

**CONTROLLED ENVIRONMENT DETERMINATION OF NITROGEN UPTAKE  
AND ITS EFFICIENT UTILISATION BY SELECTED SOUTH AFRICAN  
SUGARCANE VARIETIES**

by

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## **PREFACE**

The research contained in this dissertation was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, South Africa. The research was financially supported by.....

The contents of this study have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.



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## **DECLARATION**

I, Irvin Thabo Makhubedu, declare that:

the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

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Signed:

Date:

## **DEDICATION**

This work is dedicated to the Almighty Father for His abundant grace in seeing me through this programme, my mother Julia B Makhubedu for all she had to go through to see me educated and my two lovely children Moloko Makhubedu and Keamogetswe Makhubedu.

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## ABSTRACT

Increasing nitrogen use efficiency (NUE) of sugarcane (*Saccharum officinarum* L.) has a potential of reducing farmers input costs associated with nitrogen (N) fertilizers. Although there is evidence for some genetic variability in NUE of sugarcane in South Africa, plant growth and physiological mechanisms underlying this variability are currently unknown. The study investigated the genetic variation in sugarcane for NUE (N-uptake efficiency; NUpE x N-utilisation efficiency; NUtE) as this could provide a basis for breeding varieties with reduced N demand.

The study consisted of two separate successive pot trials at the South African Sugarcane Research Institute (SASRI) under outdoor conditions. A randomised block design preliminary trial (trial 1) was conducted in September 2013 to screen NUtE (biomass production/unit tissue N) of fifteen sugarcane varieties at three destructive (biomass) harvests that were conducted at four month intervals. Plants established from single nodal stem cuttings were, in six replicates, planted into pots that were immersed in metal troughs (5 pots/trough), which contained liquid nutrients (Schumann et al., 1998) with low (14.40 g N/pot) and high (28.80 g N/pot) N supply.

In the subsequent trial (trial 2) conducted in November 2014, eight varieties were subjected to four N treatments, a no N (0 g N/pot), low (1.94 g N/pot), medium (5.81 g N/pot) and high (11.61 g N/pot) N, herein referred to as NN, LN, MN and HN, respectively. The trial was arranged in a randomised complete block design (RCBD) design with five replications. Non-destructive measurements (stalk height, stalk population, leaf relative chlorophyll (soil plant analysis development (SPAD) and leaf N, P and K concentration) were conducted at specific time intervals. Destructive measurements (whole plant sampling) were performed at 180 days after transplanting (DAT) to determine, green leaf counts and area (GLA), shoot biomass production, biomass partitioning, root length and NUE. Nitrogen concentration (% [g N/100 g DM]) in the tissue components was determined using the LECO TruSpec N analyser at the Fertilizer Advisory Service at SASRI.

The data for trial 1 were not included in the thesis. The results of trial 2 showed that N supply significantly affected stalk height and counts, leaf counts, GLA, leaf SPAD and root length traits hence that varieties also differed significantly with respect to the physiological

measurements. Stalk height was significantly enhanced by NN and LN supply whereas stalk counts were similar among the LN, MN and HN treatments. Variety N41 had taller stalks than N12 and N37 whilst the two latter varieties had higher stalk counts than the former. There were significant N level x variety interactions with respect to green leaf counts and GLA but not for SPAD and root length. The LN treatment increased the number of leaves more than the other treatments. Variety N37 and N12 had the highest number of leaves as compared with N32. The GLA and leaf SPAD increased linearly with increasing N supply. Amongst the test varieties, N12 had significantly greater GLA and NCo376, N48 together with N41 had higher leaf SPAD values as compared with other varieties.

There was also a significant N level x variety interaction with respect to root fresh biomass and shoot, root and whole-plant dry biomass. Significant increases in shoot and whole-plant dry biomass occurred and plateaued with LN supply. Although N41 ranked the third in terms of root dry biomass, the variety ranked the highest in terms of shoot and whole-plant dry biomass. The NN and LN treated plants allocated greater proportions of biomass to the stalk component, whilst the MN and HN treated plants allocated greater biomass to green leaves.

Significant N level x variety interaction was observed for shoot N concentration and N content, NUpE, NUtE and the overall NUE. Shoot N concentration and content of test plants increased linearly with increasing N supply. Contrarily, NUpE, NUtE and NUE decreased with increasing N supply. Overall genetic variability in NUE was greater under LN supply and can be explained mainly by differences in NUtE rather than NUpE. Among the varieties, N41 had the highest NUE when compared with the N37 and NCo376 which ranked the lowest. It is concluded that N supply has a significant effect on sugarcane growth, dry biomass yield and allocation, N allocation and NUE.

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## 1. GENERAL INTRODUCTION

Nitrogen (N) is a major essential nutrient element and a constituent of proteins, nucleic acids and other organic compounds, hence, many metabolic processes are reliant on it. Although highly abundant on earth (about 78% of the atmosphere), it is considered the most limiting for plant growth and crop yields (Hirel et al., 2007; McAllister et al., 2012), because plants are unable to directly use the inert atmospheric N<sub>2</sub> molecule (Graham & Vance, 2000). Many biomass crops such as sugarcane have high N requirements which often exceed the intrinsic capacity of soils to supply N by mineralisation (Schumann, 1998). Improved yield in sugarcane cropping systems is typified by intensive synthetic N inputs whereas yield losses are associated with inadequate N inputs. Synthetic N inputs vary between 60 and 755 kg N ha<sup>-1</sup> in the top 14 sugarcane growing countries (Robinson et al., 2011).

As in many countries, sugarcane (*Saccharum* spp.) grown in the southern African region require larger quantities of N and up to 200 kg N ha<sup>-1</sup> is applied to the crop to achieve optimum biomass and sucrose yield (Schumann, 2000). This N requirement of sugarcane has been a subject of extensive research (Meyer & Wood, 1994; Meyer et al., 2007) and its optimum management and efficient utilisation remains a serious challenge. It is estimated, on average, that only 35% of applied fertiliser N is being assimilated by the sugarcane crop, depending on cultivar, N form, rate, timing, soil type and environment (Meyer et al., 2007), with the unaccounted 65% being associated with detrimental impacts on the environment. The most typical examples of such deleterious effects include eutrophication of marine and freshwater ecosystems, denitrification of N<sub>2</sub>O, leaching of nitrates and ammonia volatilization (Cassman et al., 2002).

These deleterious effects along with exorbitant prices of synthetic N fertilisers have accelerated considerable efforts to improve N-use efficiency (NUE) of cultivated sugarcane crops. Although there are numerous definitions of the concept, NUE is usually defined as harvestable yield (biomass or grain) per unit of available mineral N in the soil, which includes both residual N and applied synthetic N (Moll et al., 1982). N-use efficiency can be further divided into external efficiency (eNUE - the ability of the plant to absorb N from the rhizosphere) and internal/utilisation efficiency (iNUE - the ability of the plant to transform acquired N into harvestable yields) (Moll et al., 1982; Good et al., 2004).

Genetic variability in NUE and genotype x level of N interaction in sugarcane has been shown before by several authors (Colepeper, 1946; Inman-Bamber, 1984; Gascho, 1986; Stevenson et al., 1992). However, mechanisms underlying the genetic variability of NUE among varieties have not been extensively studied, while in grain crops the phenomenon has received substantial attention. In maize, wheat and rice, progress related to NUE includes identification of key traits related to plant performance at low N inputs (Kichey *et al.*, 2006; 2007) and to localize both genes and chromosomal regions that contribute to increased tolerance to N starvation (Laperche *et al.*, 2006; 2007). In addition, quantitative trait loci (QTL) for glutamine synthetase (GS) gene loci have already been identified in maize in relation to N remobilization from the leaf, stem and whole plant (Gallais & Hirel, 2004; Martin *et al.*, 2006). The genetic control of sugarcane productivity under N depriving conditions was studied in Australia on a biparental population of 61 progeny genotypes (Whan *et al.*, 2010). More research at the interface of biology, genetics and agronomy is required to better understand the genetic basis of NUE in sugarcane.

In South Africa, significant varietal differences in internal NUE were reported among sugarcane varieties in hydroponic pot (Schumann *et al.*, 1998) and field experiments (Weigel *et al.*, 2010) comprising of different N levels. Similar results were also reported in Australia for sugarcane grown in hydroponic (Robinson *et al.*, 2007) and field (Robinson *et al.*, 2008) experiments. Findings of those studies indicate that N-use efficiency in sugarcane is variety-specific. To date, it remains unclear whether the variation in sugarcane NUE is mainly due to varietal differences in N uptake potentials or rather differences in N utilisation efficiencies. In maize, this genetic variation in NUE at full N rates was reported to be due to variation in N uptake efficiencies, whereas at reduced rates the variation was mainly due to N utilisation efficiencies (Moll *et al.*, 1982). This observation suggests that efficiency of N use operates at both low and high N levels. Furthermore, the finding indicates that application of variable N rates to the plants may influence differential expression of several genes (Bertin & Gallais, 2000), which may contribute to either N uptake or utilisation.

Nitrogen use efficiency of sugarcane has not yet received considerable attention and it is currently poorly understood. Consequently, physiological traits contributing to NUE and their range of variation in commercial varieties is less well known (Robinson *et al.*, 2009). Evidence from recent glasshouse and field trials with low and high N levels of N indicate

some genetic variation in internal NUE (Schumann et al., 1998; Robinson et al., 2007; 2008; 2009; Weigel et al., 2010; Zhao et al., 2014; Hajari et al., 2015; Snyman et al., 2015). For example, South African sugarcane varieties (namely, NCo376, N12, N14, N16, N19, N24, N25) grown in potted sand hydroponics at three N levels showed significant varietal differences in their internal N-use efficiency (Schumann et al., 1998). Among all varieties, N12 together with N19 showed greater internal N-use efficiency (g sucrose/g accumulated N) and were 65% and 63% higher than variety NCo376 (standard), respectively (Schumann, 1998). Using sixty offspring (KQ99-) of a mapping population, Robinson et al. (2007) also found that internal NUE (g DW g<sup>-1</sup> tissue N) was on average 2-fold greater at limiting N relative to non-limiting N.

### 1.1. Rationale

Nitrogen is one of the most expensive nutrients to supply to a crop, and synthetic fertilisers represent the major cost in sugar production. Furthermore, synthetic N application is directly linked to increased sugarcane productivity and as a consequence, excessive N application is perceived as an insurance of a high yielding harvest by most farmers. Incomplete capture and poor conversion of synthetic N fertilisers pose a serious challenge to the environment and it can be an increased unnecessary input cost to farmers. To reduce pollution by nitrate leaching and maintain sufficient profit margins, the use of N fertilisers must be well managed. These objectives can be met through improved N management practices and most importantly by using sugarcane varieties with higher NUE. To achieve this goal, the identification of key traits (e.g. leaf area, biomass and N allocation, root morphology) that could potentially affect NUE at both low and high N supply is necessary.

Although there is evidence for some genetic variability in external and internal NUE (Stevenson et al., 1992; Schumann et al., 1998) of sugarcane in South Africa, the genetic and physiological mechanisms underlying this variability have never been thoroughly investigated. This knowledge gap is of significant relevance to intensive sugarcane production systems where synthetic N inputs are highly essential to ensure maximum yields and where NUE is still estimated at around 30 to 40%. Exploitation of genetic variability and identification of key traits that could potentially affect NUE, particularly under low N regime, could enhance breeding efficiency and reduce N losses. Thus, the development of rapid and

cost effective screening methods, such as outdoor hydroponic or potted sand experiment to test physiological mechanisms that control NUE and variety interactions are required.

Therefore, this study seeked to investigate the variation of NUE and identify some physiological traits (e.g. leaf area, biomass and N allocation, root morphology) that could potentially affect NUE of selected South African commercial sugarcane varieties grown in a potted sand experiment. The current study assessed (i) growth morphological characteristics (ii) dry biomass yield (iii) biomass allocation and N allocation in plant components and (iv) N uptake and utilisation efficiencies in eight commercial sugarcane varieties under low, medium and high N supply.

## 1.2. Research questions, aim, objectives, hypotheses and expected outputs

### 1.2.1. Research questions

The outdoor environment study intended to answer the following research questions regarding sugarcane production:

- i. How does the stalk yield of selected varieties respond to increasing N-rates?
- ii. What is the relationship between N supply and biomass production?
- iii. How does SPAD-based chlorophyll content of different varieties respond to changes in N supply?
- iv. How is dry biomass of the selected varieties partitioned (brown leaves, stalk, tops and green leaves) and what is the proportion of N taken up by each fraction?
- v. What is the response to N supply in terms of plant growth and development (phenology) of selected sugarcane varieties?

### 1.2.2. The aim of the study

The aim of this study was to investigate the variation in NUE and identify some physiological traits (e.g. leaf area, biomass and N allocation, root morphology) that could potentially affect NUE of selected South African commercial sugarcane varieties grown in a potted sand experiment.

### 1.2.3. Objectives

- i. To evaluate the effects of different N levels on sugarcane growth and dry biomass production.
- ii. To determine the effects of different N levels on sugarcane NUE.
- iii. To assess genetic variation among varieties with respect to growth, dry biomass production and NUE
- iv. To test the validity of the pot-trial method as a potential routine NUE screening method for sugarcane varieties.

### 1.2.4. Hypotheses

- i. There will be an effect on growth and dry biomass production of sugarcane varieties associated with N fertilisation levels.
- ii. Nitrogen rate will have an effect on NUpE, NUtE and the overall NUE of sugarcane varieties.
- iii. Sugarcane varieties show a wide genetic variation in growth, dry biomass production and NUE.

### 1.2.5. Expected outputs

- i. Data obtained in this study will improve our current knowledge of sugarcane NUE which may allow future selection of phenological traits linked to NUE
- ii. A successful and practical screening method for NUE of sugarcane could be established

## 2. LITERATURE REVIEW

### 2.1. Sugarcane botany

#### 2.1.1. Classification and varieties

Sugarcane (*Saccharum spp.*) is a monocotyledonous perennial C<sub>4</sub> grass, originating mostly in South-East Asia (Karp & Shield, 2008). Its primary cultivation is attributed to its ability to store high concentrations of sucrose in the stem, and approximately 75% of the consumed sugar worldwide is derived from sugarcane (IISD, 2014). The crop belongs to the genus *Saccharum* L., of the tribe Andropogoneae belonging to the grass family Poaceae. The tribe is very large and is comprised of many species with high economic value. The species include the tropical and subtropical grasses from the cereal genera *Sorghum bicolor* (L.) and *Zea mays* (L.) (Moore et al., 2014). There are currently six recognized species of *Saccharum*. Of the six, *S. spontaneum* and *S. robustum* are wild types whilst *S. sinense*, *S. barberi*, *S. officinarum* and *S. edule* are commercially cultivated forms (Moore et al., 2014). Commercial hybrid varieties originate from interspecific hybridization between *S. officinarum* and *S. spontaneum*.

#### 2.1.2. Sugarcane morphology

The morphology of a mature sugarcane plant is characterized by three main parts including the stalk, leaf and root system. The stalk is composed of segments called joints made up of a node and internode (Miller & Gilbert, 2010). The node is the area of the stalk in which the leaves are attached, and each internode is characterised with a bud, in which root primordia are found. The lateral buds are inserted alternately along the stalk in the axil of alternately borne leaves (Miller & Gilbert, 2010). The bud at each node is capable of sprouting into a new plant, providing a method used for vegetative propagation. A sugarcane leaf is comprised of long (1 – 2 m) leaf blades and shorter (0.2 – 0.3 m) stalk-clasping sheaths separated by a joint and are attached alternately to the nodes (Moore et al., 2014). The crop consists of approximately 10 (0.5 m<sup>2</sup> upper surface area) green leaves, depending on variety and growing conditions (Miller & Gilbert, 2010).

Various morphologically distinct types of roots can be distinguished from a sugarcane plant (Van Antwerpen, 1999; Miller & Gilbert, 2010; Moore et al., 2014). During early stages of

development, sett roots develop first from the planted material and are later replaced by more robust shoot roots. The shoot roots persist until they are replaced by the main roots during development of more shoots (tillers) (Van Antwerpen, 1999; Miller & Gilbert, 2010; Moore et al., 2014). An established sugarcane root system is comprised three main types of roots that include superficial roots, buttress roots and the rope root system.

### 2.1.3. Cultivation and propagation

#### 2.1.3.1 Cultivation

Sugarcane is a tropical plant cultivated globally under diverse climates at latitudes between 36.7° N (southern Spain) and 31.0° S (Republic of South Africa), from (0) to an altitude of 1500 m (Everingham et al., 2002; Singh, 2002; Fageria et al., 2010). It is a long duration crop and its yielding capacity is significantly influenced by temperature, moisture, relative humidity and solar radiation (Verheyen, 2010). The ideal climate for maximum production of sugar is described as a long, warm growing season and a fairly dry, sunny and cool for ripening (without frost); and a harvest season free from hurricanes and typhoons to prevent lodging (Humbert, 1968).

Temperatures between 22 to 33°C favour tillering, root and shoot growth whilst temperatures between 32 to 38°C are ideal for sprouting (germination) of vegetative stem cuttings (Srivastava & Rai, 2012). Lower temperatures in the range of 10 to 18°C decelerate sprouting and vegetative growth rate (Bull, 2000), whereas those above 38°C reduce photosynthesis (Srivastava & Rai, 2012). Lower temperatures ranging from 12 to 14°C are reported to enhance the conversion of glucose into sucrose and are therefore desirable during the final stage before harvest (ripening phase) (Fageria et al., 2011).

The sugarcane crop also requires humid conditions which improve both leaf and stalk elongation (Srivastava & Rai, 2012). Relative humidity values of about 70 to 85% are ideal for growth whilst 55 to 75% are best suited for the ripening phase. To achieve relatively high yields, the seasonal crop water requirements of sugarcane range between 1500 to 2500 mm depending on climatic conditions and length of the growing season (12 – 24 months) (Singh et al., 2006). Crop water requirement is greater during crop development and mid-season and lower during the ripening phase (NaanDanJain, 2013). Higher rainfall during ripening affects

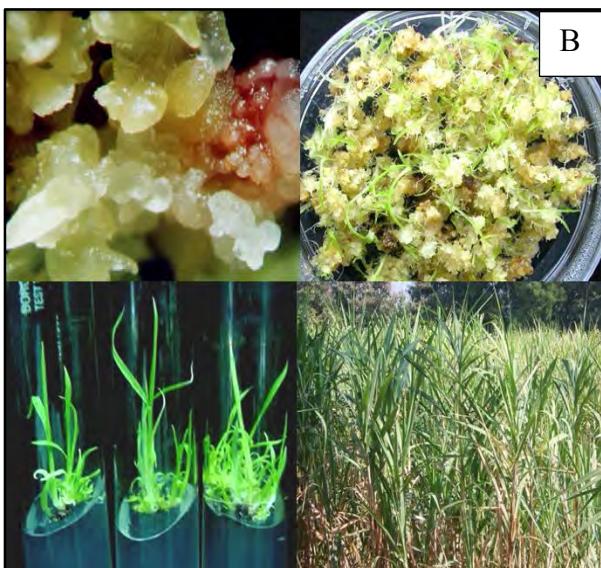
sucrose percentage in cane juice. The crop requires 12 to 14 hours of sunlight throughout the growing season (Srivastava & Rai, 2012), except during ripening when it needs 8 to 10 hours day length.

#### 2.1.3.2 Propagation

Sugarcane can be propagated *in vitro* via somatic embryos callus, axillary bud and the shoot apical meristems or from sprouting of vegetative buds (Moore et al., 2014). The former method of propagation is crucial for breeding hybrid varieties whilst the latter method is extensively used commercially. Figure 2.1 shows the two methods of propagating sugarcane.



Source: Goodman (2013)



Source: BARC (2015)

Figure 2.1. Illustration of A) vegetative sugarcane propagation from nodal stem cuttings and B) plant tissue culture or micropropagation method.

When a stem cutting (or cane sett) is planted under favorable conditions, it develops two kinds of roots. The sett roots, which arise from the root band, are thin and highly branched and their primary function is to provide water and nutrients to the young and developing shoots (Van Antwerpen, 1999; Miller & Gilbert, 2010). The sett roots are only temporary (2 to 3 months) and eventually senesce following development of shoot roots. The shoot roots develop from the lower root bands of the developing shoots and will subsequently take over

the functions of sett roots. The shoot roots are 4 – 10 times thicker than sett roots, whitish and fleshy with fewer branches (Van Antwerpen, 1999; Miller & Gilbert, 2010; Moore et al., 2014). The life span of these roots is also limited. During their development, new buds also germinate to produce shoots or tillers. Subsequently, the new tillers will develop their own roots that eventually take over the function of the original shoot roots (Miller & Gilbert, 2010).

During this period, the plant develops an increased number of tillers and subsequently, the leaf canopy expands to capture the available light. Tillers are typically produced in excess per plant but are later reduced by death due to competition for light and nutrients (Bell & Garside, 2005; Singels & Smit, 2009). Sugarcane tillers 4 to 12 stems which can grow between 3 and 5 m in height depending on the variety, management and environmental conditions. The stems mature into cane stalk which accumulate photosynthates as sucrose (Wang et al., 2013). The sucrose accumulation can reach exceptionally high concentrations of up to 18% of fresh weight basis (Inman-Bamber et al., 2011).

