD multiple range comparisons are indicated by letters

Two way ANOVA

a) Nitrogen sources :Not signifant

b) Concentrations :$F_{(2,17)} = 51.68$, $p < 0.05$

c) Interaction effects :$F_{(2,17)} = 4.94$, $p < 0.05$

4.2.2.Root weight ratio (RWR)

Figure 5 shows effect of level and nitrogen source on the dry matter partitioning to the roots in *Cynodon dactylon.* The portion of dry mass allocated to the roots relative to the whole plant mass (root weight ratio) was not significant between the two nitrogen sources. Level of nitrogen resulted in a significant effecton the ($F_{(2,14)} = 18.31$, $p = 0.0000$). Furthermore, the interaction effects of source and level of nitrogen were shown to have a significant effect on root weight ratio ($F_{(2,14)} = 10.85$, $p = 0.0014$)

4.2.3. Shoot weight ratio (SWR)

Figure 6 shows the portion of dry matter allocated to the shoots in *Cynodon dactylon* treated with nitrate or ammonium nitrogen. Sources of nitrogen showed no significant effect on the shoot weight ratio. A significant difference was found when two way

ANOVA compared the means of the shoot weight ratios at different levels of external nitrogen ($F_{(2,14)} = 28.31$, $p < 0.05$). The interaction effects between the nitrogen source and the nitrogen concentration was also was shown to be significantly different ($F_{(2,14)} = 10.85$, $p < 0.05$).

The trend in Figure 6 shows the opposite of the pattern observed in Figure 5 for the root weight ratios. At 200 ppm of the external nitrogen, the shoot weight ratio in ammonium nutrition was higher than the nitrate nutrition plants. There ratio increased as the plants received 400 ppm nitrogen in both treatment and the response was higher in the ammonium nutrition. However, high concentration of the external nitrogen showed a negative effect on the shoot weight ratio. This effect was more pronounced in the ammonium nutrition.

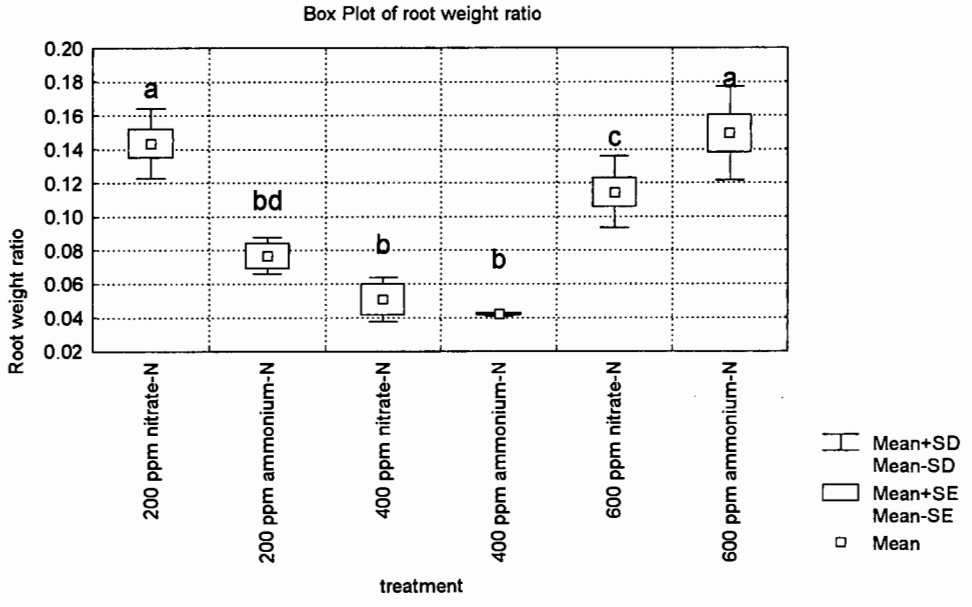

Fig. 5. Box and whisker plots showing the portion of dry mass allocated to the roots (root weight ratio) in *Cynodon dactylon* treated with different concentrations of either nitrate or ammonium nitrogen. LSD multiple range comparisons are indicated by letters to show where the difference lies.

Two way ANOVA

a) Nitrogen sources: Not significant

b) Concentrations: $F_{(2,14)} = 28.31$, $p < 0.05$

c) Interaction effects: $F_{(2,14)} = 10.85$, $p < 0.05$

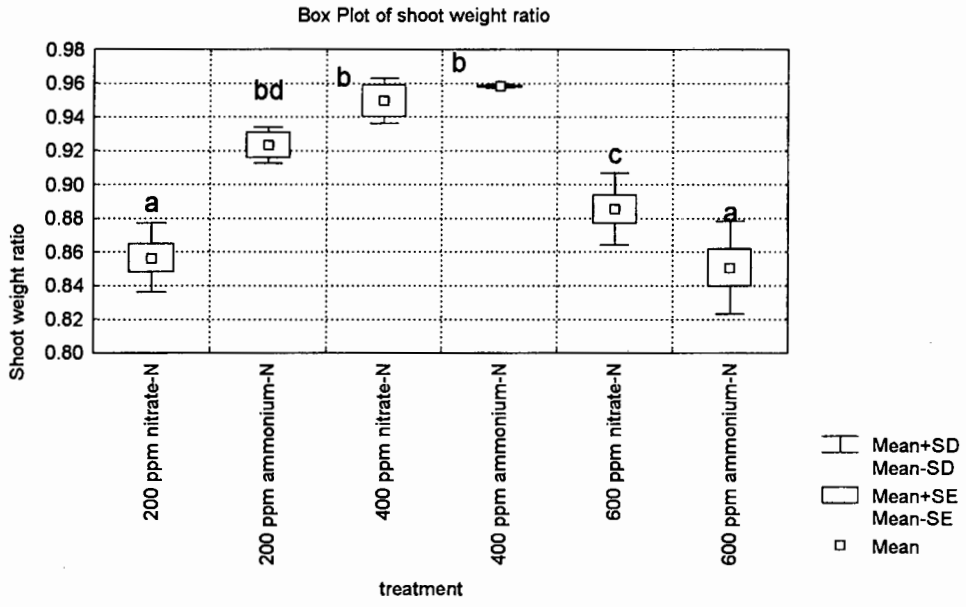

Fig. 6. Box and whisker plots showing the amount of dry mass allocated to shoots (shoot weight ratio) in *Cynodon dactylon* treated with nitrate or ammonium nitrogen. Two way ANOVA. LSD multiple range comparisons are indicated by letters.

a) Nitrogen sources: Not Significant

b) Concentrations: $F_{(2,14)} = 28.31$, $p < 0.05$

c) Interaction effects: $F_{(2,14)} = 10.85$, $p < 0.05$

4.3.Growth parameters

4.3.1 Leaf weight ratio (LWR)

The results for dry matter allocated for leaf weight are presented in Figure 7. The interspecific variation in LWR indicate that nitrate treated plants produced no special partitioning pattern in this species. However, at 400 ppm nitrogen and 600 ppm ammonium nitrogen, there was a positive response in terms of dry matter allocated for leaf development. The proportional allocation of dry matter for development of leaf weight is shown to be dependent on the type of nitrogen supplied (Two way ANOVA, $F_{(1,15)} = 12.14$; $p < 0.05$). Level of the nitrogen also showed a significant difference in the ($F_{(2,15)} = 7.23$, $p < 0.05$). The interaction effects between plants in different concentration levels of ammonium and nitrate nitrogen were also significantly different ($F_{(2,15)} = 5.72$; $p < 0.05$).

4.3.2. Leaf area ratio (LAR)

Figure 8 shows the trend in the leaf area development. Two way ANOVA showed a significant difference between the nitrogen sources ($F_{(1,12)} = 15.79$; $p < 0.05$). Level of nitrogen was also shown to be significantly different ($F_{(2,12)} = 11.24$, $p < 0.05$). Interaction effects between the nitrogen source and concentration level was found to be significantly different ($F_{(2,12)} = 6.98$, $p < 0.05$). Plants receiving nitrate were not significantly different across all the treatments. At 400 ppm and 600 ppm, the ammonium showed an increase in leaf area ratio.

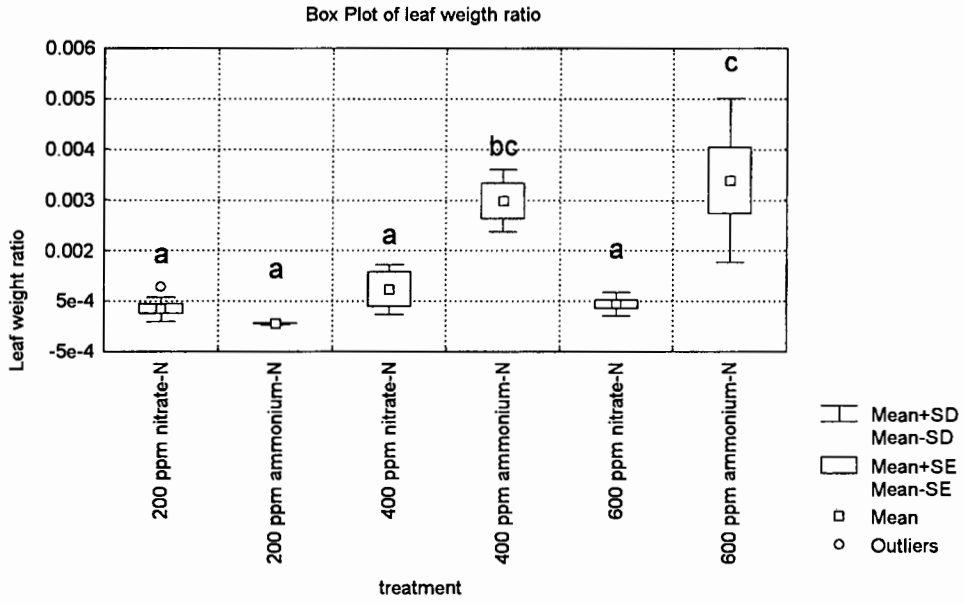

Fig. 7. Box and whisker plot showing interspecific variation in the leaf weight ratio in *Cynodon dactylon* treated with different concentrations of either ammonium or nitrate nitrogen. LSD multiple range comparisons are indicated by letters to locate the significant differences