#### 2.1.3.3. Methods of propagating sugarcane

##### 2.1.3.3.1. Conventional

Sugarcane is vegetatively propagated by means of nodal stem cuttings comprising one, two or three buds, known as “setts”, “seed canes” or “seed pieces” (Jalaja et al., 2008). Due to apical dominance, a process in which few buds at the ends germinate while interior and lower buds might remain inactive, planting the whole stalk is not recommended (Viswanathan, 2000; Baucum et al., 2009; Mukund, 2015). Two, three or six budded setts improve overall plant population and are commonly used for commercial cultivation throughout the world. The single budded setts are useful for glasshouse or pot experiments, otherwise they can be planted into trays and later transplanted to targeted sites (Baucum et al., 2009).

The cut ends of seed setts harbour various pathogens resulting in rotting of buds and root primodia. To guarantee protection, it is recommended that the setts be soaked for a certain time (e.g. 5 to 10 minutes) in a fungicide (such as methyl benzimidazole-2yl-carbamate (MBC)) prior to planting (Jalaja et al., 2008).

Commercial sugarcane is also propagated by means of stem regrowth from stools remaining underground after harvest of the previous crop.

#### *2.1.3.3.2. Micropagation*

Plant tissue culture or micropagation, has been widely adopted in commercial agriculture for fast large-scale mass production of sugarcane plant materials (Sood et al., 2006; Ali et al., 2008; Behera & Sahoo, 2009). Micropagation is an alternative method to the conventional vegetative propagation using bud setts. The *in vitro* culture method of sugarcane regenerates plantlets via somatic embryos callus, axillary bud and the shoot apical meristems (Sauvaire & Galzy, 1978; Snyman et al., 2009; Behera & Sahoo, 2009; Shimellis et al., 2014; Tolera et al., 2014). In an attempt to generate large numbers of pathogen free seedcane, the South African Sugarcane Research Institute (SASRI) has developed a rapid embryogenic propagation procedure (i.e. NovaCane®) (Snyman et al., 2009), but the technology is not yet extensively used commercially due to high cost compared with conventional planting.

Micropagation has a potential to produce some 260,000 plantlets from a single shoot apex in about six months (Hendre et al., 1983), 78,408 plantlets in four months (Lee, 1987) and about 76,500 plantlets in three months (Lal et al., 1996). The tissue culture produced sugarcane plantlets have been shown to possess some degree of superiority over the vegetatively propagated cane crop with respect to stalk yield when grown under similar conditions (Sandhu et al., 2009).

#### 2.1.3.4. Planting methods

Sugarcane can be planted either mechanically or manually but the latter method is the most commonly practiced. Although there are several techniques of planting sugarcane manually, the ridge and furrow and is the most commonly used in Asia, Africa and some South American countries (Hunsigi, 1993). The ridge and furrow planting method is adopted in areas with moderate rainfall and heavy soil with low drainage. The U- or V-shaped furrows of 30 to 40 cm depth (Mukund, 2015) are opened at intervals of 90 to 100 cm with a sugarcane ridger. Irrigation channels are formed crosswise at a distance of 20 to 25 m (Hunsigi, 1993). Prior to planting, slightly pressed setts are placed end to end on top of the ridge (Mukund, 2015). Planting of two or three eye-bud setts in an end-to-end manner

(Srivastava & Rai, 2012; Mukund, 2015) is made in either a wet or dry furrow (Hunsigi, 1993).

The wet furrow method is practiced in low to medium fertile soils. In this method, the furrows are thoroughly irrigated and setts are placed 3 to 5 cm deep. In highly fertile soils, dry furrow method of planting can be adopted. The setts are planted in dry furrows and covered with soil up to half the depth of the furrow and immediately irrigated. If heavy rains are expected during the cropping season, setts are planted half way between the ridge and furrow (Hunsigi, 1993).

The flat bed planting method is commonly used in drier areas (under rainfed conditions) where supplemental irrigation is necessary (Srivastava & Rai, 2012). The land is prepared in a fine tilth by one or two deep ploughings (Hunsigi, 1993). Shallow furrows of 5 to 10 cm depth are opened at a distance 60 to 90 cm by wooden plough (Srivastava & Rai, 2012; Mukund, 2015). The setts are placed end to end (or overlapping) in the furrow. The depth of planting is 0.25 to 5 cm and the setts are covered with soil to avoid drayage (Hunsigi, 1993). It is essential to have adequate moisture in the field at the time of planting (Srivastava & Rai, 2012).

## 2.2. World production of sugarcane

Sugarcane is an important food, bioenergy and a significant economic contributor to many countries in the tropics and subtropics (Moore et al., 2014). The crop occupies an area of about 21 million ha (Robinson et al., 2011), approximately 1.5% of the total world area used for agriculture (Moore et al., 2014). Worldwide sugarcane total production amounts to 1911 million metric tons (FAO, 2015). Sugarcane area and productivity differ widely from country to country. Out of 121 sugarcane producing countries, fifteen countries (Brazil, India, China, Thailand, Pakistan, Mexico, Cuba, Columbia, Australia, USA, Philippines, South Africa, Argentina, Myanmar, and Bangladesh) represent 86% of the total area and 87% of annual production. Out of the total white crystal sugar production, approximately 70% comes from sugarcane and 30% from sugar beet, corn, cassava, etc. (Chauhan, 2014). Brazil produces 25% of the world's cane sugar and is the largest producer and exporter.

### 2.3. Production and importance of sugarcane in South Africa

As in many countries, sugarcane production is one of the major agricultural contributors to the South African economy. South Africa is consistently ranked in the top 15 of the world's biggest producers of high quality sugar (SASA, 2013). The crop is predominantly cultivated in KwaZulu-Natal with less production in Mpumalanga lowveld and the Eastern Cape (SASA, 2013). The area under sugarcane cultivation in South Africa amounts to approximately 430 000 ha (DAFF, 2013). About 68% is grown within 30 km of the coast and 17% in the high rainfall areas of KwaZulu-Natal (DAFF, 2013) and only 15% is grown in the northern irrigated areas.

Approximately 83.8% of the total sugarcane farmers are large-scale producers which account for the total sugarcane production (SASA, 2013). The remaining 8.26% and 7.94% of the total crop is produced by small-scale growers and milling companies, respectively. Approximately 18.8 million tons of sugarcane annually which is processed at 14 regional sugarcane mills. The 14 mills produce an average of 2, 3 million tons of sugar annually (SASA, 2013). The sugar industry supplies about 76% of sugar for domestic consumption in the Southern African Customs Union (SACU) region. The remaining 14% is exported to markets in Africa, Asia and the USA. An annual average direct income of over R12 billion through revenue is generated by sugar sales in the SACU region and world export market (SASA, 2013).

The industry also contributes considerably to employment in deep rural areas. Direct employment, approximately 79 000 jobs, is mostly responsible for production and processing of sugarcane. Indirect employment is estimated at 350 000 jobs. Taken together, about 2% of South Africa's population (one million people) depend on the sugar industry for a living (SASA, 2013).

There is evidence showing a decline in total area under sugarcane cultivation which resulted in reduced yields of harvested cane, cane production and sugar production (Table 2.1). The observed steady decline in total area under sugarcane cultivation calls for an urgent transition of current agricultural management into a more resource-use efficient system that is profitable. Whilst 65% is a staggering level of N inefficiency in sugarcane systems (Meyer et al., 2007), transition to a more resource efficient system will require identification of ways to

maintain maximum yields while reducing fertilizers inputs. One way to achieve this goal is through improved N management practices (for example, N rate, N timing, N placement, and N form) coupled with the incorporation of high N-use efficient sugarcane varieties and future plant breeding efforts for more promising N-use efficient varieties.

Table 2.1. Sugarcane harvest statistics for the South African sugar industry in 2000, 2004, 2008 and 2012 (SASA, 2013).

Year	Area under sugarcane	Yields of harvested cane	Cane	Sugar
			production	production
	Ha		1000' tons	
99/00	421 637	67.74	21 223	2 531
03/04	426 861	62.64	20 418	2 419
07/08	412 979	64.17	19 723	2 281
11/12	367 301	62.06	16 800	1 832

## 2.4. Defining nitrogen use efficiency

The concept of NUE has been extensively used to study plant responses to different N availabilities in the soil (Hirose, 2011). In recent years, numerous and contradictory NUE definitions have emerged which have made the term unclear. The most conventionally used definition of the term is grain yield per unit of N available in the soil, including both residual N and fertiliser N (Moll et al., 1982). Furthermore, NUE of grain crops (such as maize, wheat or rice) is roughly determined in two ways. Firstly, it is determined from the efficiency of a plant to recover N in the soil, that is, N-uptake efficiency. Secondly, it is determined from the efficiency of a plant to transform acquired N into harvestable yield, namely N-utilisation efficiency, or physiological N-use efficiency (Moll et al., 1982).

The NUE for crops like sugarcane is expressed as the biomass and/or sucrose produced per N content of the plant biomass (Schumann et al., 1998; Robinson et al., 2007). Whether in grain or biomass crops, NUE is the product of N uptake efficiency (NUpE) and N utilisation efficiency (NUtE) which is the optimal combination between N assimilation efficiency (NAE) and N remobilization efficiency (NRE) (Good et al., 2004; Dobermann, 2005; Hirel et al., 2007; Garnett et al., 2009). These efficiencies may differ due to variation in soil type, environment and crop species type.

### 2.4.1. Methods for estimating NUE

Several indices and techniques have been developed for measuring N recovered by crops in agricultural systems (Cassman et al., 2002; Harmsen & Garabet, 2003), chiefly for the purpose of studying crop response to N availability (Dobermann, 2005). The indices include the difference (indirect) method and isotopic-dilution (direct) method (Harmsen & Garabet, 2003; Harmsen, 2003).

#### 2.4.1.1. The difference (indirect) method

The difference method is extensively used in agricultural research. Several N fertilized and unfertilized control plots are required for calculating NUE. The method determines the amount of N recovered by a crop by calculating the differences between total N content from fertilized treatments and unfertilized control plots (Roberts & Janzen, 1990; Rao et al., 1992;

Harmsen, 2003). The method assumes that N recovered from unfertilized control plots is a measure of indigenous soil N whilst that recovered from fertilized plots measures both soil and fertilizer N. The method further assumes that the addition of N to the soil does not alter the amount of indigenous soil N taken up by the plant (Hauck & Bremner, 1976). Indices of nitrogen use efficiency using the difference method are briefly described in Table 2.2.

Table 2.2. Indices of nitrogen use efficiency, their calculations using the difference method and their definitions.

Index		Calculation	Definition	References
NUpE: Nitrogen uptake efficiency	$NUpE = \frac{N_C}{N_R}$	NC, N content in aboveground tissues of unfertilized plots; NR, amount of fertiliser N applied	NUpE is defined as the efficiency of absorption or uptake of supplied N	Good et al., 2004
NtUE: Nitrogen utilization efficiency	$iNUE = \frac{Y_F}{N_F}$	YF, aboveground DM matter yield with applied N; NF, N content in aboveground tissues of fertilized plots;	iNUE is defined as the ability of plants to transforming acquired N (soil and fertiliser) into harvestable yield	Good et al., 2004; Robinson et al., 2007
AE: Agronomic efficiency	$AE = \frac{Y_F - Y_C}{N_R}$	YF, crop yield with applied N; YC, crop yield in control treatment with no N; NR, amount of fertiliser N applied	AE is defined as yield increment of crops per unit increment of N applied	Good et al., 2004; Dobermann et al., 2005
PE: Physiological efficiency	$PE = \frac{Y_F - Y_C}{N_F - N_C}$	YF, crop yield with applied N; YC, crop yield in control treatment with no N; NF, N content in fertilized plots; NC, N content in unfertilized control plots;	PE is defined as yield increment/obtained per unit of acquired fertiliser N	Good et al., 2004; Dobermann et al., 2005
PFP: Partial factor productivity	$PFP = \frac{N_F}{N_R}$	NF, N content in aboveground tissues of fertilized plots; NR, amount of fertiliser N applied	PFP is described as economic yield obtained per unit of nutrient applied	Dobermann et al., 2005

#### 2.4.1.2. The isotopic dilution (direct) method

The  $^{15}\text{N}$  isotopic dilution technique measures the amount of  $^{15}\text{N}$ -labeled N recovered in fertilized crops per unit of  $^{15}\text{N}$ -labeled N applied (Hauck & Bremner, 1976; Roberts & Janzen, 1990; Rao et al., 1992; Harmsen, 2003) as shown in Table 2.3. The technique offers several advantages over the difference method for estimation of synthetic N recovery in cropping systems. The main advantage of the technique is that it allows the distinction in the total N content between soil and fertiliser N (Trivelin et al., 1994). The technique also offers improved accuracy of large-scale field experimentation (Hauck & Bremner, 1976).

However, due to the high cost of the  $^{15}\text{N}$  labelled compounds, the technique cannot be used in large field plots (Trivelin et al., 1994), and until recently the technique had limited use (Hauck & Bremner, 1976). In addition to the high cost of  $^{15}\text{N}$  compounds, a major hindrance to more extensive use of  $^{15}\text{N}$ -trace techniques includes exorbitant cost of the equipment to measure  $^{14}\text{N}:\text{ }^{15}\text{N}$  ratios, maintenance and operation (Hauck & Bremner, 1976).

Table 2.3. Nitrogen use efficiency index, its calculation using the isotopic dilution technique and definition (Hauck & Bremner, 1976; Roberts & Janzen, 1990; Rao et al., 1992; Harmsen, 2003).

Index	Calculation	Definition
$^{15}\text{NRF}$ : $^{15}\text{N}$ isotopic recovery fraction	$^{15}\text{NRF} = \frac{Y_{xp}\text{NF}}{Y_{xf}\text{.N}_{Fi}}$	$N_F$ , total N uptake by fertilized crops at harvest; $Y_{xp}$ , the atom % excess in fertilized plots (%); $Y_{xf}$ , the atom % excess in the applied fertiliser plots (%); $N_{Fi}$ , the initial amount of N fertiliser applied $^{15}\text{NRF}$ is defined as the amount of $^{15}\text{N}$ -labeled N recovered in fertilized crops per unit of $^{15}\text{N}$ -labeled N applied (Harmsen, 2003)

## 2.5. N management and N-use efficiency in sugarcane

The South African Sugarcane Research Institute (SASRI) plays a significant role in soil fertility research on sugarcane, on-farm economics of growing sugarcane and the efficient use of synthetic fertilisers. Research data from various laboratory and field trials have contributed greatly to increased N management practices and productivity of sugarcane (reviewed in Meyer & Wood, 1994; Meyer et al., 2004). For both rain-fed and irrigated plant and ratoon cane, N fertiliser recommendations are adjusted on the basis of bioclimatic region, soil form and the capacity of soil to release N to the plant (Meyer et al., 2007).

For advisory purposes, an analytical method based on Near Infra-red Reflectance Spectroscopy (NIRS) is used by the Fertiliser Advisory Service (FAS) at SASRI to classify soils into four categories (low, moderate, high and very high) according to their potential to mineralise N from soil organic matter (Meyer et al., 1986). This system of N recommendation based on the NIRS allows for the development of site-specific N recommendations in sugarcane fields that could result in a significant reduction of N applications, thus increasing profitability.

Typically, the plant crop (i.e. sugarcane grown during its first crop cycle after planting) grown in the South African sugar industry shows less response to applied N relative to succeeding ratoon crops (Wood, 1964). Consequently, higher N applications are applied in ratoon cane due to a higher response to applied N. Depending on the soil system, recommended N rates for plant cane under rain-fed conditions range between 60 – 120 kg N/ha compared with 60 – 140 kg N/ha for irrigated cane (SASRI information sheet, 2000). One third of the recommended N is applied to the plant cane with phosphorous (P) at planting and the balance broadcasted over the row about 10 weeks later. Rates for ratoon crops under rain-fed conditions vary between 100 – 140 kg N/ha, with those for irrigated cane being 20 kg N/ha higher depending on the soil system (SASRI information sheet, 2000). For ratoon cane, all recommended rates of N are top-dressed within two weeks after harvesting the previous crop. However, split application is recommended for crops harvested in winter since plant regrowth and N uptake are decreased in winter (SASRI information sheet, 2000).

Sugarcane growers in South Africa generally apply N in the form of urea (46%), in part because of its affordability and high N concentration (Nixon et al., 2005). Elevated levels of

N loss have been previously reported where urea or ammonia based fertilisers are used compared with limestone ammonium nitrate and ammonium sulphate (Ladha et al., 2005; Nixon et al., 2005). In order to minimise N losses in the sugar industry, a new empirical ammonia volatilization model, based on soil buffer capacity, was developed to predict the potential ammonia volatilization losses from surface applied urea (Schumann, 2000). In addition to predicting losses from surface applied urea-N, the calibrated simulation model can also estimate increased volatilisation loss from band applied fertiliser or decreased loss from split application (Schumann, 2000). The FAS has implemented the method for all routine soil samples in order to provide soil-specific advice to minimise N losses (Meyer et al., 2004; 2007).

Rapid leaf analysis of macro and trace elements method is also used by the industry as a diagnostic tool to complement the soil testing method (Miles & Rhodes, 2013). The leaf analytic method is briefly described in section 2.10.1.4. Another strategy used by the industry to manage N is classifying sugarcane varieties into one of three categories using the ratio of sucrose yield to N accumulation: efficient N-use responders, inefficient non-responders or inefficient responders (Schumann et al., 1998).

### 2.5.1. Field determination of NUE in sugarcane

The responses of sugarcane varieties to adequate and high N inputs were already demonstrated in the 1940's in South Africa (Colepeper, 1946). Variety by nitrogen fertilization level interactions have since been demonstrated by Inman-Bamber (1984), Gascho et al. (1986), Stevenson et al. (1992), Schumann et al. (1998) and Weigel et al. (2010).

Nitrogen responses and NUE field trials were carried out in Mpumalanga (South Africa) with N supply rates between 0 – 150 kg N/ha (Weigel et al., 2010) on four different South African-bred sugarcane varieties. Two varieties, N19 and N32, were assessed from 2001 – 2007 (referred to as trial 1), and N25 and N36 were assessed from 2007 – 2008 (referred to as trial 2). In the first trial, data for four ratoons indicated that variety N19 was less responsive to increasing N supply and was superior in terms of internal NUE (g sucrose per N content) compared with variety N32, which was highly responsive to N increment. In trial 2, data for plant cane and the 1<sup>st</sup> ratoon showed that variety N25 was less responsive to synthetic N

increment and was higher in internal NUE in contrast to a low-N efficient variety N36 (Weigel et al., 2010). However, the relationship between both N uptake and utilisation efficiencies and biomass yield remain uncertain.

Results from these studies of NUE of sugarcane are not unique to South Africa but are comparable with international sugarcane research. In Florida, a two year field study comparing four varieties on fine sands across a range of N levels (0 – 896 kg N/ha) showed that cultivar CP 65-357 (selected from N-deficient soils) recorded the highest external and internal NUE regardless of N rate or crop season compared with the three other sugarcane varieties that were selected from N-rich soils (Gascho et al., 1986). Under controlled conditions CP 65-357 also produced higher biomass yield but did not accumulate more N than the other varieties (Gascho et al., 1986). The results may in part demonstrate the possibility of contribution of other NUE related traits in the field apart from adaptation to N-deprived soils.

In Australia, genotype by N interaction field trials compared Q117 (commercial) and five unselected genotypes for internal NUE at rates between 0 – 140 kg urea-N/ha (Robinson et al., 2008). Variety Q117 and two unselected mapping genotypes (KQ99-1387, KQ99-1484) showed a positive response to increasing N and accumulated less than 50% of the biomass under 0–N supply compared with 140 kg urea-N/ha. Genotype KQ99-1355 was less responsive to increasing N supply and at low N levels and produced up to 70% of the biomass at high N supply (Robinson et al., 2009). These genotypic variations in NUE among sugarcane varieties present opportunities for identification and genetic improvement of traits conferring high NUE. Based on available statistics in South Africa, N use in the sugar industry has declined from an average of around 2.0 kg N/t cane in 1980 to about 1.45 kg N/t in 2004 (Meyer et al., 2007). More genotype by nitrogen fertilisation level interaction trials are currently being evaluated at SASRI across different sites, including Midlands (rain-fed), Stanger (rain-fed) and Pongola (irrigated).

### 2.5.2. Controlled environmental determination of NUE in sugarcane

One published controlled environmental determination of NUE in South African sugarcane was demonstrated by Schumann et al. (1998). The outdoor hydroponic experiment compared internal NUE of seven commercial sugarcane varieties at 30, 60 and 90 mg N/pot. Nitrogen

use efficiency on the basis of sucrose yield per shoot N content showed that N12 and N19 were more N-efficient varieties at the first N increment, while N14 and N16 were the least N efficient varieties (Schumann et al., 1998). Biomass yield of the least N-efficient varieties was more pronounced at higher N supply rates. More recently, internal NUE of five transgenic lines contrasted with two popular commercial varieties was investigated for 4 months in a potted sand experiment (Snyman et al., 2015). Mean total dry matter (DM) yield at 0.4 mM N rate ranged from 24.22 – 50.18 g/pot. Transgenic line NUE23 followed by NUE57 and NUE9 accumulated the highest DM yield that was comparable to a reference high N-efficient variety, N19. Internal NUE measured on the basis of DM yield per N content (g DW g<sup>-1</sup> tissue N) was highest in line NUE9 and NUE23, whilst lowest internal NUE was recorded in the reference low N-efficient variety, NCo376 (Snyman et al., 2015).