Two way ANOVA

a) Nitrogen sources : $F_{(1,15)} = 12.14$, $p < 0.05$

b) Nitrogen concentrations : $F_{(2,15)} = 7.23$, $p < 0.05$

c) Interaction effects : $F_{(2,15)} = 5.72$, $p < 0.05$

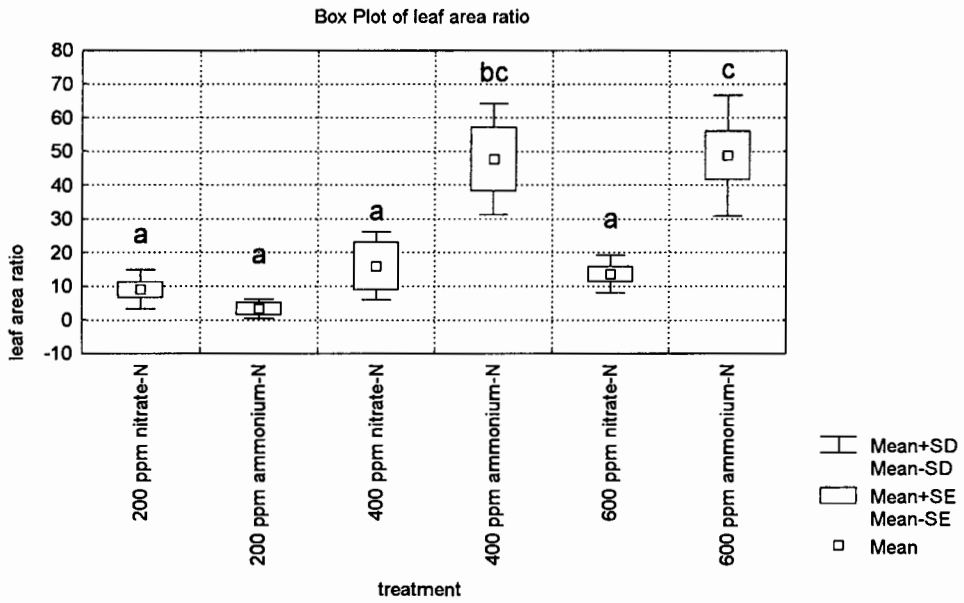

Fig. 8 Box and whisker plot showing the trend in the leaf area development in *Cynodon dactylon* treated with different concentrations of either nitrate nitrogen or ammonium nitrogen. LSD multiple range comparison are indicated by letters to locate the difference.

Two way ANOVA

a) Nitrogen sources : $F_{(1,12)} = 15.79$, $p < 0.05$

b) Concentration levels : $F_{(2,12)} = 11.24$, $p < 0.05$

c) Interaction effects : $F_{(2,12)} = 6.98$, $p < 0.05$

4.4. CHEMICAL ANALYSIS

4.4.1. Leaf nitrogen concentration

Leaf nitrogen concentrations for plants grown in different level of either ammonium or nitrate nitrogen are presented in Figure 9. Two way ANOVA showed nitrogen uptake was significantly higher in plants treated with nitrate nitrogen ($F_{(1,17)} = 6.74$, $p < 0.05$). Different levels of external nitrogen was found to have no significant effect on the nitrogen uptake by *Cynodon dactylon*. Furthermore, the interaction of nitrogen source and level was not significantly different.

4.4.2. Leaf phosphorus concentration

Figure 10 shows effects nitrogen sources on the uptake of phosphorus in *Cynodon dactylon*. Statistical analysis of the data using two-way ANOVA at 5% probability or greater revealed that phosphorus uptake was significantly higher in the ammonium nutrition ($F_{(1,18)} = 4.99$; $p < 0.05$). However, changes in the levels of nitrogen showed no significant effect on the uptake of phosphorus. The interaction between plants grown in both nitrogen sources and concentrations were also not significantly different. The trend in Figure 10 shows that there was a tendency for phosphorus uptake to increase with increase in concentration of the external nitrogen.

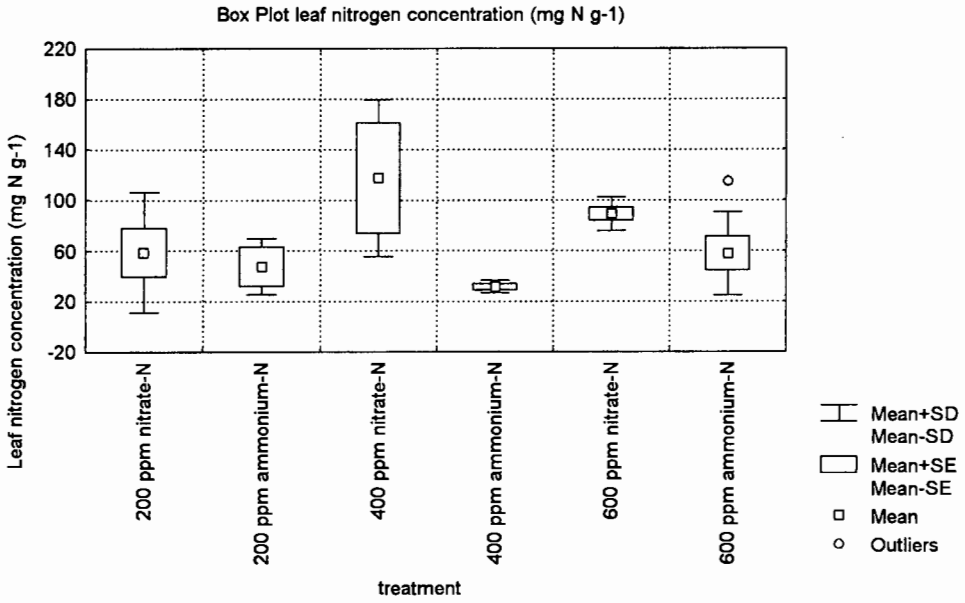

Fig. 9. Nitrogen concentration values in leaves of *Cynodon dactylon* fed treated with different concentrations of either nitrate or ammonium nitrogen. LSD multiple comparisons are indicated by letters.