Robinson et al. (2007) also studied genetic variation of 3-month old sugarcane varieties at low (0.4 mM) and high (10 mM) N supply. Results of the study showed that internal NUE (g DW g<sup>-1</sup> tissue N) was on average 2-fold greater at limiting N supply relative to non-limiting N supply with biomass yields of 190 and 90 g DW g<sup>-1</sup> N, respectively. Within N treatments, iNUE of genotypes varied up to 2-fold at both low (143 – 303 g DW g<sup>-1</sup> N) and high (53 – 110 g DW g<sup>-1</sup> N) N supply (Robinson et al., 2007). A 2-year pot trial study conducted in Florida assessed the genotypic variation of three sugarcane varieties in response to four N supply rates on sandy soils (Zhao et al., 2015). Mean shoot DM yield response of varieties for both 2009 and 2010 increased sharply with increasing N supply from 0 – 225 kg/ha. Agronomic N-use efficiency was 68.3, 92.3 and 102 g DW g<sup>-1</sup> N for variety CP 80-1743, CP 01-2390 and TCP 87-3388, respectively (Zhao et al., 2015). The genetic variability among sugarcane varieties demonstrate possible opportunities for identification of physiological traits contributing strongly to N-use efficiency. Quantification of these traits can improve our knowledge of NUE and help in further classification of sugarcane varieties into NUE categories.

## 2.6. Factors affecting N-use efficiency by sugarcane

Synthetic N application is required as basic practise to optimise sugarcane yields. Approximately, only 35% of applied N is recoverable in aboveground biomass and the balance is lost from the plant-soil system (Meyer et al., 2007). The ways in which N can be lost from the environment are transformation dependent processes (nitrification,

denitrification, and volatilization) and poor synchrony between soil N supply and crop N demand (leaching, soil erosion and surface runoff). However, the most commonly reported way in which N is lost from the South African sugarcane cropping systems is N volatilization (Schumann, 2000).

### 2.6.1. Nitrogen volatilization

Conventional N carriers (urea; 46% N) are commonly used by growers in the South African sugar industry (Schumann, 2000; Nixon et al., 2005) and account for approximately 60% of the N fertilisers used in Brazil (Cantarella et al., 2008). Limestone ammonium nitrate (LAN; 28% N), ammonium nitrate (AN; 34% N) and ammonium sulphate (AS; 21% N) are other important and less volatile N sources. However, in the view of relatively high costs and less N concentrations in AN, LAN and AS, urea has a low price per unit N (Schumann, 2000; Cantarella et al., 2008). Several studies have reported significant volatilization losses of N from sugarcane fields, particularly when urea is used as a source of N (Cantarella et al., 2008; Pereira et al., 2009; Nascimento et al., 2013). In fact, when urea is spread on soil or brown leaf residue surface, large quantities are rapidly transformed into ammonia by catalytic actions of urease enzyme ( $\text{NH}_3\text{-N}$ ) (da Silva Paredes et al., 2014). More than 50% of the transformed  $\text{NH}_3\text{-N}$  is volatilised from surface-applied urea and the remaining N may be nitrified and lost into the environment as leaching and denitrification (Schumann, 2000).

Nixon et al. (2005) demonstrated that surface application of urea N on sugarcane brown leaf residue results in higher ( $46.8 \text{ kg N ha}^{-1}$ ) (29%) volatilization losses of N as compared with (<1%) lost from LAN-N and AS-N. Volatilization losses were generally more pronounced within the first two weeks of the experiment. In another study, it was demonstrated that surface application of urea at rates of  $80 - 100 \text{ kg N ha}^{-1}$  resulted in volatilisation losses of 1 – 25% of the applied N (Cantarella et al., 2008). Noteworthy, insignificant amounts of N was lost when AN or AS were applied. The rate of  $\text{NH}_3\text{-N}$  volatilization depends on temperature, soil pH, soil moisture, soil texture and the quantity of brown leaf residue blanket (Schumann, 2000).

## 2.7. Strategies to improve NUE in sugarcane

### 2.7.1. Improved N management practices

#### 2.7.1.1. Nitrogen rate

Low N application often results in decreased sugarcane yields. Excessive N inputs have been shown to decrease N-use efficiency and sucrose yields (Muchow et al., 1996; Meyer & Wood, 2001; Meyer et al., 2007; Lofton & Tubana, 2015), increased lodging and susceptibility to insect pests and diseases (Meyer et al., 2007). To effectively manage N in sugarcane and improve NUE, all sources of N should be taken into account when determining N fertiliser application rate. Optimal N fertiliser application rate should also incorporate many factors such as soil type, crop age, varieties, climate, and length of growing cycle or season (Wiedenfeld, 1995; Wood et al., 1996).

Current N recommendation guidelines for the South African sugar industry for plant and ratoon cane are adjusted according to soil form (type), bioclimatic region and capacity of the soil to release N to the plant (Meyer & Wood, 1994; Meyer et al., 2007). For various reasons such as spatial variability of soils, testing of soil samples remains a mandatory pre-planting practice in the South African sugarcane agriculture. To maximise profit margins and production efficiency, growers rely on soil sampling and testing for designing fertilization programs. Soil samples are tested at FAS in order to provide insight into the general fertility status and also to provide site-specific N recommendations.

#### 2.7.1.2. Nitrogen timing

In addition to N rate, timing of fertiliser application can improve NUE and eventually maintain low levels of inorganic N in soil. This is achieved by delaying N application to coincide with a period of rapid growth and N uptake. For example, during very cold winters in South Africa sugarcane regrowth is very slow and subsequently crop N uptake is substantially reduced, and splitting N application into two distinct periods (6 – 8 weeks apart) will increase NUE (SASRI information sheet, 2000). Contrarily, spring and summer harvested cane accumulate N rapidly and synthetic N fertilisers are applied within two weeks of harvest. This variation in crop N uptake is integrated in fertiliser management strategies to inform farmers' decision about the timing and rate of N fertiliser.

#### 2.7.1.3. Leaf testing

Nutrient analysis of the top visible dewlap (TVD or third) leaf blade has been extensively used as a diagnostic tool to complement soil testing in sugarcane production (McCray & Mylavarapu, 2010; Miles & Rhodes, 2013). Leaf analysis provides an indication of the crop nutritional condition at the time of sampling (McCray et al., 2010; McCray & Mylavarapu, 2010; Miles & Rhodes, 2013). Furthermore, the method demonstrates the extent to which applied nutrients have been captured by the crop, and determines if the presence of deficiencies or imbalances limit crop growth (Miles & Rhodes, 2013).

To cater for shortages or imbalances showed by leaf analysis, the FAS at SASRI has established a set of N and K (potassium) recommendations (Miles & Rhodes, 2013). As in other sugarcane producing countries, the X-ray fluorescence spectrometry method has been extensively used to control the nutrient status of sugarcane in the South African sugar industry and is widely recognised as a way of improving N-use efficiency. Leaf sampling, tissue nutrient analysis and the subsequent interpretation of results may support decisions regarding fertiliser recommendations for optimum growth and yield of sugarcane (McCray et al., 2010). Sampling of TVD takes place when the crop is actively growing (Miles & Rhodes, 2013). However, the period of sampling varies depending on the geographical area and the crop age. For instance, about 40 leaves (30 cm in length) are collected randomly throughout the field at 3 – 5, 4 -7 and 4 – 9 months in the Northern irrigated, coastal lowlands and midlands, respectively (Miles & Rhodes, 2013). The midrib of collected leaves is stripped out and only the leaf blades are retained and sent to FAS for leaf analysis.

#### 2.7.2. Selection and breeding for NUE

Direct selection and breeding for NUE-linked traits in sugarcane that are more efficient at capturing and transforming N into biomass (sucrose) can decrease applied N losses in sugarcane cropping systems. Traits related to efficient N uptake and metabolism have been suggested as selection parameters (Agrama et al., 1999). Several promising morphological traits associated with adaptation under N-limiting conditions include root architecture (root to shoot ratios; root vigour, root length density, increased storage capacity; Garnett et al., 2009) or senescence and remobilization of N (Coque et al., 2006; Hirel et al., 2007; Kant et al.,

2011). However, breeders rarely consider root characteristics as selection criteria due to difficulties involved in intact root excavation and lack of rapid and cost-effective screening methods. Furthermore, root physiology of sugarcane is poorly understood relative to other crops (Otto et al., 2014), mainly because of the long crop cycle (Matsuoka & Garcia, 2011), difficulty in extracting and quantifying root mass.

Generally, synthetic N applications in sugarcane systems are applied during the early stages of crop development. Thus, breeding for root morphology, architecture and rapid N transport could be important for immediate N capture and storage. Using molecular tools several positive coincidences between QTLs for N-uptake and QTLs for root architecture traits were observed, suggesting that breeding programs targeting to increase the NUE should breed for a root system more efficient at taking up N (Coque et al., 2008). Coque & Gallais (2006) found that growing maize under limiting and non-limiting N supply, changes in root architecture had a major influence on grain yield. Such evidence demonstrates the underlying importance of root traits in yield and NUE, thus, should be targeted for NUE breeding programs.

## 2.8. Physiological variables in relation to sugarcane growth and NUE

Previous studies have highlighted the importance of leaf area development for maximization of solar radiation interception and for improvement of cane yields (Inman-Bamber, 1994; Sinclair et al., 2004; Streck et al., 2010). Green leaf area (GLA), leaf N concentration, photosynthetic capacity, Rubisco content, canopy N contents and N allocation and root morphology (Robinson et al., 2008; 2014; Zhao et al., 2014) have been targeted in attempts to improving N-use efficiency of sugarcane under low N regimes.

Growth responses of sugarcane to different N levels have been extensively studied (Schumann et al., 1998; Robinson et al., 2008; 2009; Weigel et al., 2010; Zhao et al., 2014; Lofton & Tubana, 2015). Under conditions of low N regime, sugarcane plants reduce leaf N concentration and leaf area, which in turn decrease radiation interception and photosynthetic rates (Ludlow et al., 1991; Ranjith & Meinzer, 1997; Allison et al., 1997; Sinclair et al., 2004; Robinson et al., 2014; Zhao et al., 2014). There is also evidence showing that low leaf N content towards the end of the harvesting season may reduce carboxylation capacity and ultimately biomass and sucrose yield (Wood et al., 1996). A decline in SLN from 2.0 – 0.7 g N m<sup>-2</sup> was demonstrated to result in decreased photosynthetic rates from 40 – 0.5 µmol CO<sub>2</sub>

$\text{m}^{-2}\text{s}^{-1}$  in sugarcane cultivar NCo310 (Ludlow et al., 1991). On average, maximum photosynthetic capacity of sugarcane typically ranges between 30 – 45  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$  and where leaves are supplied with excess N, the peak response of photosynthetic rates can increase up to 45 – 57  $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$  (Sage et al., 2014).

In a study with *Phalaris arundinacea* and *Carex stricta*, supplying low (0.15 mM) N rate resulted in 50% reduction in leaf N content followed by a significant decline in photosynthetic capacity and photosynthetic nitrogen use efficiency (PNUE) as compared with plants receiving high (15 mM) N rates (Holaday et al., 2015). The decline in leaf N concentration and subsequent decline in photosynthetic capacity observed in C4 plants proves that it is possible to improve NUE by selection of varieties that can allocate N to chlorophyll or carboxylation enzymes.

Nitrogen allocation in aboveground components has been sought as a physiological trait for quantification and selection of high N-use efficient sugarcane. Nitrogen allocation parameters of six genotypes from a mapping population contrasted with a commercial variety were investigated for 7 months under glasshouse conditions supplied with low, intermediate and high N rates (Robinson et al., 2008). Reduced N allocation ranging from 16 – 29% to the stalk was observed under low N rates compared with 38 – 54% stalk N allocation under adequate N rates (Robinson et al., 2008). Elevated concentrations of N in stalk following application of 268 kg/ha urea-N have been shown to reduce stalk sucrose content and N-use efficiency, measured as (biomass/N content) or (sucrose/N content) (Muchow et al., 1996). Low N allocation to the stalk under low N inputs suggest that greater N is allocated to the leaves and photosynthetic parameters which may contribute significantly to greater biomass production and N-use efficiency.

Sugarcane growth and productivity may be also influenced by root system properties because of their effects on the uptake of soil available N. To date, very few studies have focussed on genotypic variation of root systems and none have focussed on the effect of root system properties on NUE (Robinson et al., 2009). Root responses to localized nutrient supply play a crucial role in nutrient capture by crops and can potentially improve nutrient-use efficiency (Shen et al., 2011). Although there is controversy regarding responses of root growth to reduced N supply, plants respond to low N supply with increased root growth, roots branching, root hair length and root hair density (Gojon et al., 2009). In a glasshouse study

conducted in Hawaii, a drought-tolerant cultivar showed increased dry-matter allocation to roots with decreasing N supply in comparison with drought susceptible variety (Ranjith, 2006).

Root biomass responses to N fertilization in a ratoon crop were also demonstrated in seven South African sugarcane varieties (Schumann et al., 1998). At high (90 mg) N fertilisation, variety N25, N14 and N24 showed the most vigorous stimulation of shoot growth and accumulated greater concentrations of N. In contrast, root biomass accumulation was also stimulated only in variety N25 and N14 but not in N24. These observations may suggest that, at a high level of N nutrition, greater exploitation of soil volume and N uptake by roots contributed most strongly to plant growth in N25 and N14 whereas at medium (60 mg N) fertilisation, N utilisation contributed most strongly to shoot growth and NUE of N12 and N19 (Schumann et al., 1998).

### 3. MATERIALS AND METHODS

#### 3.1. Study area

The study was conducted at the South African Sugarcane Research Institute (SASRI) shade-house in Mount Edgecombe, South Africa ( $29^{\circ}42'0''$  S;  $31^{\circ}2'0''$  E). The shade-house was fitted with clear polycarbonate roofing and walls of 40% white-shade cloth (Figure 3.1). The total area of the shade-house facility was 14 m x 25 m x 3.3 m.



Figure 3.1. Pots filled with sand in the shadehouse fitted with clear polycarbonate roofing and walls of 40% white-shade cloth at SASRI.

#### 3.2. Soil properties

The thoroughly leached river sand, from the Umgeni river, was passed through a 2.38 mm sieve. The physical and chemical properties of the potted soil were assessed at the SASRI Fertiliser Advisory Services (FAS) (Table 3.1). The particle size distribution was done following the method described by Bouyoucos (1962). The soil was potted in 20 L well drained black plastic pots with a diameter of 0.28 m and a height of 0.32 m. Prior to planting,

the soils were irrigated using tap water until water drained from the drainage holes beneath the pots.

Table 3.1: Physical and chemical analysis of Umgeni River sand used in the potted-sand trials (trial 1 and 2).

Soil character	Trial	
	Trial 1	Trial 2
<b>Particle size distribution (%)</b>		
Clay	nd*	3
Silt	nd*	2
Sand	nd*	95
<b>Chemical composition</b>		
K (mg/L)	12	17
Ca (mg/L)	160	198
Mg (mg/L)	65	64
Na (mg/L)	16	26
Exch. Acidity (cmol/L)	0.02	0.05
Total cations (cmol/L)	1.45	1.72
Acid saturation (%)	1.4	2.9
pH (CaCl <sub>2</sub> )	5.59	5.12
Zn (mg/L)	0.4	0.8
Cu (mg/L)	0.4	0.7
Mn (mg/L)	4.2	13.7
Fe (mg/L)	21	60
Si (mg/L)	12	9
NIRS Clay Estimate (%)	17	6
OM (%)	0.6	1.6
Sample density (g/mL)	1.47	1.47

\*nd = not determined

### 3.3. Varieties and production of plant materials

Plant material of all varieties was derived from seedcane obtained from the Kearsney research station nursery (S 29 17 48 / E 31 16 06). Fifteen sugarcane varieties (NCo376, N12, N19, N25, N27, N31, N32, N36, N37, N39, N40, N41, N48, N49 and N52) were used as test plants in the first experiment (trial 1). The varieties were selected because they are currently under evaluation for N-use efficiency under field conditions in the coastal, midlands (rain-fed) and irrigated regions of South Africa. From the fifteen varieties screened in trial 1, eight varieties were selected for further analysis of physiological traits contributing to genetic variation in

internal NUE under four N-supply regimes in the subsequent pot trial (trial 2). The eight sugarcane varieties (N12, N19, N31, N32, N37, N41, N48 and NCo376) used in trial 2 were selected based on their differences in internal NUE (g sucrose/ g N<sup>-1</sup>) from a pot experiment (Schumann et al., 1998) and preliminary data from field evaluations. Based on shoot biomass production per N content, the varieties are ranked into categories of high (N12, N19, N41 and N48), intermediate (N31 and N37) and low (NCo376 and N32) internal N-use efficiency (Schumann et al., 1998; Weigel et al., unpublished data).

In both trials (trial 1 and trial 2), sugarcane plants were established from disease-free single nodal stem cuttings (setts) of about 2.5 cm in length. Setts of all varieties were cut from the mature central section of the stalk and inspected for bud quality before being dipped into a fungicide (50 mL ERIA ([difenoconazole [62.5 g/L] and carbendazim [125 g/L]; ©Syngenta) in 10 L water) for 15 minutes. The pre-treatment of 2.5 cm sugarcane nodal stem cuttings with ERIA fungicide and establishment of sugarcane plants in both trial 1 and 2 are illustrated in Figures 3.2 and 3.3. In trial 1, sugarcane plants were established by planting setts directly into moist potted sand. Plantlets used in trial 2 were pre-germinated in 96-well polystyrene seedling trays covered with sterile vermiculite and kept well-watered in a germination room at 34°C. Following “shoot emergence” at 7 days after planting (DAP), plantlets were allowed to grow for a further 14 days in the germination room before being transferred to the shade-house. Germinated plantlets were allowed to acclimatize to the ambient conditions for 6 days prior to transplanting into 20 L pots.



Figure 3.2. Pictorial demonstration of (a) single budded stem cuttings (setts) of about 2.5 cm in length dipped in ERIA fungicide, (b) direct planting of three setts into pots filled with sand and (c) healthy sugarcane plantlets inside pots placed linearly into steel trays.

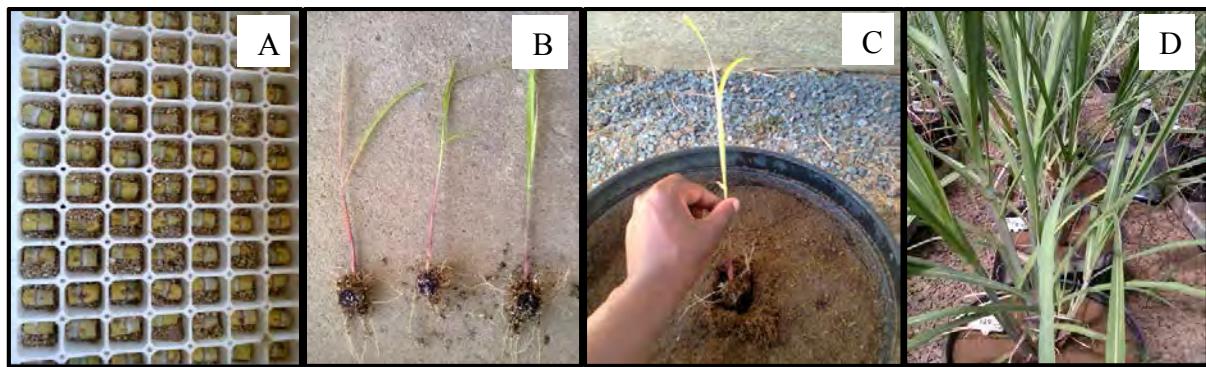


Figure 3.3. Pictorial demonstration of (a) pre-germination of fungicide treated setts (2.5 cm in length) in 96-well polystyrene seedling trays, later covered with sterile vermiculite, (b) carefully removed germinated setts (plantlets) from trays, (c) transplanting of plantlets in pots filled with sand and (d) well established tillering sugarcane plantlets in pots placed linearly into steel trays.

### 3.4. Experimental designs and N treatments

#### 3.4.1. Trial 1.

A preliminary outdoor potted sand trial was conducted to assess the genetic variability in N-use efficiency of fifteen commercial varieties at two contrasting N-supply regimes (Figure 3.4). Planting of setts in pots commenced on the 25 September 2013.

##### 3.4.1.1. Trial design

Setts of all varieties were planted into a total of 180 x 20 L well drained black plastic pots. Three setts were planted per pot. After planting, five pots were placed linearly into a total of 36 galvanized metal troughs (2 m long × 0.4 m wide × 0.1 m deep) as shown in Figure 3.4. A 250 µm thick black polyethylene sheeting was placed over the troughs with holes cut to fit tightly around the pots to prevent algal growth and to reduce evaporation during irrigation. The trial consisted of two nitrogen levels and was arranged in a random design with six replications.