Two way ANOVA

a) Nitrogen sources: $F_{(1,17)} = 6.74$, $p < 0.05$

b) Nitrogen level :Not significant

c) Interaction :Not significant

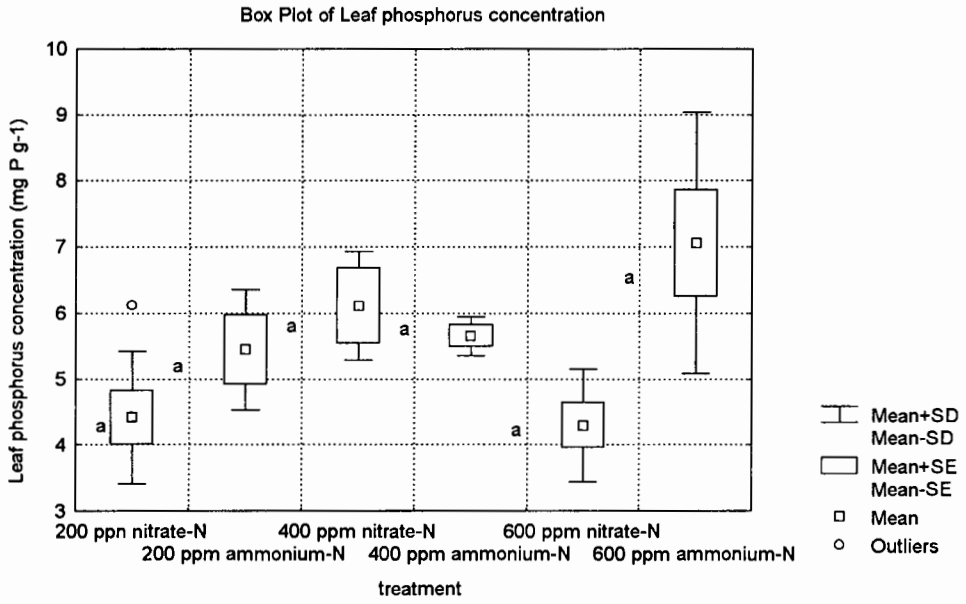

Fig. 10. Plots showing effects of ammonium nitrogen or nitrate nitrogen on phosphorus uptake by *Cynodon dactylon*. LSD multiple range comparisons are indicated by letters.

Two way ANOVA

a) Nitrogen source :$F_{(1,18)} = 4.99$, $p < 0.05$

b) Nitrogen level :Not significant.

c) Interaction :Not significant

4.4.1. Nitrogen to phosphorus ratio

Ratio of nitrogen to phosphorus in the leaves of *Cynodon dactylon* treated with either ammonium or nitrogen is presented in Figure 11. This ratio was significantly higher in the in the nitrate nutrition than the ammonium nutrition ($F_{(1,17)} = 13.25$, $p < 0.05$). There was no significant difference on this ratio at different external nitrogen concentration.

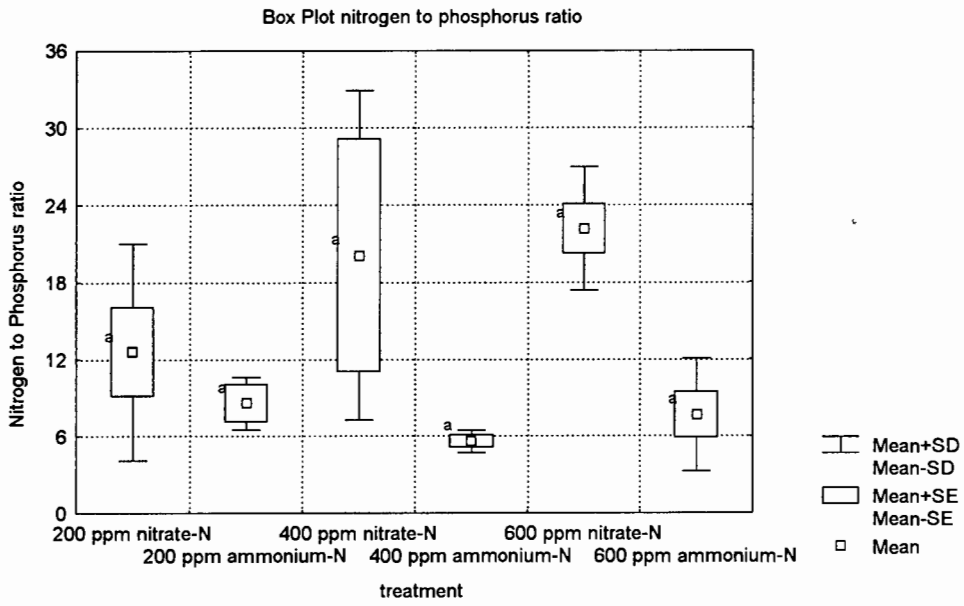

Fig. 11. Effects of level and source of nitrogen on the nitrogen to phosphorus ratio in *Cynodon dactylon*..LSD multiple range comparisons are indicated by letters on the plots.

Two way ANOVA

a) Nitrogen source : $F_{(1,17)} = 13.25$, $p < 0.05$

b) Nitrogen level : Not significant

c) Interation : Not significant

5. Discussion

Biomass

Synoptic review of the results obtained shows that ammonium nutrition plants yielded almost four times more on the aboveground and on the total plant mass at 200 ppm and 400 ppm of the applied nitrogen (Figs 1 and 2). There was a marked reduction in productivity at the highest nitrogen concentration (600 ppm) in the ammonium nutrition. The nitrate nutrition plants yielded less and did not show any change in productivity at different nitrogen levels. However, belowground yields of *Cynodon dactylon* showed a decline as the external nitrogen level advanced (Fig. 3). Although there was reduction in belowground productivity, the effect was highly significant at the highest concentration and the effect was more on the ammonium nutrition plants. These observations show a positive response of *Cynodon dactylon* to ammonium nutrition in terms of aboveground as well as total mass productivity. However, decline in productivity on the ammonium nutrition at 600 ppm nitrogen concentration shows that ammonium nitrogen may have reached toxic levels and hence reduced growth compared to the 200 ppm and 400 ppm. Therefore, this observation suggests toxicity of ammonium ion rather than nitrogen deficiency. Furthermore, poor production from the nitrate nutrition suggest that *Cynodon dactylon* does not prefer nitrate nitrogen regardless of the external nitrogen supply.

A central problem in integration, at the whole plant level is to establish what consequences the metabolic activities in one organ have on the balance of the system. Several theoretical models for growth processes take into account the root and shoot interactions, in particular, Davidson (1969) showed that the relationship can be obtained by looking at the balance between the growth of shoot and roots. This model requires a large number of growth parameters which can sometimes be difficult to determine experimentally. To avoid some of these difficulties, this study compared the shoot to root ratio to establish this balance and the biomass allocation in relation to the total plant production. Davidson (1969), argued that photosynthate is partitioned to roots in inverse proportion to their functional activity, thus providing work done by the roots (root weight x rate of absorption) and by leaves (foliage weight x rate of photosynthesis and transpiration). Using this model it was observed that as the

external nitrogen was increased, the shoot to root ratio was also increased (Fig 4). This shows there was more allocation of the dry matter to the shoots at the expense of the roots. However, the ratio was higher in the ammonium series than the nitrate at comparable external nitrogen supply. This confirms the observation made with the yields above. Similarly, the negative effect on the aboveground production at high external nitrogen supply (600 ppm) is also revealed by this ratio. This mean that toxic effects of high nitrogen concentration forced plants to reduce the aboveground productivity.

Dry matter partitioning between either the aboveground or the belowground mass relative to the total mass produced by the plant (Figs 5 and 6 respectively) further confirm observations made in the shoot to root ratios These results suggest that, increase in nitrogen (at least from 200 ppm to 400 ppm) presumably increases the efficiency of foliage, and hence increases the imbalance between the root and foliage functioning. This imbalance was more from the ammonium nitrogen meaning efficient utilisation of this form of nitrogen for aboveground yield increase. This further give an insight that ammonium nutrition enhanced photosynthesis hence why we observed high yields from this treatment at low and optimum nitrogen supply.

The results as stated above appear to be in agreement with results obtained by various investigators with other plants. Tolerance of plants to increased available nitrogen as discussed in the introduction appear to be controlled by the nitrogen content in the soil (Wiltshire, 1973) and the sensitivity of grasses to varying nitrogen levels (Roux, 1954; Jong and Roux 1955; Warren, 1966; Wiltshire, 1972; Wolfson, 1988). Although, secondary or climax grasses were not available for comparison in this experiment, it has generally been found that ammonium adapted plants tend to respond positively to increased levels ammonium as nitrogen source than the nitrate nitrogen by giving high foliage yields. For example, Roux (1954) has shown that dominance of *Eragrostis* in the primary succession stage can be explained by its ability to respond to increased level of available nitrogen as well as its ability to withstand high salinity. This opinion is supported by the results of this study using *Cynodon dactylon*, a member of the primary stage in succession.

Evidence from Robson and Parsons (1977) suggests that increase in the aboveground dry mass is a result of an increase in leaf area. They found that the leaf area in plants receiving a low concentration of nitrogen (3 $mg.l^{1}$) was lower than the plants receiving high nitrogen levels (300 $mg.l^{-1}$). This difference was ascribed to the greater allocation of dry weight to leaf growth in high nitrogen plants. This argument is support by the data in this study. Leaf area was higher in plants receiving high nitrogen concentration than those receiving lower nitrogen (Fig. 8). The increase in leaf area was closely related with an increase in leaf mass (Fig. 7) The larger yields from the ammonium nutrition can be associated with the larger leaf area as compared to the nitrate nutrition plants.