##### 3.4.1.2. N treatments

Plants were subjected to two N treatments: low (90 mg N/L) and high (180 mg N/L) N supply, subsequently referred to as low N and high N supply, respectively (Table 3.2). To prepare a nutrient stock solution, all macronutrient reagents listed in Table 3.2 were dissolved in 20 L

water. Nitrogen was added to the stock solution in the form of lime ammonium nitrate (LAN) and ammonium sulphate ( $[NH_4]_2SO_4$ ). Nitrogen was the only variable nutrient and adequate amounts of other macronutrients (P, K, Ca, Mg and S) were applied equally to all N treatments. Trace elements (Fe, Mn, Zn, Cu, B and Mo), from Micronutrient Hydroponic Seedling mix (©Hygrotech), were also added to the nutrient stock solution at rates of 1 g L<sup>-1</sup> (Table 3.2). A stock solution (2 L) containing macro- and micro-nutrients was added to 18 L of water to make a 20 L nutrient solution. The solution was then added to each trough. Another 20 L of fresh tap water was added to make a total of 40 L per trough. Treatment solutions were prepared and applied every fortnight, and in alternate weeks, tap water was supplied into the troughs.

During the course of the experiment, plants were sprayed for 15 days with dursban insecticide (2 mL L<sup>-1</sup> water) (75 % w/w chlorpyrifos; Dow AgroSciences Ltd) whenever aphids and other pests (scale, leafhoppers) were visible. Only two replicate pots were maintained for 360 days after planting (DAP) whilst two replicate pots were harvested after 120 DAP and another two at 240 DAP as shown in figure 3.6.

Table 3.2: Nutrient solution used to irrigate potted sugarcane plants in trial 1 and 2 (nutrient solution was modified from Schumann et al., (1998)).

N level	Nutrient	Trial 1 Mass (g/20 L)	Trial 2 Mass (g/20 L)
<b>Macronutrients</b>			
<b>No N</b>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	N/A	0
	LAN	N/A	0
<b>Low</b>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	82.8	20.7
	LAN	65	16.3
<b>Medium</b>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	N/A	62.1
	LAN	N/A	48.8
<b>High</b>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	166	124.2
	LAN	130	97.5
	MgSO <sub>4</sub> .7H <sub>2</sub> O	170.3	170.3
	K <sub>2</sub> SO <sub>4</sub>	141.4	141.4
	CaCl <sub>2</sub> .2H <sub>2</sub> O	112.1	112.1
	KCL	29.4	29.4
	Superphosphate	67	67
<b>Micronutrients</b>			
		Mass (g/20L)	
	Fe	1.46	1.46
	Zn	0.19	0.19
	Cu	0.03	0.03
	Mn	0.38	0.38
	B	0.48	0.48
	Mo	0.05	0.05

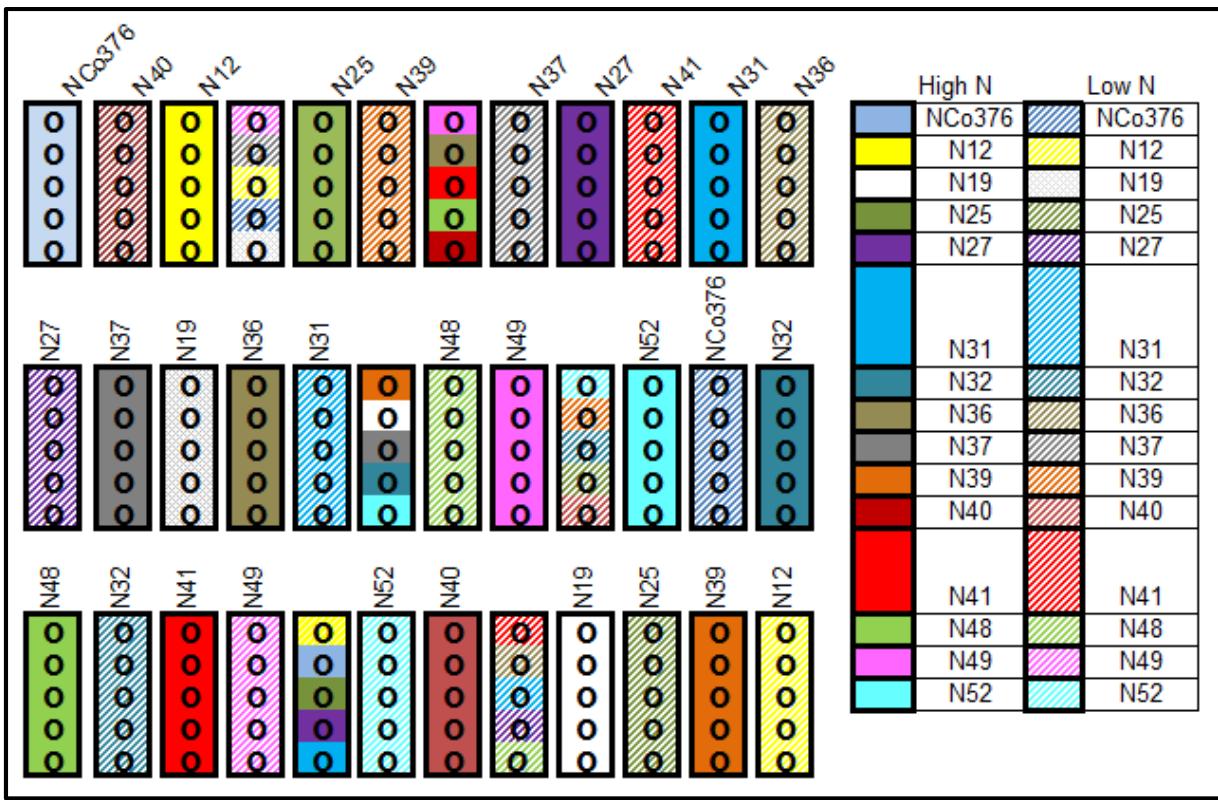


Figure 3.4. Illustration of trial 1 layout and arrangement of varieties in pots and N treatments in the shade-house. Unlabelled trays contain 5 pots, each with a different variety.

### 3.4.2. Trial 2.

An outdoor pot trial study to identify and characterize phenotypic traits linked to N-use efficiency of eight sugarcane varieties at four contrasting N supply regimes (Figure 3.5) was used. Pre-germination of setts commenced on the 23 October 2014 whereas transplanting of plantlets was conducted on the 20 November 2014.

### 3.4.2.1. Development of trial 2 design

Performance, practicability and the partly statistical limitations of pot trial 1 are discussed below and an improved follow-up trial (trial 2) was designed. The validation and improvement of the pot trial method was one of the objectives of this thesis. The following paragraph summarizes the limitations of trial 1 and the changes that were introduced in trial 2.

- i. Germination rate: the direct planting of the single budded setts resulted in variable germination rate. Consequently, there were already varietal differences occurring from the start of the experiment just due to variable germination rates. In the subsequent pot trial 2, plants were pre-germinated and only one plantlet was planted per pot.
- ii. N rates: measured N concentrations in plant parts from trial 1 showed that the selected N rates in the 1<sup>st</sup> trial (50%N and 100%N) both led to a luxury consumption of N, indicating that the effect of N deficiency was somewhat not detected. It is well documented in literature that internal NUE and genetic variation in iNUE of various plant taxa, including sugarcane, is greater under conditions of low N supply. Therefore in the subsequent pot trial 2, four N rates were included viz. 0% N, 25 % N, 75% N and 150% N, subsequently referred to as no N, low N, medium N and high N, respectively.
- iii. Irrigation: Nutrient solution and pure water were continuously supplied into 50 L troughs where potted plants are placed. At harvest, a black colouration was noticeable on roots together with an unpleasant smell. Central to this observation may be poor aeration at the lower surface of the submerged pots. Therefore as an improvement to this method, pots were watered from the top.
- iv. Number of replications: For both N treatments, three destructive harvests were conducted periodically throughout the year at four months intervals. Two plants per N treatment were randomly harvested at each sampling period. In most cases, the mean variation between replicates was greater than 30%, thus limitations for statistical evaluations. Treatments (varieties) were not randomised within reps. In the second trial however, a RCBD with five blocks (replications) was used.

- v. Time of destructive harvest: by means of the whole trial performance and the results of the three destructive harvests for trial 1, a period of 180 DAP has been identified for a once off destructive harvest. Reasons being, that at later times both growth and N-uptake by the plants slow down and a lot of interfering external factors (e.g. pest and diseases, risk of lodging) are limiting trial performance.

#### 3.4.2.2. Trial design

After acclimatization, plantlets from the eight selected sugarcane varieties were transplanted into a total of 160 x 20 L pots at 10 cm depth. Only one plantlet was transplanted per pot. Immediately after transplanting, eight potted plants were placed linearly into two conjoined galvanized metal troughs (2 m long × 0.4 m wide × 0.1 m deep) as shown in Figure 3.5. Varieties were randomly allocated within an N-level. The N levels were also randomly allocated in each block (rep). The upper portions of the pots were covered with a “circular cut” 250 µm thick black polyethylene sheets around the primary shoot to reduce moisture loss and algal growth. The trial consisted of four different N levels and was arranged in an RCBD design with five replicates.

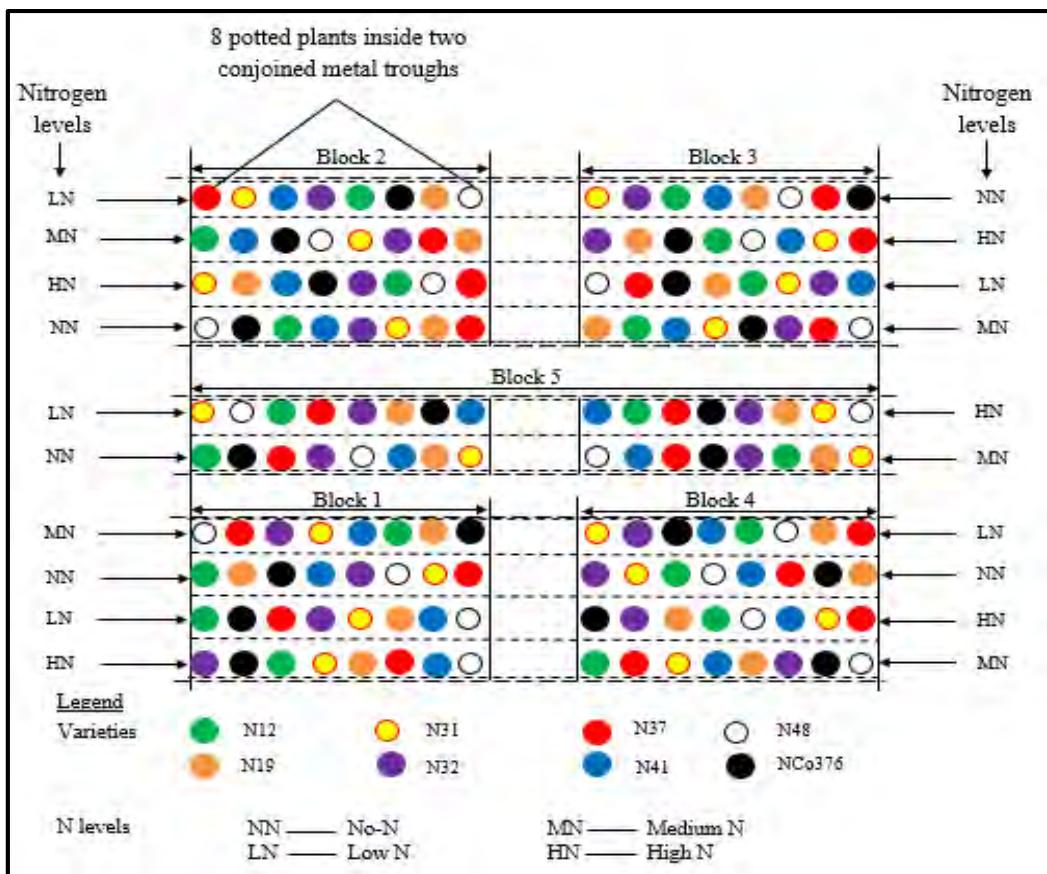


Figure 3.5. Illustrated representation of trial 2 layout and arrangement of sugarcane varieties together with N levels.

### 3.4.2.3. N treatments

Transplanted plantlets were subjected to four N treatments: no (0 mg N/L), low (22 mg N/L), medium (67 mg N/L) and high (134 mg N/L) N supply in the form of LAN and  $(\text{NH}_4)_2\text{SO}_4$ , subsequently referred to as NN, LN, MN and HN, respectively. Nutrient stock solution was prepared as briefly described in section 3.4.1.2. A stock solution (1 L) containing macro- and micronutrients chemical was added to 19 L water to make a 20 L nutrient solution.

Pots were watered from the top with 2 L of respective nutrient solutions. At the beginning of the trial (leaf and shoot development stages), irrigation was restricted to once a week with a 2 L container. Later, at 90 days after transplanting (DAT), the 250  $\mu\text{m}$  thick black polyethylene sheets around the primary shoots were removed to allow further development of multiple shoots. The irrigation schedule was also attuned to three times per week using a 2 L container.

The nutrient solution volumes were selected to ensure that soil hydration was maintained, no leaching occurred, and all of the added solution was retained within the pot.

During the course of the experiment, plants were sprayed with insecticide (chlorpyrifos 2 mL L<sup>-1</sup> water) on monthly basis to prevent infestations of aphids and other pests (scale, leafhoppers). All plants were harvested at 180 DAT.

### 3.5. Measurements

3.5.1. Trial 1. In the preliminary trial (trial 1), destructive sampling of two replicate pots was conducted periodically. At each sampling period, whole plant sampling was conducted and sampled plants were differentiated into components as shown below (Figure 3.6).

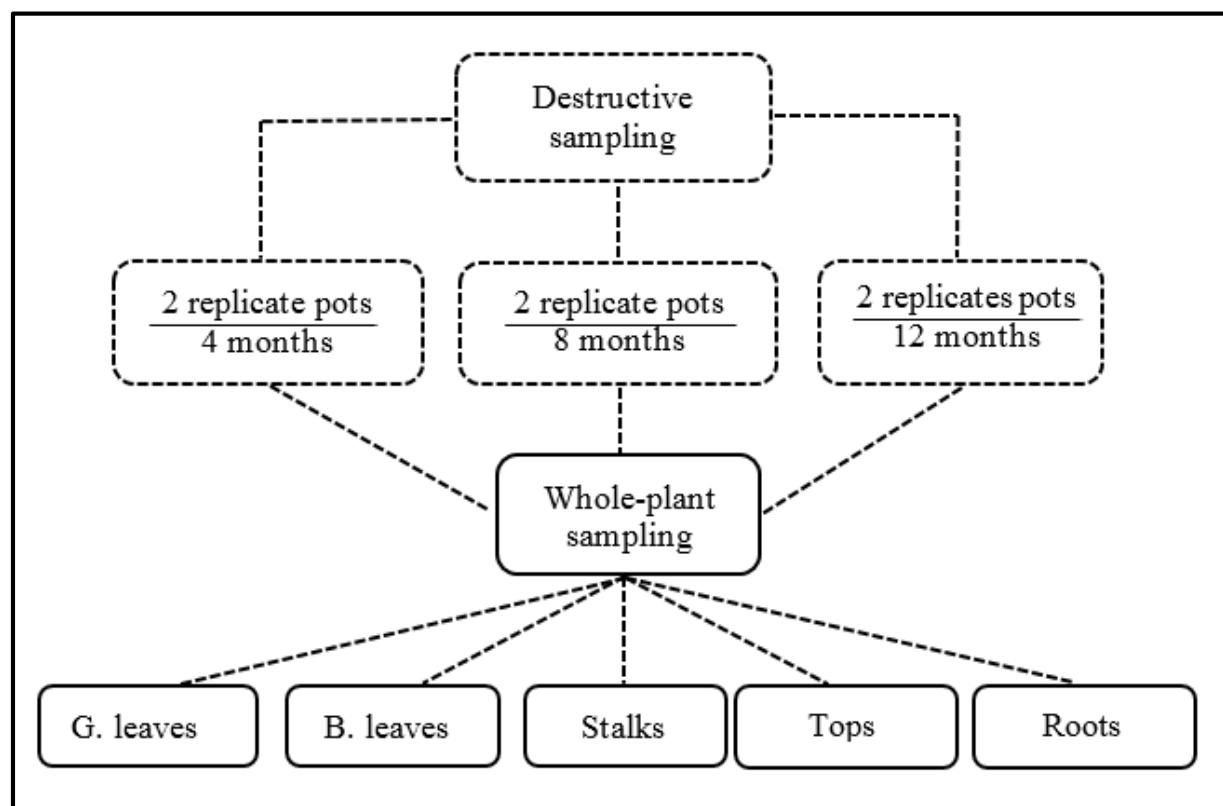


Figure 3.6. Demonstration of destructive harvests conducted periodically throughout the year at four month intervals and biomass components that were sampled. G (green) and B (brown).

For both low and high N supply, three destructive harvests were conducted periodically throughout the year at 120, 240 and 360 DAP (Figure 3.6). Two plants per N treatment were

randomly harvested at each sampling period. For each harvest, the aboveground biomass was clipped near the soil surface and separated into green leaves (photosynthetic leaf blades), brown leaves, tops (leaf sheath and undeveloped leaves) and stalks. Roots were recovered from pots and washed gently over a fine mesh screen. The shoot components (green leaves, brown leaves, tops and stalks) and roots were weighed and put into brown perforated bags. Stalk samples were crushed at SASRI millroom prior to oven-drying. The shoot components and roots were then oven-dried at 60°C for 72 h and weighed again for determination of dry matter yield.

**3.5.2. Trial 2.** In the subsequent trial (trial 2), non-destructive measurements were conducted at specific time intervals. Destructive sampling was conducted from all pots at 180 DAT and harvested plants were differentiated into tissue components as shown below (Figure 3.7).

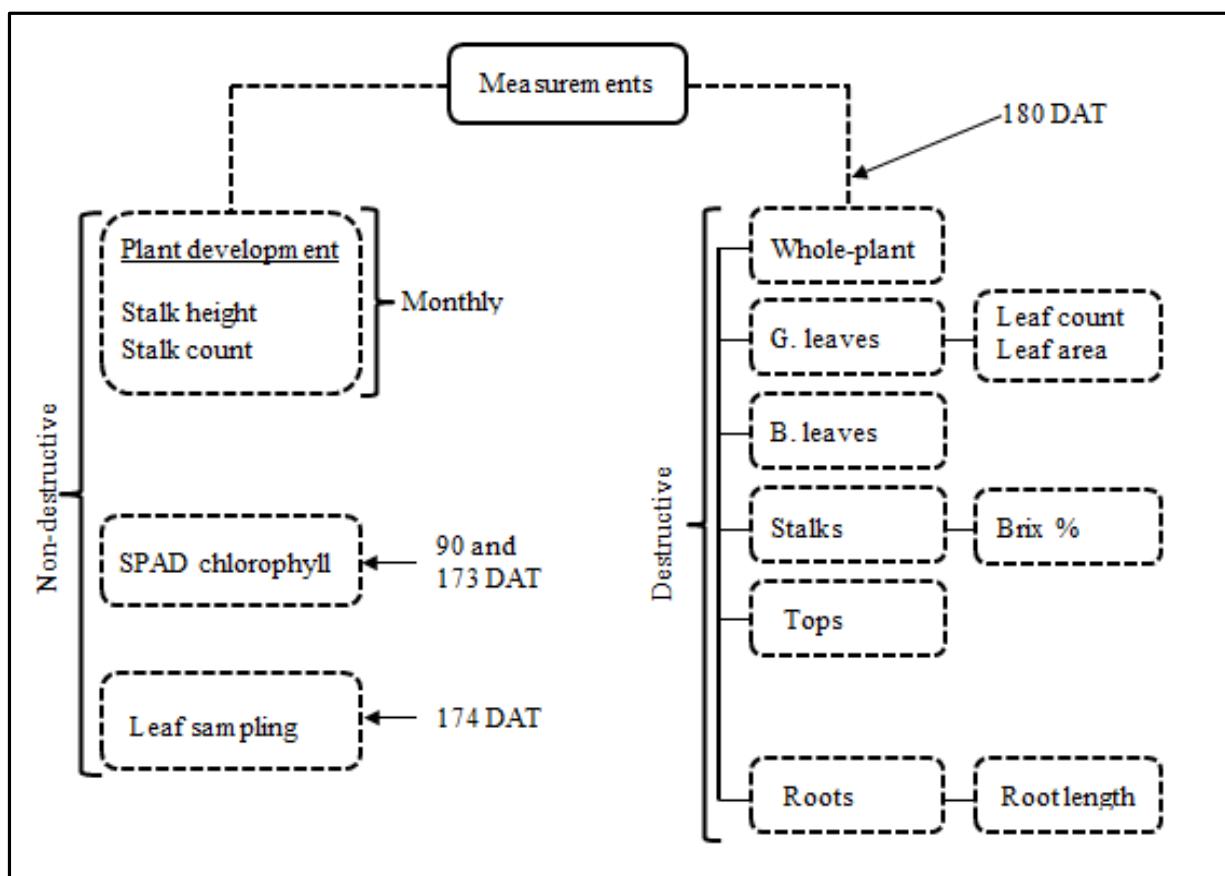


Figure 3.7. Summary of non-destructive measurements at specific time intervals (DAT) and destructive (biomass) measurements at final harvest (180 DAT).