The physiological advantage of the ammonium adapted plants on preference to ammonium nitrogen over nitrate is not fully understood, therefore the results from this study on *Cynodon dactylon* may not be able to answer this question directly. However, the argument behind energy conservation when ammonium is metabolised by plants should in part be able to explain why ammonium adapted species such as *Cynodon dactylon* under study respond positively when fertilised with ammonium. Ammonium is readily absorbed by plants and may be assimilated more efficiently energetically than nitrate (Lewis, 1986). This is because before nitrate can be metabolised by plants, it has to be reduced to ammonium. This process has been shown to be an energy consuming and hence it brings a significant loss of energy from the plant's overall economy, the energy that could be used to stimulating productivity (Lewis 1986). Based on this reason, ammonium nutrition has an advantage since no apriori reduction is required before metabolised by plants, as a result quite a large energy is conserved. That is probably why they can tolerate high levels of ammonium nitrogen.

Despite a positive response of *Cynodon dactylon* to increase in ammonium nitrogen, excessive nitrogen fertilisation appeared to be toxic to this species. Toxicity of ammonium to plants has been shown to result from two main factors:

(i). Assimilation of ammonium ions leads to the production of hydrogen ion (eqn 1) which are excreted by the root into the external medium resulting in the acidification of the root environments (Kirkby and Mengel 1967).

$NH_4^+ \Longrightarrow NH_3 + H^+$ 1

This lowering of pH, may occur at metabolic sites (Kirkby and Mengel) and hence may reduce the photosynthetic CO_2 fixation. Reduction in the photosynthic CO_2 fixation would therefore reduce production in the leaves hence less yield. Therefore, low yields obtained at very high ammonium fertilisation show that sensitivity of this species is limited by excessive supply of ammonium nitrogen which increases the acidity to above its tolerance level.

(ii) accumulation of non-assimilated ammonium can build up to toxic levels in the plant's tissue and affect metabolic process (Haynes and Goh, 1978). It is therefore, possible that since the external nitrogen was very high, a lot of ammonium was being absorbed and the rate of absorption was higher than assimilation hence the ammonium became toxic for plants at this nitrogen level. It has been shown that ammonium uptake depends on the availability of good carbohydrate supply (Nightingale, 1937). These carbon skeletons are shown to be necessary for the production of amino molecules from ammonium which would otherwise build up to toxic levels in the root cells (Wolfson, 1988). It is argued that the efficient assimilation and detoxification of ammonium in the roots is related to the ability of the plants to sustain adequate supply of carbohydrate to the root system from the shoot (Wolfson 1988). Although concentrations of total non structural carbohydrates were not measured in this experiment, it can be assumed that there was a drop in carbohydrate supply in the roots as a result of reduced photosynthesis due to acidification of the root system which probably affected metabolic process, when plants were excessively fertilised with ammonium nitrogen. This mean that ammonium that was accumulated in the tissues and was not assimilated by plants due to lack of carbohydrate. Therefore, the assumed changes in the carbohydrate level may have accounted for the observed decrease in the dry weights at this nitrogen level.

Wiltshire (1972) suggested that, it is difficult to directly distinguish effects of source from effects of level where sources may differ in availability.. However, from *Cynodon dactylon* investigated here, it is clear from the results that excessive external nitrogen reduced the performance of this plant. This is because, at this level both sources of nitrogen showed the same effect. It is only that the effects were more pronounced on the ammonium nitrogen because of the negative effect of this iom when it is accumulated in the plant tissue. Thus, more than the source, it was the level of nitrogen that affected the yields.

Most of the studies done on sensitivities of grasses to nitrogen source have always considered low concentration mostly ranging from 10 ppm nitrogen to 400 ppm nitrogen at most. A complete picture of nitrogen tolerance of various grasses was only obtained by Warren (1966) by using a wide range of levels of nitrogen sources from as low as 2 ppm nitrogen similar to those found under natural conditions as well as extremely high nitrogen level (800 ppm). Similar to this, the present study was also interested on how this species would respond at those very high external nitrogen fertilisation.

Nitrogen uptake

Nitrogen concentrations in the leaves were significantly higher in the nitrate treatment compared to the ammonium treatment. This result shows a negative relationship with the dry weights yields because higher yields have been obtained from the ammonium than the nitrate treatments. This result should not be surprising because, higher external nitrate concentrations have been shown to cause rapid nitrate uptake from the roots environments, concentrating it in the root tissue or in xylem sap. Nitrate being less toxic than ammonium can be accumulated in large concentrations in the plant's tissue or translocated and need not be reduced until it is assimilated. Many authors (e.g. Basset et al. 1970) have investigated the relationship between nitrogen fertiliser application and its uptake and it was found that plants can take up nitrogen faster than is needed for the current growth and accumulate nitrogen in their tissues. This

accumulation normally occurs when the supply of nitrogen exceeds demand and may be independent of the growth rates (Millard, 1988). With this note, it possible to say that the high concentrations observed in the nitrate treated plants was the soluble nitrogen which was not assimilated. Therefore, this nitrogen was not available for yield production. If *Cynodon dactylon* was assimilating nitrate efficiently we would observe high yields from this treatment. However, low yields show the inability of this species to efficiently assimilate nitrate despite the high concentrations observed in plants from this treatment. On the other hand ammonium being a readily available nitrogen form, was taken up and assimilated quickly for plant's structural development especially the aboveground structures. This is true because determining the amount allocated for leaf area development, was markedly higher in the ammonium nutrition than the nitrate nutrition.

This observation on the effects of nitrogen source on uptake of other nutrients is in agreement with Wiltshire (1972) who showed yields of all the species they studied were negatively correlated with that nitrogen concentration when plants were treated with ammonium or nitrate as nitrogen sources. Wiltshire (1972) suggested that this observation can be related to inability of those plants to metabolise nitrate as readily as ammonium.

On the other hand the lower concentrations of ammonium shows that since ammonium does not require reduction before utilisation by plants it stands an advantage of being assimilated quickly for the biomass production. However, Roux (1955) observed high nitrogen levels especially from the ammonium treatments in sand culture for *Eragrostis curvula* a primary stage grass, but did not report nitrogen contents of the plants.

Phosphorus uptake

The absorption of either ammonium ion or nitrate ion have resultant effects on the absorption and accumulation of other ions in order to maintain electroneutrality between the cell and the external medium. With the nitrate nutrition, high amounts of (NO_3^-) are absorbed and with the ammonium nutrition, high amounts of cations (NH_4^-) are absorbed. In order to reduce the deleterious effects of ammonium on the plant

which might be caused by imbalance of electroneutrality between the cell and medium, diffusible anions such as the phosphate ions (PO_4^-) must accompany ammonium ion absorption (Kirkby and Mengel 1967). Sideris and Young (1946) reported greater values of phosphorus in *Ananas comosus* from the ammonium treatments than in the nitrates. They suggested that this observation may be due to the fact that PO_4^- are attracted electrostatically by the ammonium ions in the ammonium series. Similar result has been obtained in this experiment. Concentration of phosphorus was significantly higher in the ammonium and limited in the nitrate treatment. There was an indication of a corresponding rise in concentration of phosphorus in the shoots as the external nitrogen concentration advances. Therefore this mean that despite the known deleterious effects of ammonium ions in plants *Cynodon dactylon* has the ability to take up phosphorus and respond to changes in external nitrogen. This maintenance of electroneutrality through absorption of PO_4^- contributes to the observed tolerance of this species to increasing ammonium fertilisation. This observation is in agreement with Spratt (1974) who indicated that application of ammonium or nitrate changed the uptake of other phosphorus on wheat (*Triticum aestivum*) plant. They found that application of ammonium nitrogen increased the uptake of phosphorus at silage stage meaning that plants that effieciently utilise ammonium nitrogen also take up phosphorus efficiently. This effect is well illustrated by the N:P ratio (Fig. 11) which was significantly higher in the nitrate series. This indicate that uptake of nitrate had a depressant effect on the uptake of phosphorus in *Cynodon dactylon*. On the other hand, the low N:P ratio in the ammonium nutrition shows that uptake of phosphorus in *Cynodon dactylon* was enhanced when fertilised with ammonium nitrogen.

6. Conclusion

In general the data reported here emphasise the dual nature of the influence of the form of nitrogen on *Cynodon dactylon*. High foliage yields of *Cynodon dactylon* with ammonium nutrition show that this species prefer ammonium nitrogen counter to nitrate nitrogen. However, sensitivity to nitrogen source is limited by very high application of both sources of nitrogen, with ammonium nitrogen showing the most deleterious effects on the performance of this species in terms of productivity.

On the other hand, productivity was not highly stimulated from nitrate nutrition suggesting less preference of this type of nitrogen.. However, Jong and Roux (1955) had observed the abnormal behaviour of *Cynodon dactylon* in that small additions of ammonium sulphate did not stimulate growth of this species. Although, they found that this grass showed a fair degree of tolerance at higher concentration levels they did not report the actual values. However, from this study, it has been observed nitrogen levels going as high as 600 ppm are toxic to this plant. This effect is assumed to result from acidification of the nutrient medium which goes beyond the tolerance capacity of this species. The ability of this species to take up ammonium has a positive effect on the uptake of other nutrients such as phosphorus.

In general we can conclude that *Cynodon dactylon*, a primary stage grass in succession, favours ammonium to nitrate fertiliser in terms of productivity and effects on the uptake of phosphorus. However, excessive fertilisation with the former nitrogen form can result in deleterious effects on this species hence reduction in productivity. As an important pasture grass, ammonium fertiliser is recommended to livestock farmers if productivity is to be increased from this species. This is based from the fact fertilising this species with ammonium nitrogen can be economical in the sense that smalll additions of this nitrogen form stimulate productivity more than the nitrate can do.

Acknowledgements

I am grateful to my supervisor Prof. W. D. Stock for his guidance and encouragement he gave me throughout this project.

I would also like to thank my sponsor Southern African Botanical Diversity Network Project for the financial support while persuing BSc. Honours in Botany at the University of Cape Town, without which I could not have had an opportunity to engage myself in this type of work.

References

Alexander, M. (1965). Nitrification. In: Soil nitrogen, pp 307-343 (Eds. Bartholomew, W. V. And Clark, F. E.). American Society of agronomy No. 10.