### 3.5.2.1. Non-destructive measurements

#### 3.5.2.1.1 *Stalk heights and counts*

Plant growth was assessed for each treatment solution by examining the stem height (cm), using a ruler from the root collar to the terminal bud, and the number of shoots per pot was obtained by manual counting. The measurements were taken at 30 day intervals.

#### 3.5.2.1.2. *SPAD chlorophyll*

A Chlorophyll Meter, SPAD-502 (SPAD-502, Minolta Co., Japan), was used to take dimensionless Soil Plant Analysis Development (SPAD) values to estimate leaf chlorophyll content. Measurements were made at a central point on the leaflet between the midrib and the leaf margin. Three SPAD measurements were taken from the primary shoots on the top visible dewlap (TVD). The three measurements were averaged to a single SPAD value for each N treatment. The SPAD measurements were taken at 120 and 173 DAT.

#### 3.5.2.1.3. *Leaf sampling*

The third fully expanded leaf from the top of the plant was sampled at 174 DAT from all pots. Approximately 30 leaves were sampled from all treatments. The sampled leaves were held together in a bundle and the top and bottom parts were chopped off with secateurs leaving a central portion of roughly 30 cm in length. The midrib was stripped off by tearing and discarded as reported by Muchojov et al. (2005), and only the leaf blade was retained (SASRI Information Sheet, 2000). The collected leaf samples were correctly labelled and sent to FAS. The harvested TVD leaf samples were oven-dried at 75°C for 48 hours at fertiliser advisory services (FAS), and then finely ground in a leaf mill by passing them twice through a 0.5 mm perforated screen. Concentrations of N, P and K in leaf samples were determined using X-ray fluorescence spectrometry.

### 3.5.2.2. Destructive measurements

At 180 DAT, stalk material from plants in all pots were cut near the soil surface and separated into green leaves, brown leaves, tops and stalks. The number of green leaves was recorded, and the green leaf area (GLA) was determined using a LI-3100 electronic leaf area meter (Li-

cor, Inc. Lincoln, Nebraska, USA). Stalk length was measured using a measuring tape, and the number of stalks were counted manually and recorded. The number of nodes and internodes of individual stalks were recorded. Internode numbers 2, 4 and 6 of every primary shoot were separated from each stalk and used for determination of Brix percentage (%) using a refractometer (Refracto 30GS). Cane juice from internode numbers 2, 4 and 6 was squeezed into the measuring cell of the brix refractometer using a water-pump playa.

Roots were carefully removed from all pots and washed gently over a fine mesh screen. The length of the longest roots was measured for all treatments using a ruler. Afterwards, all the plant materials were weighed and put into brown perforated bags. The samples were oven-dried at 60°C for 72 h and weighed again for determination of dry matter yield. The stalk samples were ground to a coarse powder using a pestle and mortar.

### 3.6. Determination of N concentration (%) in plant components

Prior to analysis of N concentration in tissue components, the oven-dried samples were milled at FAS using a leaf grinder and then oven-dried again at 75°C for 24 h to ensure water content close to zero. Approximately 0.15 g of ground sample was encapsulated in tin foil and placed into sample carousel of TruSpec N® instrument. Nitrogen concentration (% [g N/100 g dry mass tissue]) in tissue components was determined using the automated thermal combustion technique (LECO TruSpec N analyser, LECO Corporation, Michigan, USA).

### 3.7. Calculations of NUE

Nitrogen content ( $\text{g.shoot}^{-1}$ ) was computed as a product of mineral N concentration and leaf biomass (Pederson et al., 2002).

$$\text{N content} = \text{mineral element (\%)} \times \text{DM yield (g)}$$

Nitrogen uptake efficiency (NUpE) was calculated as the ratio of N content and N supplied (Good et al., 2004).

$$\text{NUpE} = \frac{\text{N content}}{\text{N supply}}$$

Nitrogen utilisation efficiency (NUtE; g DW/g N) was calculated as the ratio of dry matter accumulation and N content (Good et al., 2004; Robinson et al., 2007).

$$\text{NUtE} = \frac{\text{Dry biomass yield}}{\text{N content}}$$

Overall NUE was computed as the product of uptake efficiency (NUpE) and utilization efficiency (NUtE) (Good et al., 2004; Snyman et al., 2015).

$$\text{NUE} = \text{NUpE} \times \text{NUtE}$$

### 3.8. Data analyses

All data collected for trial 2 were tested for normality using the Shapiro-Wilk test prior to analyses. Analysis of variance (ANOVA) was done using the GenStat software package (version 14; VSN International, UK). Either a 1-Way or 2-Way ANOVA was carried out to compare treatment means. Where significant differences were found, the Duncan Multiple Range Test (DMRT) was used to separate treatment means at  $p<0.5$ .

## **4. RESULTS AND DISCUSSION**

### **4.1. Plant growth and development**

#### **4.1.1. Stalk height**

There were no significant N level x variety interactions detected with respect to stalk height (Table 4.1). However, there were highly significant differences for both main effects in terms of stalk height (Table 4.1, Figure 4.1 & 4.2). Averaging across all varieties, the stalk height increased as the age of the crop advanced but decreased with increasing N supply (Figure 4.1). It has been shown in literature that increasing N supply promotes stalk height of sugarcane (Aktar & Silva, 1999; Akhtar et al., 2000; Zhao et al., 2014). However, in this study taller stalks were recorded in plants grown with NN and LN supply whereas shorter stalks were recorded when plants were grown at MN and HN supply. Average stalk heights recorded at harvest (180 DAT) were 115.6 cm, 110.1 cm, 95.9 cm and 99.0 cm for plants receiving NN, LN, MN and HN supply, respectively (Table 4.1). The stalk heights obtained in this study were shorter < 130 cm as compared ± 300 cm often reported for sugarcane grown under field conditions. This result could be attributed to the fact that the study varieties were harvested at 180 DAT.

The response to N supply found in this study is very unusual for the sugarcane crop. The result corroborates the findings of Allison & Pammerer, (2002) who observed higher stalk heights of two contrasting sugarcane varieties at low N supply compared to high N supply at 173 DAT. The finding can be ascribed to reduced stalk dry biomass content of plants in response to increasing N supply as has been reported by Muchow et al. (1996).

The test varieties also differed markedly in their response to increasing N level (Table 4.1; Figure 4.2). Averaging across all N levels, maximum stalk height recorded at harvest was 126.8 cm followed by 113.5 cm, 110.5 cm and 110.2 cm for N41, N32, N31 and NCo376, respectively. Lowest stalk height measured at harvest was 87.8 cm and 89.2 cm for N37 and N12, respectively (Table 4.1). Variety, N41 followed by NCo376, N31 and N32 showed the highest stalk growth throughout the experiment relative to other varieties. In contrast, N12 and N37 had significantly shorter stalks throughout the measurement dates (Figure 4.2).

Shorter stalk height in N12 could be attributed to slow germination and establishment of a canopy in this study (Ramburan, 2014).

Table 4.1: Response of sugarcane stalk height (of a primary tiller) for eight varieties to N supply levels. Mean values in columns followed by different letters are significantly different at \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ . NS = not significant.

Treatment	Stalk height (cm)				
	60 DAT	90 DAT	120 DAT	150 DAT	180 DAT
<b>N level</b>					
No N	32.5c	56.0a	83.2a	106.9a	115.6a
Low	36.3a	55.1a	81.1a	101.2a	110.1a
Medium	34.6b	49.8b	72.2b	86.3b	95.9b
High	34.0bc	50.0b	69.9b	85.9b	99.0b
LSD <sub>(0.05)</sub>	1.6	4.2	4.8	7.5	7.4
<b>Variety</b>					
N12	28.7d	43.2e	63.2d	81.9cd	89.2d
N19	31.9c	48.4de	73.1c	92.9bc	106.4bc
N31	37.5b	59.5ab	83.0ab	100.5b	110.5b
N32	38.1ab	54.9bc	83.5ab	100.2b	113.5b
N37	33.7c	47.3de	66.2cd	79.5d	87.8d
N41	40.0a	62.1d	89.4a	115.0a	126.8a
N48	32.5c	49.9cd	71.9b	88.5cd	96.8cd
NCo376	32.6c	56.6ab	81.7b	102.1b	110.2b
LSD <sub>(0.05)</sub>	2.3	5.9	6.8	10.6	10.5
<b>2-Way ANOVA</b>					
<i>F-statistics</i>					
N level	6.9***	4.8**	14.7***	15.6***	12.3***
Variety	20.8***	9.5***	14.3***	9.6***	12.5***
N level x variety	0.8NS	0.8NS	0.8NS	0.6NS	0.6NS

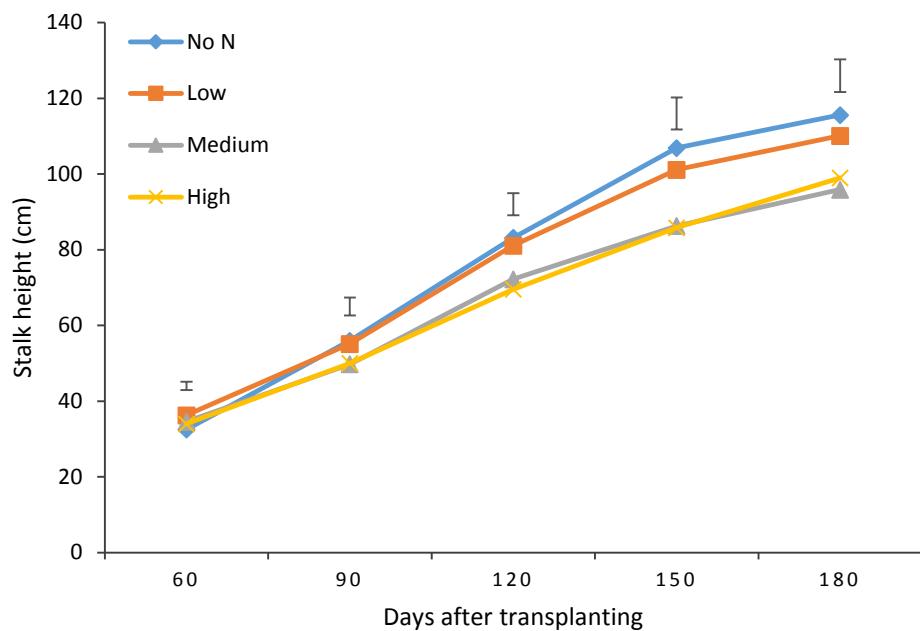


Figure 4.1: Responses of sugarcane stalk height during growth as affected by N supply level. Data points are means of all test varieties and vertical bars represent the values of LSD at  $p \leq 0.05$  level.

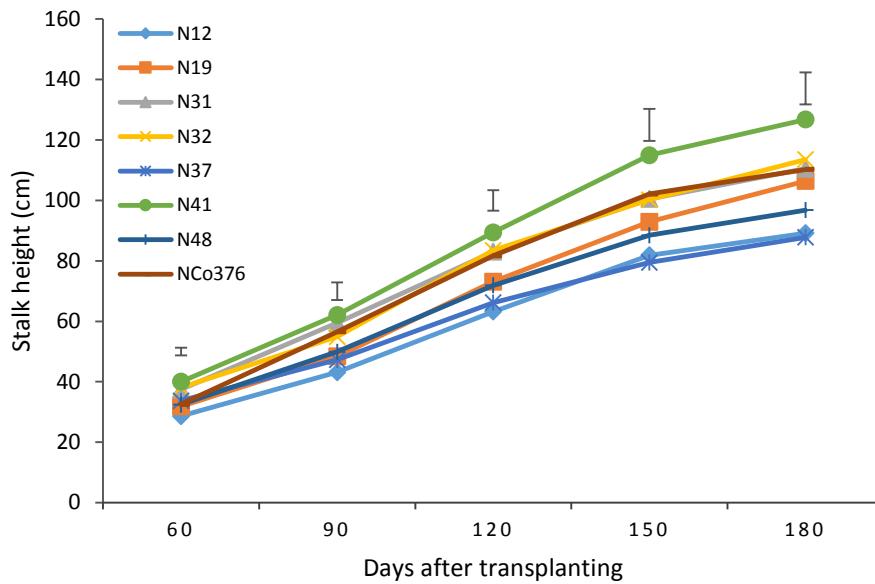


Figure 4.2: Responses of sugarcane stalk height during growth as affected by variety. Data points are means of N levels and vertical bars represent the values of LSD at  $p \leq 0.05$  level.

#### 4.1.2. Tiller count

There were no significant N rate x variety interactions detected with respect to stalk counts (Table 4.2; Figure 4.3 & 4.4). However, significant differences were detected for the main effects. Except at 120 DAT, where all plants reached their peak tillering stage, the stalk counts of the LN, MN and HN treated plants were statistically similar across all measurement dates (Table 4.2; Figure 4.3). At 120 DAT, sugarcane plants produced the highest number of stalks in pots that were supplied with MN followed by HN and LN (Table 4.2). The NN treated plants, however, produced the lowest number of stalks relative to other N-treated plants throughout the measurement dates (Figure 4.3). All plants reached their peak tillering stage at 120 DAT. It was previously demonstrated that sugarcane plants reach the peak tiller number, irrespective of variety or the crop start date, at the same thermal time (Singles et al., 2005).

Regardless of N level, a decline in number of stalk counts was observed starting from 120 DAT until harvest when the plants attained a full canopy cover. The observed behavioural characteristic of sugarcane plants is well documented in literature. Akhtar & Silver (1999) and Akhtar et al. (2000) also observed a reduction in number of stalk counts following a peak growth period due to competition for resources and mortality of newly developed tillers. It was also found that the highest tiller mortality was among the tillers formed at 45 days after planting (DAP) and those formed after 120 DAP (Vasantha et al., 2012). With certainty that competition for moisture and nutrients was eliminated by the nature of this pot trial, and therefore the reduction in stalk counts from the peak tillering stage can possibly be attributed to competition for light (Singles & Smit, 2009).

During the peak tillering stage, perhaps throughout the experiment, N12 produced the highest number of stalks (Figure 4.4). The highest number of stalks produced by N12 could be linked to its inherently high yielding capacity in terms of stalk population ( $> 145\ 000$  per hectare) (Ramburan, 2014). N48 recorded the lowest stalk count (5.8 per pot) at peak tillering as compared with N12 (9.4 per pot). At harvest, N12 followed by N37 and N19 produced the highest number of stalks whilst N41 followed by NC0376 and N48 produced the lowest stalk counts at harvest (Figure 4.4).

Table 4.2: Response of sugarcane stalk counts (tiller numbers) during growth as affected by four N supply levels. Mean values in columns followed by different letters are significantly different at \*\*\* $p\leq 0.001$ . NS= not significant.

Treatment	Stalk counts (no.pot <sup>-1</sup> )				
	60 DAT	90 DAT	120 DAT	150 DAT	180 DAT
<b>N level</b>					
No N	3.4b	3.5b	3.9c	3.7b	3.2b
Low	4.8a	5.9a	7.5b	7.4a	6.8a
Medium	4.8a	6.3a	8.5a	7.9a	7.3a
High	4.7a	6.0a	8.0ab	7.7a	7.0a
LSD <sub>(0.05)</sub>	0.4	0.5	0.8	0.8	0.7
<b>Variety</b>					
N12	4.5a	6.4a	9.4a	9.0a	8.6a
N19	5.0a	5.7abc	7.6b	7.2b	6.1c
N31	4.8a	5.6bc	7.0bc	6.6bc	5.6cd
N32	4.5a	5.0de	6.1c	5.7c	5.4cd
N37	4.5a	6.0ab	7.6b	7.8b	7.5b
N41	4.6a	5.0de	6.0c	5.6c	4.8d
N48	3.6b	4.7d	5.8c	5.7c	5.3cd
NCo376	3.7b	5.1cd	6.4bc	5.8c	5.0cd
LSD <sub>(0.05)</sub>	0.6	0.7	1.2	1.2	1.1
<b>2-Way ANOVA</b>					
<i>F-statistics</i>					
N level	20.4***	53.7***	48.1***	43.5***	52.2***
Variety	5.4***	5.9***	8.0***	8.3***	12.6***
N level x variety	0.9NS	1.1NS	0.9NS	1.3NS	1.1NS

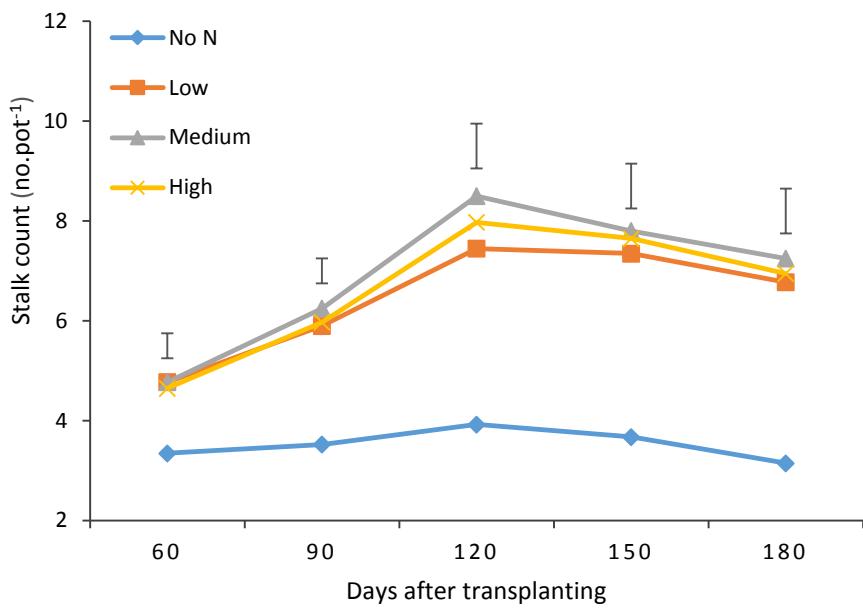


Figure 4.3: Responses of sugarcane stalk height during growth as affected by N supply level. Data points are means of all test varieties and vertical bars represent the values of LSD at  $p \leq 0.05$  level.

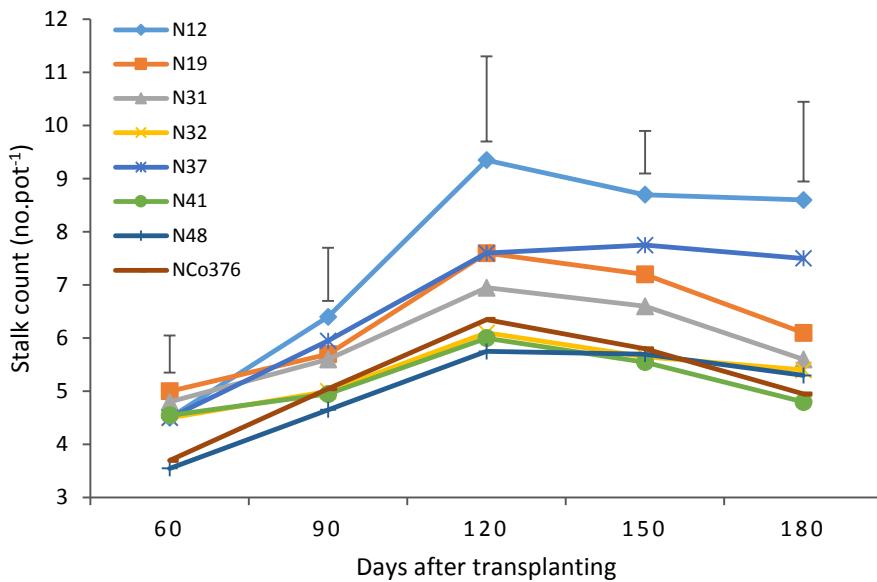


Figure 4.4: Responses of sugarcane stalk height during growth as affected by variety. Data points are global means of all N levels and vertical bars represent the values of LSD at  $p \leq 0.05$  level.

#### 4.1.3. Green leaf number

The number of green leaves determined at harvest was significantly increased with LN supply (Table 4.3). In fact, the LN treatment increased the number of green leaves by 197.7% in comparison with the NN treatment. However, further N increment resulted in 12.2% decline in green leaf number for both MN and HN supplied plants (Table 4.3). The lowest number of green leaves was recorded in NN control treatments. Since MN and HN treatments supplied adequate amounts of N, the cause of reduced number of green leaves is most likely as a result of increasing shading of older leaves by the fully developed leaf canopy. The results could also be attributed to high mortality of tillers produced after 120 DAT in plants receiving MN and HN supply.

While some studies suggest that leaf senescence is accelerated by localized N supply (Ohyama, 2010), plants supplied with LN, in this study, maintained more green leaves than did those receiving MN and HN supply. Moreover, under conditions of LN supply, plants translocate N from older to young and developing leaves. This process results in chlorophyll degradation followed by leaf senescence. However, in this experiment, it appears that the LN treated plants maintained a homeostatic leaf N concentration by maintaining a higher number of leaves per plant with reduced leaf area. The findings of this study concur with studies that have recorded increased growth rate and delayed senescence in plants supplied with optimum N (Muchow, 1988; Uhart & Andrade, 1995).