Altona, R. E. (1972). The influence of soil fertility in secondary succession. *Proceedings of Grassland Society of South Africa* 7:20-22.

Arnon, D. I. (1937). Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen-ion concentration, Manganese, Copper and Oxygen supply. *Soil Sci.* 44:91-121

Basset, D. M., Anderson, W.D. and Werkhoven, C. H. E. (1970). Dry matter production and nutrient uptake in irrigated cotton (*Gossypium hirsutum*). *Agron. J.* 62:299-303.

Bonner, J. (1946). The role of organic matter, especially manure, in the nutrition f rice. *Bot. Gaz.* 108:267-279.

Bremner, J. M. (1965). Total nitrogen. In: *Methods of soil analysis,* Part 2. Chemical and microbiological properties. Pp.1149-1178. C A. Black et al. (Ed). American Society o Agronomy, Inc. Publishers, Madison, Wisconsin,

Brockington, N. R. (1961). Studies of the growth of a *Hyparrhenia*-dominant grassland in Northern Rhodesia. II. Fertiliser responses. *J. Br. Grassld.Soc.* 16:54-60

Cox, W. J., and Reisenauer, H. M. (1973). Growth and ion uptake by wheat supplied nitrogen as nitrate or ammonium or both. *Plant and Soil* 38:363-380.

Davidson, R. L. (1969). Effect of root/leaf temperature differentials on root/shoot ratios in some pasture grasses and clover.*Ann. Bot.* 33:561-9

Ghosh, B. P. and Burris, R. H. (1950). Utilization of nitrogenous compounds by plants. *Soil Sci.* 70: 187-203.

Hall, T. D., Meredith, D. & Altona, R. E. (1949). A comparison of four nitrogenous fertilisers on the veld. *South African Journal of Science*, 45:100-105.

Haynes, R. J., and Goh, K. M. (1978). Ammonium and nitrate nutrition of plants. *Biol. Rev.* 53:465-510

Hewitt, E. J. (1952). *Sand and water culture methods*. Technical Communication no. 22, of the Commonwealth Bureau of Horticulture and Plantation Crops. East Malling, Maidstone, Kent. C. A. B.

Jong and Roux (1955). A further investigation of the nitrogen sensitivity of veld grasses. *S. Afr. J. Sc.* 52:27-28

Kirkby, E. A. and Mengel, K. (1967). Ionic balance in different tissues of the tomato plant in relation to nitrate, urea or ammonium nutrition. *Plant Physiol.* 42:6-14

Lewis, O.A.M. (1986) *Plants and Nitrogen*. Studies in Biology No. 166. Edward Arnold.

Millard, P. (1988). The accumulation and storage of nitrate by herbaceous plants. *Plant, Cell and Environ.* 11:1-18

Nightingale, G. T. (1937). The nitrogen nutrition of green plants.. *Bot. Rev.* 3:85-194

Nittler, L. W. And Kenny, T. J. (1976). Effect of ammonium to nitrate ratio on growth and anthocyanin development of perennial ryegrass cultivars. *Agron. J.* 68:680-682

Novoa, R. and Loomis, R. S. (1981). Nitrogen and plant production. In: *Soil water and nitrogen in Mediterranean-type environments*. pages 177-204. Eds. Monteitth, J. and Webb, C.

Robson, M. J. And Parsons, A. J. (1977). Nitrogen deficiency in small closed communities of S24 ryegrass. I. Photosynthesis, respiration, dry matter production and partition. *Ann. Bot.* 42:1184-1197.

Roux, E. R. (1954). The nitrogen sensitivity of *Eragrostis curvula* and *Trachypogon plumosus* in relation to grassland succession. *South African Journal of Science*, 50:173-176.

Scarsbrook, C. E. (1965). Nitrogen availability. In: *Soil nitrogen*, pp 481-502. Eds. Bartholomew, V. W. and Clark, E. F. Madison: USA, American society of Agronomy.

Sideris, C. P. And Young, H. Y. (1946): Effects of nitrogen on growth and ash constituents of *Ananas comosus* (L). Merr. *Pl. Physiol.* 21:247-270

Smith, V. R. (1980). Aphenol-hypochlorite determination of ammonium-nitrogen in Kejldahldigests of plant tissue. *Comm. Soil. Sci. Plant anal.* 11:709-722

Spratt, E. D. (1974). Effects of ammonium and nitrate forms of fertiliser N and their time of applicationon utilization of N by wheat. *Agron. J.* 66:57-61

StatSoft, Inc. (1996). STATISTICA for Windows [Computer program manual]. Tulsa.

Warren, M. (1966). A study of soil nutritional and other factors operating in secondary succession in highveld grassland in the neighbourhood of Johannesburg. Ph.D. thesis, University of the Witwatersrand, Johannesburg.

Wiltshire, G. H. (1972). Response of highveld grass species to ammonium and nitrate nitrogen. *Proceedings of the Grassland Society of southern Africa*, 13:99-102.

Wiltshire, G.H. (1973). Response of grasses to nitrogen source. Journal of Applied *Ecology,* 10:429-435

Wolfson, M. M. (1988). The effect of inorganic nitrogen on growth, morphology and some aspects of the physiology of Digitaria eriantha (Steud). Ph.D. thesis University of Witwatersrand, Johannesburg.