There was a significant interaction between N level x variety interaction with respect to green leaf number measured at harvest (180 DAT) (Figure 4.5). Although the number of leaves was increased by the LN treatment, the highest number of leaves was achieved by N37 when treated with HN supply. When treated with LN supply, N19 followed by N41 and N12 achieved the highest number of leaves compared to N32 and N48 that had the lowest number of leaves. Variety N12 and N19 recorded the highest green leaf numbers relative to their counterparts in response to the NN treatment (Figure 4.5).

#### 4.1.4. Green leaf area (GLA)

There were highly significant differences for the main effects of N level and variety in terms of GLA (Table 4.3). Green leaf area measured at harvest was significantly increased by increasing N supply. In comparison with the plants grown with NN, the GLA of plants supplied with LN, MN and HN increased significantly by 222.9%, 254.7% and 273.1%, respectively. Maximum GLA was recorded at HN ( $11525.1 \text{ cm}^2$  per pot) supply followed by MN ( $10956.1 \text{ cm}^2$  per pot) supply. A similar result where application of moderate (160 kg N/ha) and high (200 kg N/ha) N produced higher leaf area than the control was observed by Abayomi et al. (1987) in a single sugarcane cultivar. The result can be attributed to the fact that increased N availability enhances green leaf area in plants (Lawlor, 2002).

Plants that were supplied with LN recorded  $9974.9 \text{ cm}^2$  per pot as compared with  $3088.7 \text{ cm}^2$  per pot recorded in NN treated plants (Table 4.3). Reductions in GLA, photosynthesis, plant development and biomass production under LN and compared with NH supply have been reported in literature (Abayomi et al., 1987; Zhao et al., 2005). Significantly reduced GLA in NN treated plants observed in this experiment could be ascribed to N deficiency associated with reduction in chlorophyll content, reduced stomatal conductance and photosynthesis (Zhao et al., 2005).

The effect of N level x variety interaction was highly detected with respect to GLA (Table 4.3; Figure 4.6). Among the test varieties, N12, N48 and N37 recorded significantly greater GLA relative to their counterparts when treated with HN (Figure 4.6). With MN supply, variety N12 followed by N48 and N41 recorded the highest GLA as compared with other varieties. The LN treatment increased GLA in N12 and N19. In contrast, N41, NCo376 and recorded the lowest GLA when grown at HN, MN and LN supply, respectively (Figure 4.6).

#### 4.1.5. SPAD-based chlorophyll content

Findings from the SPAD measurements conducted at 120 and 174 DAT revealed no significant N level x variety interaction (Table 4.3). At 120 DAT, there were highly significant differences detected for the main effects of N level only, however, at 174 DAT both main effects of N level and variety were highly significant (Table 4.3). Averaging across

the varieties, the SPAD values increased with both plant age and increasing N supply, and the highest SPAD values for both measurement dates were found in plants supplied with HN followed by MN relative to LN and NN supplied plants. Among the varieties, NCo376 followed by N48 and N41 recorded the highest leaf SPAD values at 174 DAT as compared with other varieties. Significantly lower leaf SPAD values were recorded by N32 (Table 4.3).

The result obtained from this study is an indication that increased N availability improves chlorophyll in sugarcane. The result is in agreement with recent findings of Xiong et al. (2015) who demonstrated an increase in SPAD values with increasing N supply in seven monocots and dicots. Similar increase in leaf SPAD readings found in this study has been reported before in sugarcane (Zhao et al., 2014), rice (Yang et al., 2014), maize (Tajul et al., 2013), short-season cotton (Feibo et al., 1998) and winter wheat (Zhao et al., 2005).

Lower chlorophyll (SPAD-based) content of all the NN treated plants could presumably be due to N stress existence. It was also shown in cereal crops that lower rates of N supply results in reduced chlorophyll contents (Wang et al., 2014), that could explain lower SPAD values of NN treated plants (solely dependent on soil N). Findings of this study suggest that the SPAD-502 meter, which is widely used to monitor leaf N status of crops by measuring leaf chlorophyll content (Peng et al., 1996; Bausch & Diker, 2001; Fontes & de Aruju, 2006), could be used as a sensitive tool to monitor leaf N status of sugarcane.

#### 4.1.6. Root length

The effect of N level x variety interaction on root length was not significant (Table 4.3). However, the main effects of N level and variety were significant. Although the root dry biomass accumulation was suppressed by NN and LN supply (Table 4.5), the roots were significantly longer than those treated with MN and HN supply (Table 4.3). Compared with the NN treated plants, root length of plants supplied with LN increased by 8.9%, but further N increments to MN and HN resulted in significantly reduced root length by 13.4% and 18.7%, respectively.

Averaged across all N levels, N19 followed by N37, N12 and N32 recorded the highest root lengths when compared with other varieties. In contrast, NCo376, N31 and N48 recorded the lowest root lengths (Table 4.3). The results of this study with respect to root responses to N

fertiliser are similar to those reported by other researchers. For example, Beatty et al. (2010) also reported much longer roots of barley with reduced dry biomass content when plants were grown with low N compared to high N supply, thus corroborating the results found here. In maize, low N supply was found to increase axile root elongation, mature cell length and extend cell elongation zones (Gao et al., 2015).

It is well documented in literature that plant roots employ a variety of acclimation strategies, including altering root traits for better nutrient salvaging, to mitigate the limitations of low nutrient availability (Goron et al., 2015). This hypothesis was also demonstrated in sugarcane by Otto et al. (2009) who recovered approximately 50%, 34% and 15% of roots of unfertilized cane between 0 to 20 cm, 20 to 40 cm and 40 to 60 cm depths, respectively. With N fertilization, only 13% of roots were recovered between 40 to 60 cm whilst 17% and 70% were recovered in the 20 to 40 cm and 0 to 20 cm depths, respectively. This morphological root trait has been linked to increased biomass accumulation and NUE of various plant taxa.

Table 4.3: Green leaf count, green leaf area (GLA), SPAD chlorophyll and root length of sugarcane varieties subjected to four N supply levels. Mean values in columns followed by dissimilar letters are significantly different at  $*p \leq 0.05$ ,  $**p \leq 0.01$  and  $***p \leq 0.001$ . NS = not significant.

Treatment	G. leaf count	G. leaf Area	SPAD reading		Root length
	no.plant <sup>-1</sup>	cm <sup>2</sup> .plant <sup>-1</sup>	120 DAT	174 DAT	cm
<b>N level</b>					
No N	17.6c	3088.7d	28.3d	32.5c	53.6b
Low	52.4a	9974.9c	35.4c	38.4b	58.4a
Medium	46.1b	10956.1b	38.3b	40.1b	50.6bc
High	46.0b	11525.1a	42.6a	43.1a	47.5c
LSD <sub>(0.05)</sub>	4.2	385.7	2.4	2.6	3.9
<b>Variety</b>					
N12	46.9a	10490.9a	34.6a	38.4bc	54.1ab
N19	40.8bc	9086.6bc	37.5a	38.9bc	57.4a
N31	39.5bc	8702.4bcd	36.1a	36.1cd	49.7b
N32	35.0d	8386.0c	35.3a	34.4e	52.6ab
N37	46.1a	8627.3bcd	35.6a	36.3cd	55.2ab
N41	38.5bcd	8338.9c	38.2a	41.0ab	51.4ab
N48	37.8cd	9480.1b	36.7a	41.3ab	50.3b
NCo376	39.6bc	7977.5d	36.2a	42.0a	49.5b
LSD <sub>(0.05)</sub>	5.9	545.4	NS	1.8	5.5
<b>2 Way ANOVA</b>					
<b>F-statistics</b>					
N level	96.5***	808.4***	48.1***	41.1***	11.2***
Variety	3.3**	16.7***	0.9NS	7.9***	2.1*
N level x variety	2.5***	5.3***	0.7NS	1.0NS	1.3NS

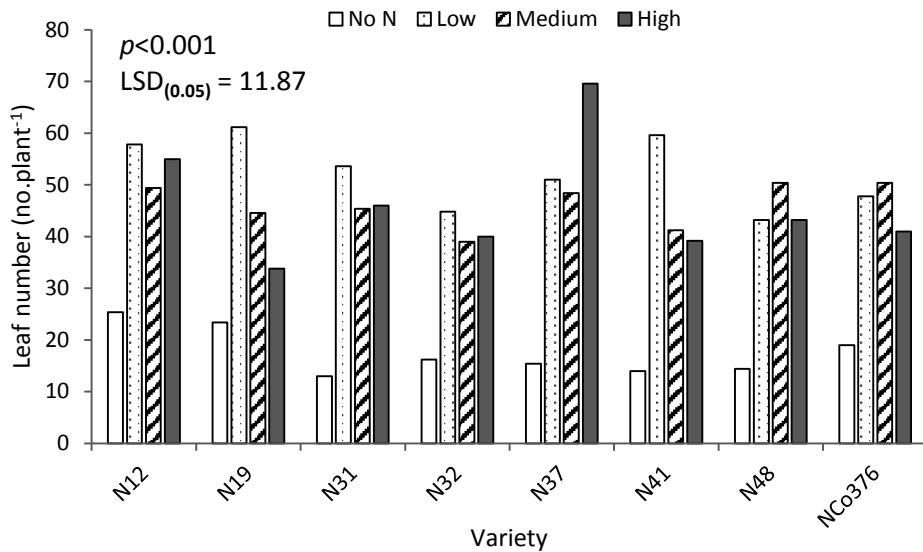


Figure 4.5: The effects of N level x variety interaction on leaf counts of eight sugarcane varieties subjected to four N supply levels.

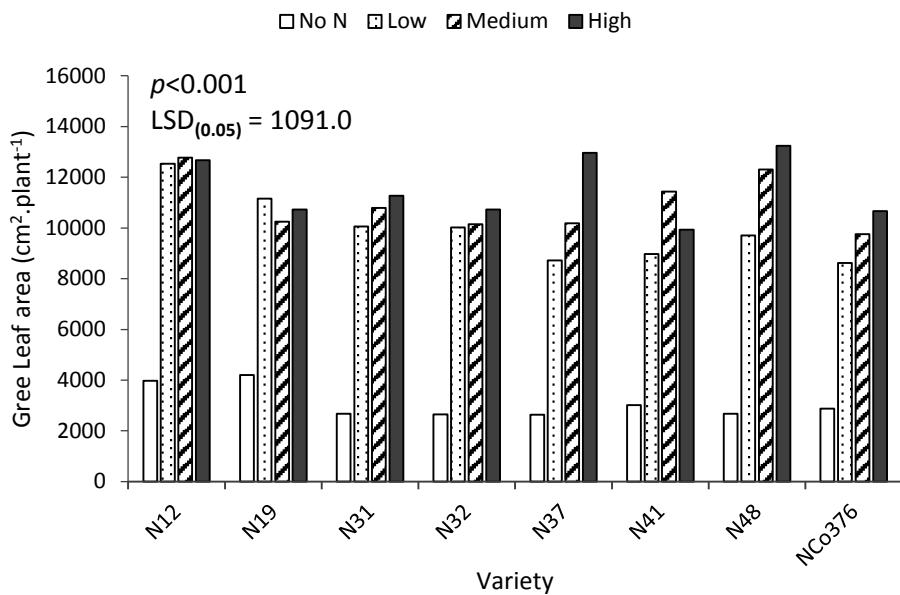


Figure 4.6: The effects of N level x variety interaction on leaf area of eight sugarcane varieties subjected to four N supply levels.

## 4.2. Leaf nutrient analysis

### 4.2.1. Nitrogen

Nitrogen concentration of the top visible dewlap (TVD) leaf (Schroeder et al., 1999), a sensitive indicator of N status in sugarcane, varied among the eight test sugarcane varieties at 174 DAT (Figure 4.7). With the exception of NCo376, N19 and N41 which showed no further increment at HN supply, the N concentrations in the TVD leaf of all varieties increased with increasing N supply. At NN and LN application, the leaf N concentration of all varieties was well below the minimum threshold value of 1.7% (Miles & Rhodes, 2013).

As NN treated plants depended solely on soil available N, it was certainly expected that leaf N concentration will fall below the threshold value. With the LN treated plants which produced higher dry biomass, it could be possible that N concentration below the threshold value was probably due to the effect of N dilution (Jarrell & Beverly, 1981). However, at MN and HN supply, all varieties accumulated the optimum leaf N concentrations within the range of 1.8 to 2.2% (Figure 4.7). Among the varieties, NCo376 and N48 accumulated the highest N concentrations across all N levels compared with N37 that accumulated the lowest leaf N concentrations at low and medium N supply.

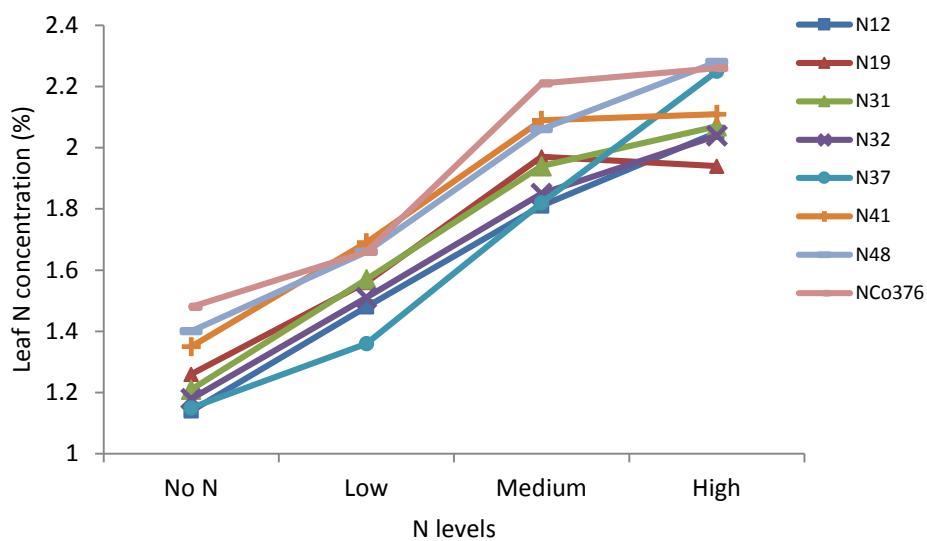


Figure 4.7: Differences in leaf N concentration at 174 DAT of eight sugarcane varieties in response to increasing N supply.

#### 4.2.2. Phosphorous

The P concentration was positively influenced by increasing N supply, but the effect was apparent only at MN and HN levels (Figure 4.8). The leaf P concentration of the NN and LN treated plants were similar, suggesting that P uptake in sugarcane is enhanced by elevated levels of N supply. When grown with MN supply, N32 followed by N41 and N31 registered the highest leaf P concentrations relative to their counterparts. At HN supply, N31 and N32 accumulated the highest leaf P concentration in comparison with NC0376 that accumulated the lowest leaf P concentrations. The leaf P concentrations across the N supply levels were generally above the threshold value of 0.19% for the seven test varieties and 0.16% for N12 (Miles & Rhodes, 2013). The results thus suggest that the amount of P (superphosphate) used in this study was sufficient as it appears that P deficiency was not a problem within the duration of the trial.

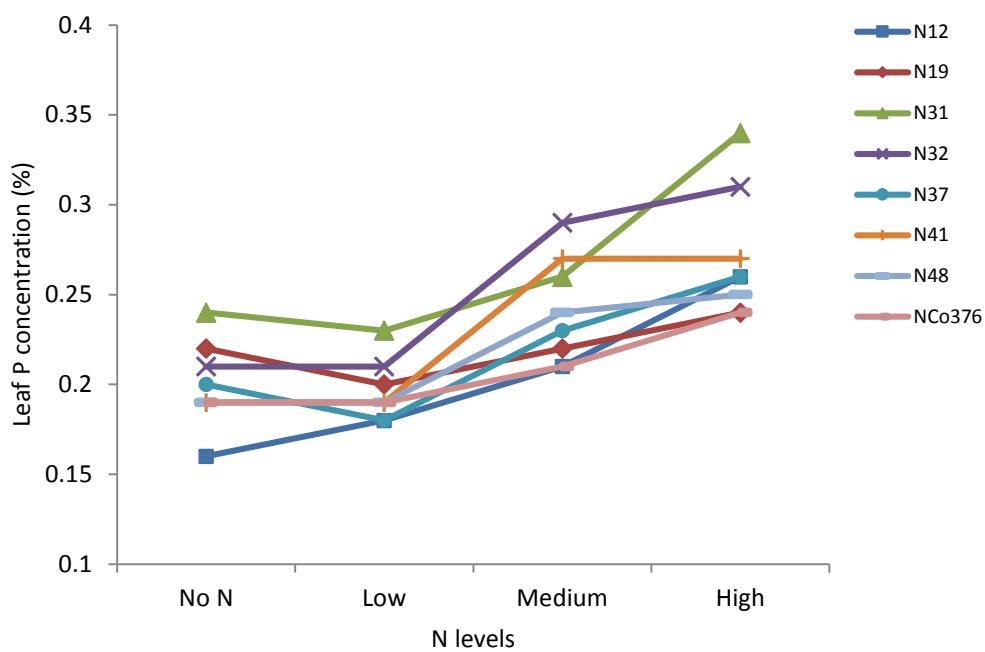


Figure 4.8: Differences in leaf P concentration at 174 DAT of eight sugarcane varieties in response to increasing N supply.

#### 4.2.3. Potassium

With respect to the trend in potassium uptake, the response to increasing N on K concentration was variable between the varieties. Varieties N41 and NC0376 showed no further increment in K concentration between MN and HN supply (Figure 4.9). The variety that responded most vigorously to K concentration in response to increasing N supply, was N32. The leaf K concentration was above the threshold value of 1.05%, even in the NN treatment (Miles & Rhodes, 2013). Similar to P concentrations, the leaf K concentrations across the N supply levels were generally above the threshold value of 1.05% for all the test varieties (Miles & Rhodes, 2013). The results indicate that the constant level of K supply to all N treatments provided adequate K nutrition.

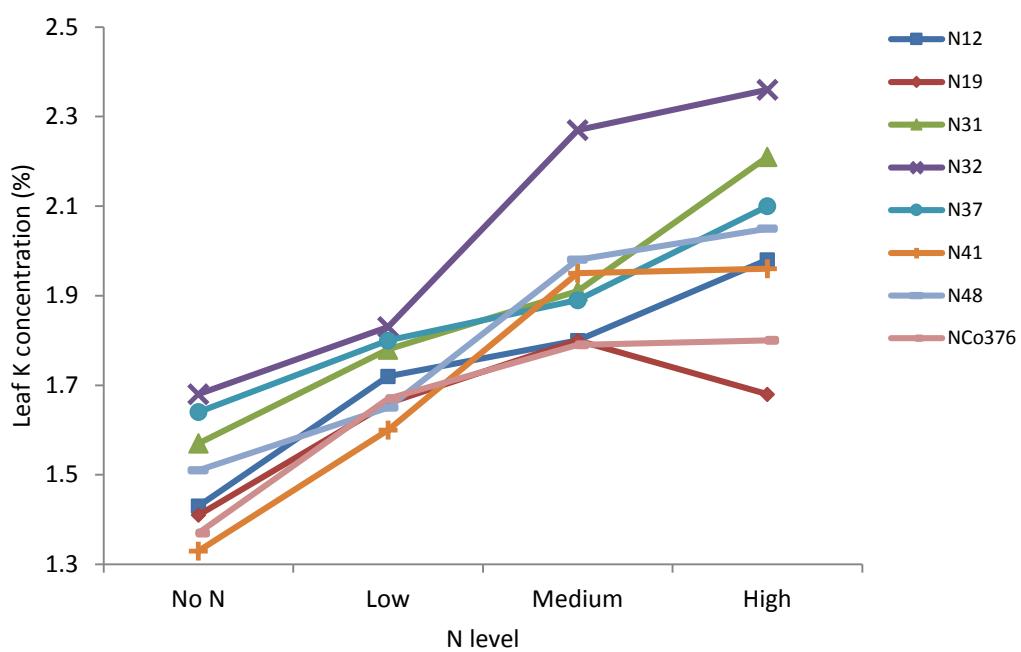


Figure 4.9: Differences in leaf K concentration at 174 DAT of eight sugarcane varieties in response to increasing N supply.

#### 4.3. Fresh and dry biomass production at final harvest

##### 4.3.1. Fresh biomass yield

Fresh biomass yield of 180 day old sugarcane varieties is shown in Table 4.1. In exception of root fresh biomass, statistical analysis showed no significant N level x variety interaction with respect to shoot and whole-plant fresh biomass yield (Table 4.4). However, the main effects of N level and variety were highly significant in terms of shoot and whole-plant fresh biomass yield. Shoot and whole-plant fresh biomass yield responded to the first N increment (from NN to LN) and then showed no further responses to increasing N supply. Compared with plants supplied with NN, average shoot fresh biomass of varieties increased by 119.5%, 92.8% and 85.8% for LN, MN and HN supply, respectively (Table 4.4). Whole-plant fresh biomass yield of respective LN, MN and HN treated plants increased by 126.1%, 114.3% and 105.8% when compared with the NN treated control counterparts.

The root fresh biomass yield increased with increasing N supply up to MN but declined by 4.8% at HN supply (Table 4.4). The interactive effect of N level x variety for root fresh biomass yield is illustrated in Figure 4.10A. Among the varieties, N37 and N32 ranked the highest in terms of root fresh biomass production when treated with HN and HN supply, respectively (Figure 4.10A). However, when treated with LN supply, N19 followed by N12 were superior over other varieties in terms of fresh root biomass production (Figure 4.10A). In contrast, NCo376 recorded the lowest root fresh biomass production at LN, MN and HN supply relative to other varieties. Variety N37 together with N41 recorded the lowest root fresh biomass production when treated with NN (Figure 4.10A).

##### 4.3.2. Dry biomass yield

Significant differences in the effects of N level, variety and N level x variety interaction for shoot and whole-plant dry biomass yield were found (Table 4.4; Figure 4.10B, C & D). Shoot dry biomass yield of NN treated plants increased by 133.6% following LN supply, however, further N supply resulted in 13.5% and 17.9% decline in shoot dry biomass yield for MN and HN treated plants (Table 4.4; Figure 4.10B). In comparison with the NN treated plants, whole-plant dry biomass of the LN treated plants was increased by 127.8% but further N increment to respective MN and HN treatments resulted in a decline of 8.7% and 13.4% compared to LN treatments (Table 4.4). Among the varieties, N41 produced the highest shoot

dry biomass across all N levels (Table 4.4; Figure 4.10B). This variety was followed by N48, N31 and N32 when grown with LN, MN and HN supply, respectively. In contrast, N37 ranked the least shoot biomass producer when treated with LN and the same can be said for NCo376 when treated with MN and HN supply (Figure 4.10B).

Similarly N41 also ranked the highest in terms of whole-plant dry biomass yield across all N levels (Table 4.4; Figure 4.10D). Variety, N37, NCo376 and N19 ranked the lowest biomass producers under LN, MN and HN supply, respectively (Figure 4.10D). Although a linear positive responsive behaviour of dry biomass productivity as a function of N rate has been demonstrated in sugarcane (Wiedenfeld & Enciso, 2008; Azzay et al., 2008; Zhao et al., 2014), in this study however, the dry biomass production of N supplied plants plateaued with the LN supply. The result suggests that the level of N supplied to the LN treated plants was the maximum N concentration required to attain maximum biomass yields.

Lack of biomass response to further N increment reported here could also be largely associated with reduced stalk dry biomass content of plants at MN and HN supply (Table 4.5), as was previously reported by Muchow et al. (1996). Improved biomass production at LN supply was mainly due to greater allocation of dry biomass content to stalk tissue which somewhat overcompensated for reduced leaf dry biomass production. The finding of this study is in agreement with previous studies that have demonstrated lack of response of plant cane to increasing N supply (Inman-Bamber, 1984; Muchow et al., 1996; Wiedenfeld, 1997; Muchovej & Newman, 2004; Robinson et al., 2007; Franco et al., 2010). Two studies conducted in South Africa have attributed the lack of N response to the study being conducted on a virgin soil (Inman-Bamber, 1984; Weigel et al., 2010) whilst some studies have attributed the low or lack plant cane response to N supply to biological nitrogen fixation through endophytic associations (Boddey et al., 1991; 2003; Urquiaga et al., 1992).

The effects of N level x variety interaction with respect to root dry biomass production was significant (Table 4.4; Figure 4.10C). The root dry biomass yield increased with increasing N supply up to MN and declined with further N increment (Table 4.4). Variety N37 accumulated the highest root dry biomass yield when grown at MN and HN supply compared with other varieties (Figure 4.10C). At LN supply, N41 accumulated the highest root dry biomass relative to its counterparts. Variety N48 accumulated the lowest root dry biomass when grown

NN and LN supply whilst N19 and NCo376 produced the lowest root dry biomass when grown with MN and HN supply, respectively (Figure 4.10C).

Table 4.4: Variation in fresh (FM) and dry biomass (DM) yields and root: shoot ratio of commercial sugarcane varieties subjected to four N supply regimes at final harvest (180 DAT). Mean values in columns followed by dissimilar letters are significantly different at \* $p\leq 0.05$ , \*\* $p\leq 0.01$  and \*\*\* $p\leq 0.001$ . NS = not significant.

Treatment	Fresh biomass (g.plant <sup>-1</sup> )			Dry biomass (g.plant <sup>-1</sup> )		
	Shoot	Root	Total FM	Shoot	Root	Total DM
<b>N level</b>						
No N	677.8c	164.3c	842.1c	174.3d	56.5b	230.8c
Low	1487.8a	416.3b	1904.0a	407.2a	118.6a	525.8b
Medium	1307.1b	497.6a	1804.7ab	352.4b	127.6a	479.9a
High	1259.7b	473.9a	1733.6b	334.4c	121.1a	455.5b
LSD <sub>(0.05)</sub>	87.1	51.4	107.0	16.8	14.5	22.9
<b>Variety</b>						
N12	1167.5bc	351.7bc	1519.2cd	314.6bc	92.8c	407.5bc
N19	1163.9bc	377.2abc	1541.1bcd	299.1c	93.5c	392.6c
N31	1238.9ab	414.3ab	1653.2abc	326.9b	117.4ab	444.2ab
N32	1232.8ab	452.8a	1672.2ab	332.9b	109.6ab	442.5ab
N37	1038.7c	449.1a	1487.8cd	297.6c	133.5a	431.1b
N41	1361.7a	378.9abc	1740.6a	359.3a	115.5ab	474.8a
N48	1126.3bc	366.9abc	1493.2cd	317.0bc	92.8c	409.8bc
NCo376	1134.9bc	326.9c	1461.8d	289.2c	92.5c	381.6c
LSD <sub>(0.05)</sub>	123.2	72.7	151.4	23.7	20.5	32.4
<b>2-Way ANOVA</b>						
<b>F-statistics</b>						
N level	127.0***	69.4***	164.9***	277.4***	41.1***	256.8***
Variety	4.8***	2.7*	3.6***	7.2***	4.8***	7.1***
N level x variety	1.0NS	2.2**	1.8NS	2.1**	2.0*	2.8***

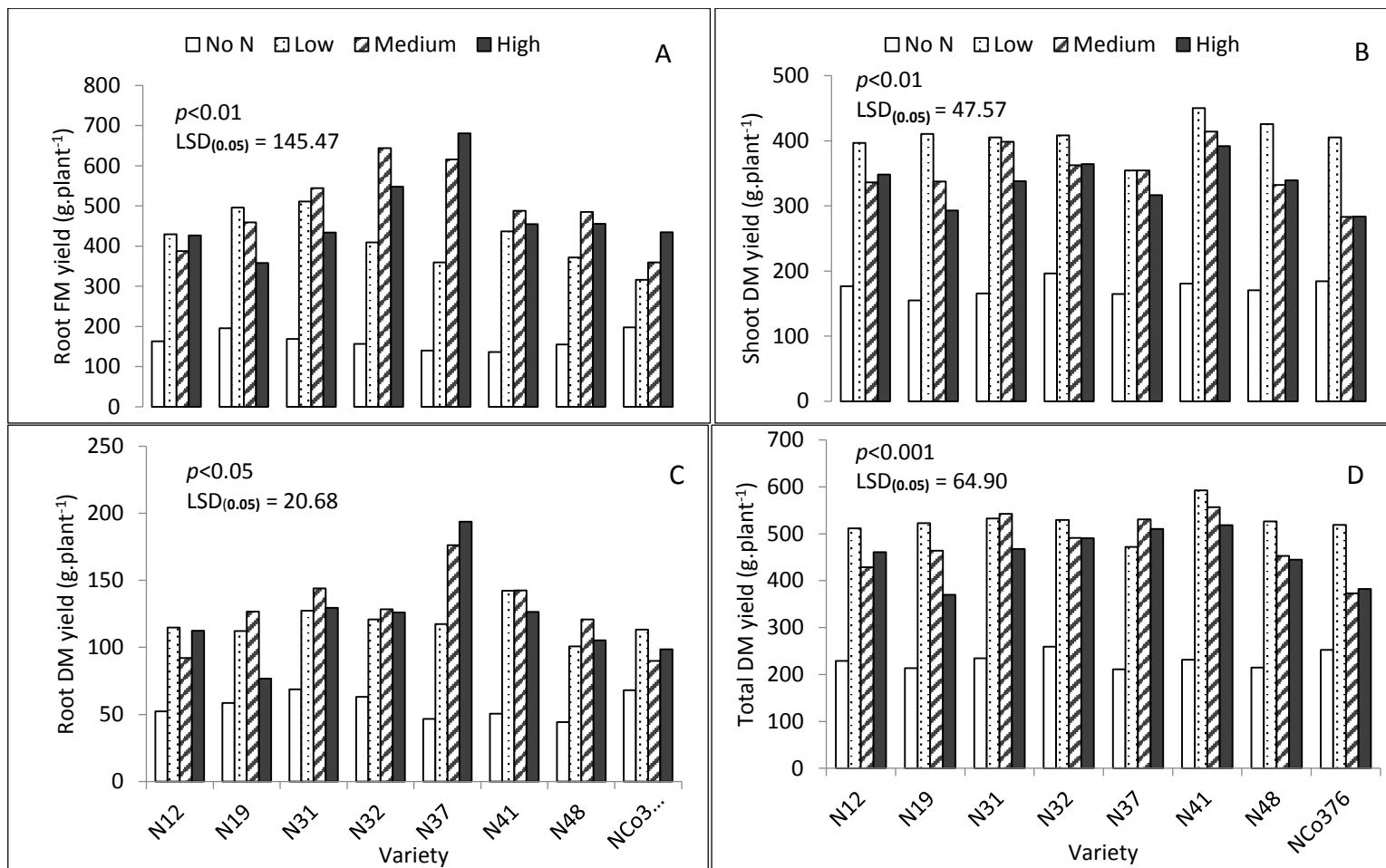


Figure 4.10. Effect of N x variety interaction on (A) fresh root biomass (FM) yield, (B) dry shoot, (C) dry root and (D) total dry biomass (DM) of 180 day old sugarcane varieties grown with no, low, medium and high N supply. Data are means of five replications.

#### 4.3.3. Biomass accumulation in shoot fractions (g)

The main effects of N level and variety with respect to brown residue biomass, stalk, tops and green leaves were all significant (Table 4.5). Brown residue biomass increased by 60.9% with addition of LN, but declined by 2.1% and 9.4% with further N increment to MN and HN supply, respectively. Among the varieties, N32 and N31 produced greater brown biomass residue as compared with other varieties. Variety, NCo376 produced the least brown residue biomass.

Significant N level x variety interactions were detected in terms of stalk and tops fractions (Table 4.5; Figure 4.11; Figure 4.12). Compared with the NN treated plants, biomass accumulated in stalk of LN treated plants increased by 120.4% but further additions of N resulted in 30.5% and 38.4% decline of stalk biomass of respective MN and HN treated plants. When grown at all N supply levels, variety N41 accumulated significantly higher stalk biomass relative to other varieties (Table 4.5; Figure 4.11). The variety (N41) was followed by N48 and NCo376 at LN supply whilst at MN and HN supply it was followed by N31 and N32 in terms of stalk biomass production. N37 accumulated the least stalk biomass when grown at LN and HN supply and the same can be said for NCo376 and N48 when grown with MN supply.

Tops biomass production of the NN treated plants was increased by 157.8% when plants were treated with LN supply (Figure 4.12). The same fraction was decreased by 5.5% and 8.8% when plants were grown at MN and HN supply, respectively. Among the varieties, N12, NCo376 and N37 produced the highest tops biomass at LN supply whilst at MN and HN supply N37 and N48 ranked the highest tops biomass producers. Variety N31 and N32 produced the least tops biomass at LN and MN supply whilst N19 accumulated the least tops biomass with HN supply (Figure 4.12)

Green biomass production was linearly related to increasing N supply (Table 4.5). In comparison with plants treated with NN, green leaf production was increased by 2.6-, 2.8- and 2.9-fold for the LN, MN and HN treated plants. Averaging across the N levels, N12 followed by N48 and N37 produced the highest green leaf biomass as compared with other varieties. Variety, NCo376 produced the lowest green leaf biomass (Table 4.5).

#### 4.3.4. Percentage (%) biomass allocation to shoot fractions

With the exception of green leaves, there were no significant N level x variety interactions detected with respect to dry biomass partitioning to brown leaves, tops and stalks (Table 4.5). The main effects of N level and variety were highly significant for tops and stalks fractions (Table 4.5). Although there were no effects of N levels on brown residue biomass production, there were varietal differences. Among the varieties, N19 followed by N32, N31 and N37 generally produced greater brown residue biomass across all N levels as compared with N41, N48 and NCo376 that produced the lowest brown leaf biomass (Figure 4.13A – D).

Supplying NN and LN to plants resulted in 48.0% and 45.1% dry biomass allocation to stalk whilst increasing N supply to MN and HN resulted in 35.9% and 33.1% biomass allocation to stalk (Table 4.5). Among the varieties, N41 followed by N32, NCo376 and N31 allocated more biomass to stalk in comparison with other varieties. Lower biomass allocated to stalk can be ascribed to reduced stalk dry biomass content with increasing N supply, as was demonstrated by Muchow et al. (1996). Applications of LN, MN and HN to plants increased dry biomass allocation to tops by 11.9%, 22.6% and 23.8% as compared with the NN treated plants (Table 4.5). Averaging across all N levels, N37 followed by N12, N48 and NCo376 allocated higher biomass to tops as compared with N31 which allocated the least biomass to tops.

The interaction between N level and varieties was highly significant with respect to biomass allocation to green leaves (Table 4.5; Figure 4.14). Biomass allocation to green leaves increased with increasing N supply and was 18.9%, 20.9%, 26.4% and 28.9% for the NN, LN, MN and HN, respectively. The functional aspect of this N response may be linked higher N availability which improved green leaf area. When treated with HN supply, variety N12 and N37 allocated greater biomass to green leaves as compared with other varieties (Figure 4.14). In contrast, N41 consistently allocated lower biomass to leaves when treated with NN, LN, MN and HN supply (Figure 4.14).

Table 4.5: Percentage (%) biomass allocation patterns to brown leaf, stalk, tops and green leaf of sugarcane varieties subjected to four N supply regimes. Mean values in columns followed by dissimilar letters are significantly different at  $*p \leq 0.05$ ,  $**p \leq 0.01$  and  $***p \leq 0.001$ . NS= not significant.

Treatment	Shoot	B. leaf		Stalk		Tops		G. leaf	
	g DW	g DW	%	g DW	%	g DW	%	g DW	%
<b>N level</b>									
No N	174.3d	27.9b	16.3a	84.3d	48.0a	29.4c	16.8b	32.8c	18.9d
Low	407.2a	60.9a	15.1a	185.8a	45.1a	75.8a	18.8a	84.7b	20.9c
Medium	352.4b	59.6a	17.0a	129.2b	35.9b	71.6ab	20.6a	92.0a	26.4b
High	334.4c	55.2a	17.3a	114.5c	33.1b	69.1b	20.8a	95.5a	28.9a
LSD <sub>(0.05)</sub>	16.8	5.6	NS	14.5	3.2	6.1	1.9	5.7	1.5
<b>Variety</b>									
N12	314.6bc	46.6cd	15.0bc	111.5cd	36.0cd	68.7a	21.6a	87.8a	27.4d
N19	299.1c	53.4bc	19.0a	117.8bcd	39.1bc	54.3c	17.9bc	73.5cd	24.0bc
N31	326.9b	58.6ab	18.2ab	140.2b	42.9b	55.0c	16.9c	73.0cd	22.0c
N32	332.9b	63.3a	18.7a	136.4b	41.1b	58.4bc	18.0bc	74.8bcd	22.2c
N37	297.6c	53.1bc	18.2ab	96.6d	33.4d	68.2a	22.3a	79.6abc	26.2ab
N41	359.3a	49.3cd	13.8c	178.0a	50.0a	62.5abc	17.1bc	69.7d	19.2c
N48	317.3bc	42.8de	14.6c	125.3bc	39.8bc	65.7ab	20.2ab	83.2ab	25.4ab
NCo376	289.2c	40.1e	14.1c	121.6bc	41.9b	59.1abc	20.1ab	68.4d	24.0bc
LSD <sub>(0.05)</sub>	23.7	7.9	3.2	20.5	4.5	8.6	2.8	8.1	2.1
<b>2-Way ANOVA</b>									
<b>F-statistics</b>									
N level	243.1***	52.2***	1.2NS	60.8***	36.2***	94.0***	6.9***	186.8***	76.5***
Variety	6.3***	6.6***	3.5***	9.9***	8.7***	3.2**	4.2***	4.9***	12.3***
N level x variety	1.8*	1.4NS	0.9NS	2.0**	1.2NS	1.7*	1.4NS	1.4NS	2.3***

(%) Fraction = (each plant organ dry weight  $\div$  shoot dry weight)  $\times 100$

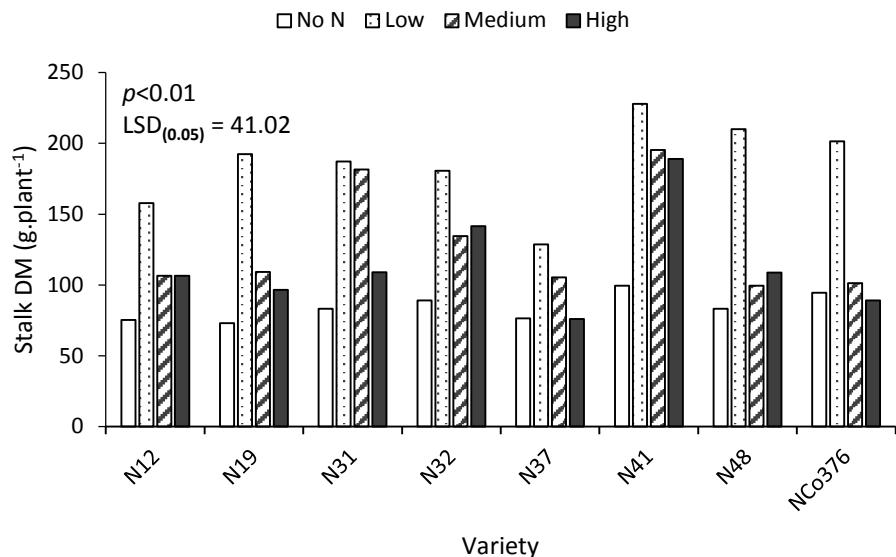


Figure 4.11. The interactive effects of N level x variety on stalk dry biomass allocation of eight sugarcane varieties subjected to four N supply levels.

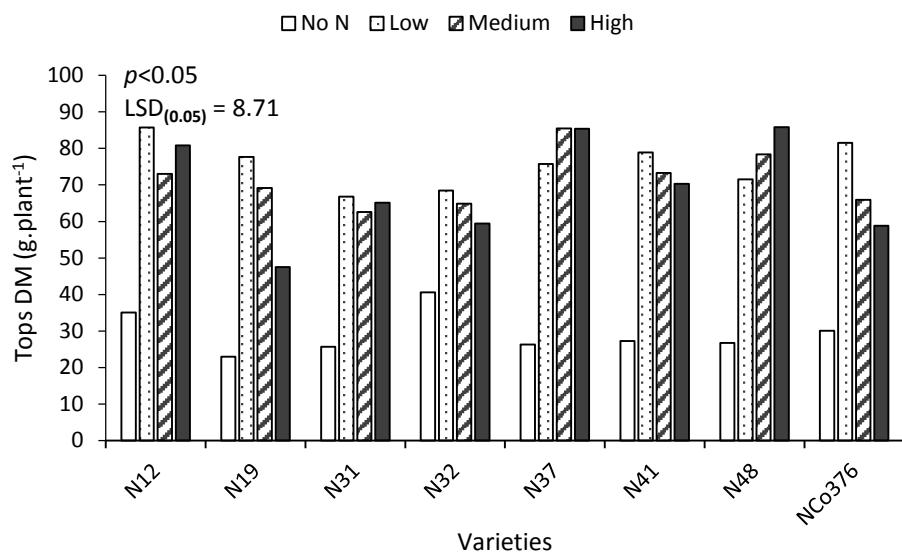


Figure 4.12. The interactive effects of N level x variety on tops dry biomass allocation of eight sugarcane varieties subjected to four N supply levels.

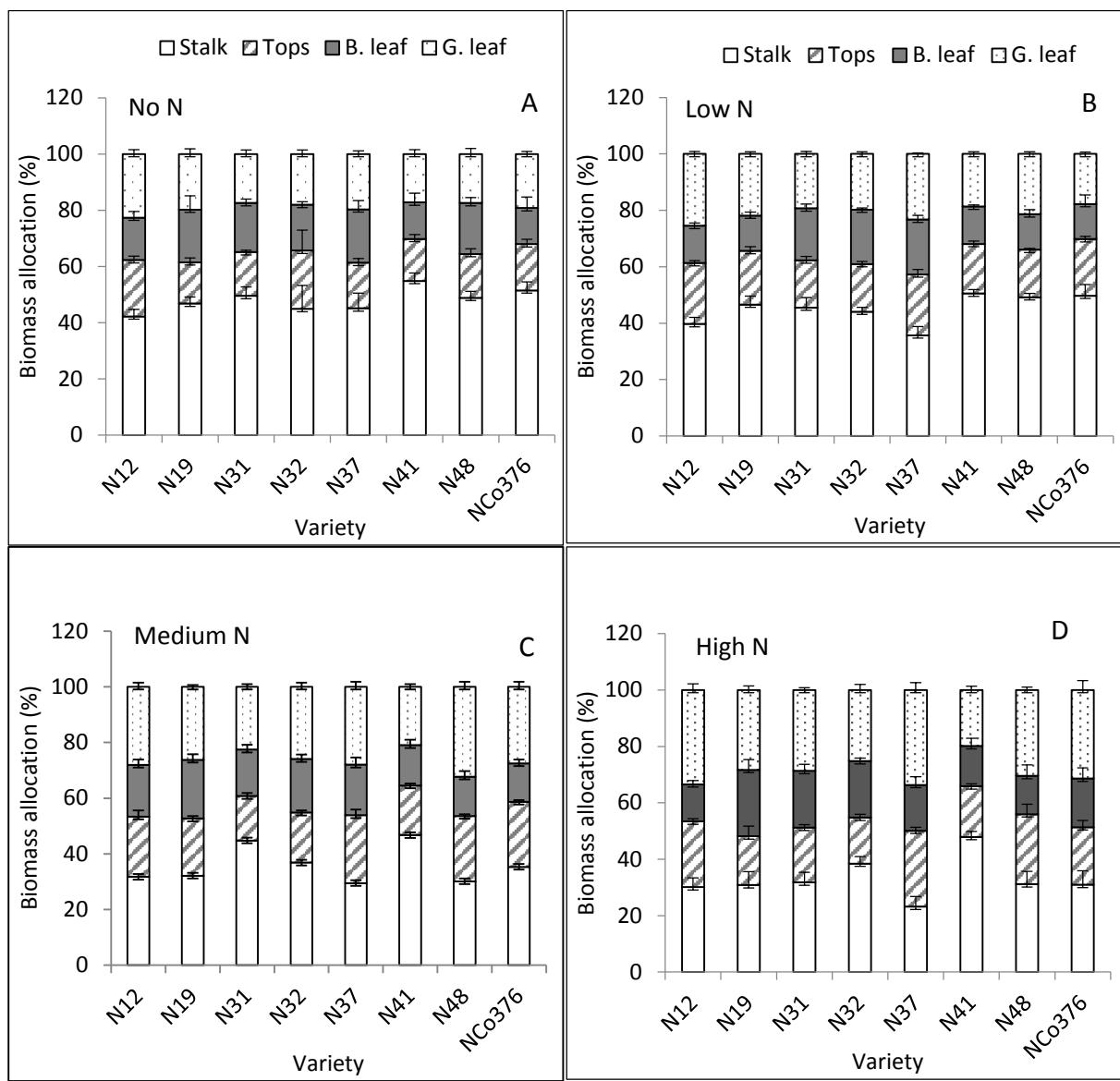


Figure 4.13. Proportion of biomass allocated to B. leaf, stalk, tops and G. leaf of 180 day old sugarcane varieties grown with (A) no, (B) low, (C) medium and (D) high N supply. Values are representing means of five replicates with bars indicating standard error.

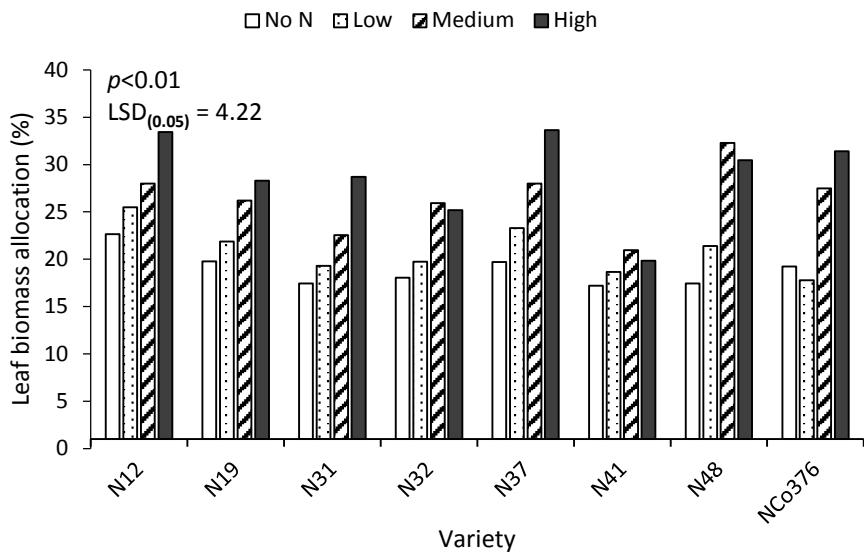


Figure 4.14. The interactive effects of N level x variety on leaf biomass allocation of eight sugarcane varieties subjected to four N supply levels.

#### 4.4. Nitrogen concentration, N content, NUpE, NUtE and NUE

##### 4.4.1. N concentration in shoot

There were significant N level, variety and N level x variety interactions with respect to total shoot N concentrations (Table 4.6; Figure 4.15). In comparison with the NN supplied plants, total shoot N concentration of the respective LN, MN and HN supplied plants increased by 45.2%, 151.7% and 219.4% (Table 4.6). Within varieties, N37 followed by N31 and NC0376 recorded the highest shoot N concentration at HN supply relative to N41 that ranked the lowest in terms of shoot N accumulation (Figure 4.15). At MN supply, N19, N37, N48 and NC0376 accumulated the highest shoot concentration whilst the shoot N was accumulated in highest concentrations at LN supply by N19 and N37. Variety N12 and N31 were the lowest shoot N accumulators under LN supply as compared to their counterparts (Figure 4.15).

The low shoot N concentration in the NN treated plants was largely due to N deficiency as plants depended solely on soil available N. There are reports of many crops showing a sharp decrease in shoot N concentration following an increase in biomass (Greenwood, 1990; Justes et al., 1994). As this was found to be the case for the LN treated in this study, the decline in N concentration was associated with plant growth as part of a dilution phenomenon of plant nitrogen by carbon assimilates (Greenwood et al., 1991). The results support the notion that, when potted plants are irrigated with a solution containing a fixed amount of nutrients, the available N-to-biomass ratio decreases with duration of the experiment (Farage et al., 1998).

The study showed that increasing N supply had significant effect on shoot N concentration but a corresponding increase in dry biomass production was not found (Table 4.6). Wood et al. (1996) proposed that higher N accumulation of N in sugarcane ratoon crop was related to biomass production. In that study, maximum N accumulation reported for plant cane was unrelated to biomass production (Wood et al., 1996). The responsive nature of sugarcane to increasing N supply found in this study with respect to shoot N concentration is also similar to that reported for plant cane by Muchow et al. (1996) and the responsive behaviour was associated with luxury N consumption. This implies that growing sugarcane plants under MN and HN supply resulted in the continued uptake of N beyond the level required to achieving maximum growth (Greenwood et al., 1990; Lamine & Gastal, 1997).

Table 4.6. Shoot nitrogen concentration and content, NUpE (N content/ N supply), NUtE (Dry biomass yield/ N content) and NUE (NUpE x NUtE) of commercial sugarcane varieties subjected to four N supply regimes. Asterisks in columns indicate significance level \* $p\leq 0.05$ , \*\* $p\leq 0.01$  and \*\*\* $p\leq 0.001$ , NS = not significant.

Treatment	Shoot N concentration mg.g <sup>-1</sup>	Shoot N content g.shoot <sup>-1</sup>	NUpE	NUtE g DW g <sup>-1</sup> N	NUE
<b>N level</b>					
No N	20.0a	4.6d	-	-	-
Low	29.2b	13.2c	12.2a	34.6b	210.4a
Medium	50.6c	24.1b	6.1b	20.1c	60.8b
High	64.3d	33.5a	3.6c	16.0d	28.8c
LSD <sub>(0.05)</sub>	2.5	0.9	0.2	1.0	4.9
<b>Variety</b>					
N12	40.7bc	18.0a	7.0a	24.1abc	195.3bc
N19	42.0b	18.4a	7.4a	22.7bcd	197.1bc
N31	39.9bc	19.6a	7.2a	24.6ab	204.8a
N32	39.1bc	18.7a	7.2a	24.6ab	203.2a
N37	46.1a	21.5a	7.6a	20.9d	181.1c
N41	37.1c	19.2a	7.7a	25.6a	225.2a
N48	40.4bc	17.9a	7.5a	23.5bc	204.3a
NCo376	42.7ab	17.7a	7.1a	22.3cd	188.5bc
LSD <sub>(0.05)</sub>	3.5	NS	NS	1.7	8.1
<b>2-WAY ANOVA</b>					
<i>F-statistics</i>					
N level	518.5***	522.7***	851.4***	696.8***	2991.6***
Variety	4.6***	2.0NS	0.9NS	6.1***	5.1***
N level x variety	2.4**	2.5***	2.4**	1.7NS	2.5**

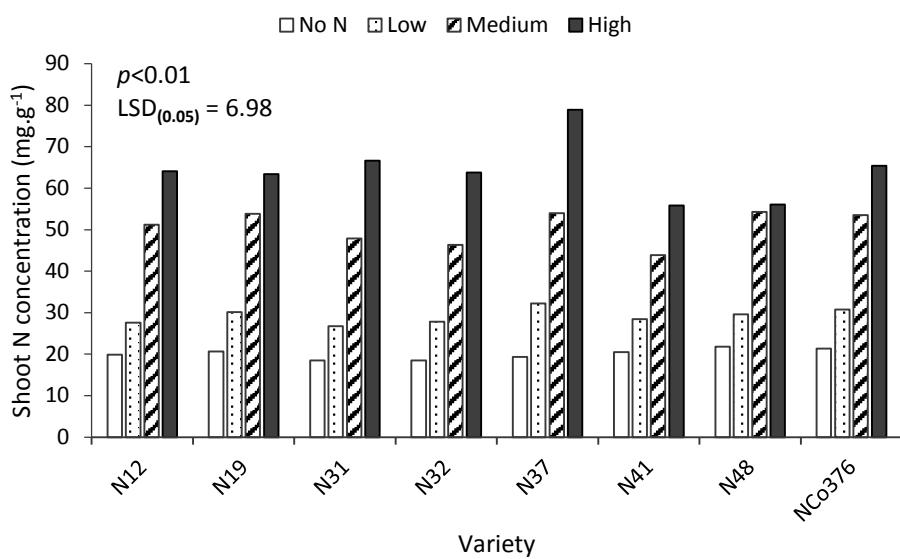


Figure 4.15. The effects of N level x variety interaction on total shoot N concentrations of sugarcane varieties subjected to four N supply levels.

#### 4.4.2. Shoot N content (N uptake)

Although there were no differences in shoot N content among the varieties, significant differences among the N treatments were detected (Table 4.6). Total shoot N content of plants increased with increasing N supply. Application of LN, MN and HN to plants resulted in 4.0-, 5.0- and 6.0-fold increase in shoot N content, respectively, as compared to those treated with NN supply (Table 4.6). The low shoot N content in NN treated plants could be due to N deficiency as they depended solely on soil N. Reduced shoot N contents in plants treated with LN could be N dilution associated with increasing dry biomass production (Greenwood et al., 1991). The shoot N contents are comparable to those reported for seven ratooning South African sugarcane varieties subjected to low (30 mg N/pot), medium (60 mg N/pot) and high (90 mg N/pot) N supply (Schuman et al., 1998).

The effects of N level x variety interactions with respect to total shoot N content were significant (Figure 4.16). Among the varieties, N37 followed by N32, N31, N12 accumulated the highest shoot N content when treated with HN supply as compared to other varieties (Figure 4.16). When supplied with MN, variety N37 followed N31 accumulated the highest shoot N content relative to other varieties. Variety N41 ranked the highest in terms of shoot N content when treated with LN relative to other varieties (Figure 4.16).

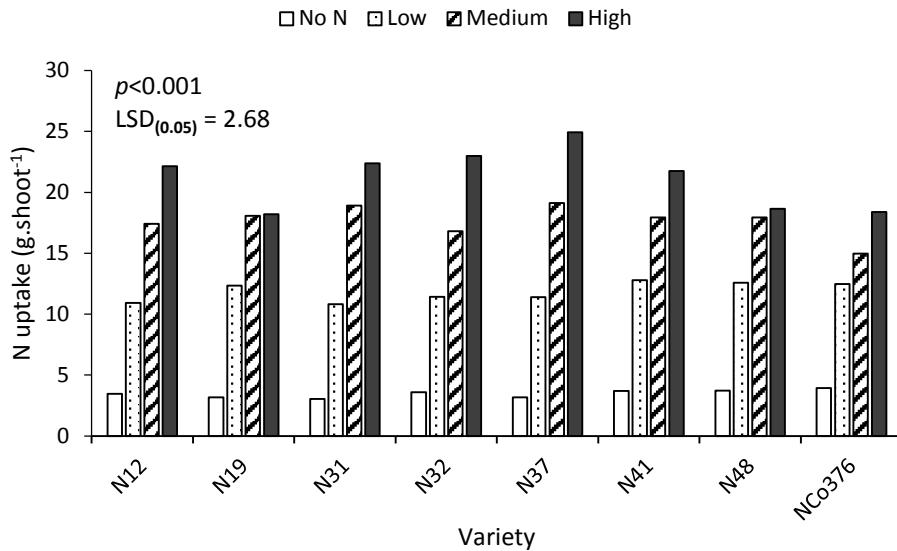


Figure 4.16. The effects of N level x variety interaction on total shoot N uptake of sugarcane varieties subjected to four N supply levels.

#### 4.4.3. N uptake efficiency (NUpE)

Data for NUpE (N content/N supply) of test plants is shown in Table 4.6 and Figure 4.17. Although there were no varietal differences with respect to NUpE, the main effect of N level and N level x variety interaction was significant (Table 4.6). Averaging across varieties, the NUpE decreased significantly with increasing N supply. The NUpE of the LN treated plants was 2-fold and 3-fold greater than the MN and HN treated plants, respectively (Table 4.6). The NUpE obtained in this were lower as compared to those reported for seven South African sugarcane lines supplied with 0.4 mM N (Snyman et al., 2015). The current results are in agreement with the findings of Moll et al. (1982) and Sinebo et al. (2004) that also reported higher NUpE with low N application but declined significantly with increasing N level.

Amongst the varieties, N41 followed by N48, NCo376 and N19 were superior over their counterparts with respect to NUpE when supplied with LN (Figure 4.17). When treated with MN, N37 and N31 ranked the highest in terms of NUpE. Variety, N37 ranked the highest in NUpE relative to other varieties when treated with HN level (Figure 4.17). Previous studies with maize and *Arabidopsis* have reported that under high N supply genetic variation in NUE is explained by genotypic variation in NUpE (Moll et al., 1982; Bertin & Gallais, 2001;

Coque et al., 2008). However, the genetic variation in NUpE obtained in this study was relatively lower in all N supplied plants.

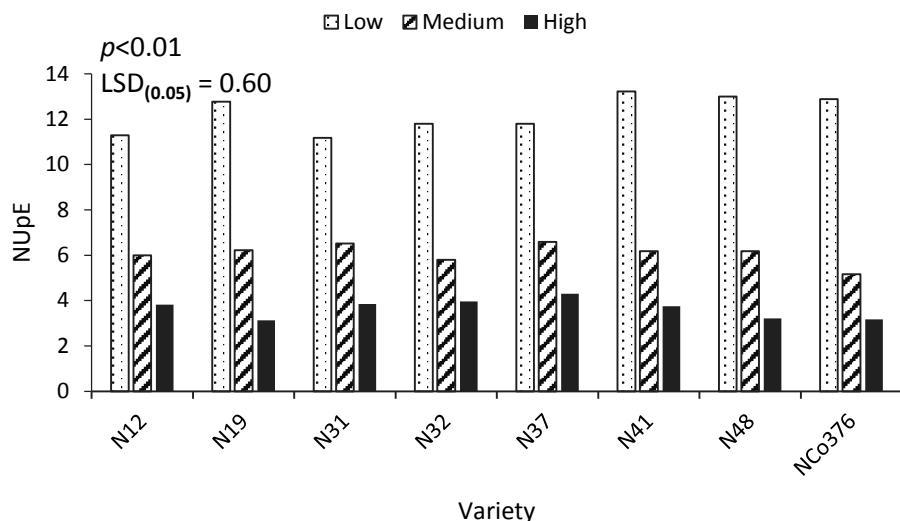


Figure 4.17. The effects of N level x variety interaction on NUpE of eight sugarcane varieties subjected to four N supply levels.

#### 4.4.4. Nitrogen utilisation efficiency (NUtE)

The effects of N level x variety interactions in terms of NUtE ( $\text{g DW. g}^{-1}$  tissue N) were not significant (Table 4.6). However, the main effects of N level and variety were highly significant with respect to internal N-use efficiency (NUtE) (Table 4.6). Average dry biomass yield obtained in this study was highest ( $525.8 \text{ g.plant}^{-1}$ ) at LN treated plants as compared with 479.9 and  $455.5 \text{ g.plant}^{-1}$  obtained for the MN and HN treated plants, respectively. This indicates that NUtE is always higher when plants are grown with LN treatments and decreases with increasing N input (i.e. from MN to HN supply) (Table 4.6). The decrease in NUtE with increasing N supply obtained in this study has been reported before in sugarcane (Schuman et al., 1998; Robinson et al., 2007; 2008; Weigel et al., 2010; Snyman et al., 2015) and various plant taxa (Bertin & Gallais, 2000; Coque et al., 2008; Chardon et al., 2010). In comparison

with plants treated with LN, the NUE of MN and HN treated plants declined by 41.9% and 53.8%, respectively (Table 4.6).

Some studies have demonstrated considerable genetic variability among varieties for NUE for a given level of N supply (Bertin & Gallais, 2000; Coque et al., 2008; Chardon et al., 2010). Lower genetic variance among varieties is often observed under stressed conditions (Gallais & Coque, 2005). Robinson et al. (2007) demonstrated some considerable variation in NUE of sugarcane using a diverse sugarcane plant material. More recently, Hajari et al. (2014) reported higher genetic variation in NUE of sugarcane varieties under lower N input as compared with higher N input. In this study however, the magnitude of genetic variation amongst varieties for NUE was relatively lower ranging from 31.2 to 37.5, 18.6 to 23.4 and 13.1 to 18.1 g DW g<sup>-1</sup> tissue N for the respective LN, MN and HN treated plants (Table 4.6).

Robinson et al. (2007) reported mean NUE values of 223 and 82 g DW g<sup>-1</sup> N of 3-months old Australian sugarcane genotypes grown at low (0.4 mM) and high (10 mM) N supply, respectively. Similarly, NUE mean values of 148 g DW g<sup>-1</sup> N of 4-months old South African sugarcane lines were also reported at low (0.4 mM) N supply (Snyman et al., 2015). Lower NUE obtained in this study could be due to higher shoot N contents (3 to 29 g/plant) as compared to 0.17 to 0.32 mg/plant reported by Robinson et al. (2007) and Snyman et al. (2015).

Preliminary findings of NUE field trials conducted in South Africa screening similar varieties used in this study have ranked N41 and N48 as high N efficient and NCo376 as low N efficient based on the ratio of biomass to N content. Similarly, variety N41 ranked the highest in terms of NUE followed by N31, N48 and N32 in comparison with their counterparts. In contrast, N37 and NCo376 ranked the lowest in terms of NUE relative to other varieties (Table 4.6). The findings obtained in this study in part corroborate the results reported in the above mentioned NUE screening field trials. This finding highlights in part the successes of the newly developed method for screening sugarcane varieties for NUE. Further improvement of this method could be helpful for future experiments targeting NUE of sugarcane.

#### 4.4.5. Nitrogen use efficiency

There were significant N level, variety and N level x variety interaction with respect to the overall NUE computed as the product of NUpE and NUtE (Table 4.6; Figure 4.18). In comparison with the LN treated plants, the average NUE of MN and HN treated plants declined by 3.5- and 7.3-fold, respectively. The result could be attributed to common knowledge that N-use efficiency decreases when the level of fertilization increases (Gallais & Coque, 2005). Since biomass production of all test varieties did not respond positively to increasing N supply, it was expected that the NUE will decrease with increasing N supply. However, the negative effect of reduced biomass on NUE was relatively small as compared with the dominant effect of luxury N uptake observed with MN and HN supply.

Although NUE is rarely computed as the product of NUpE and NUtE as was the case in this study, the trends of NUE found in this study are consistent with recent reports of Robinson et al. (2007), Zhao et al. (2014) and Hajari et al. (2014) who reported decreased NUE of sugarcane with increasing N rate in a pot trial.

When treated with LN, N41 followed by N48 ranked the highest in terms of NUE as compared with other varieties (Figure 4.18). Variety N37 ranked the lowest in terms of NUE (Figure 4.18). The variation in NUE could be attributed to morphological characteristics displayed by varieties which contributed significantly to high biomass production. For example, N41 had the tallest stalks and recorded the highest stalk dry biomass production whilst N37 had shorter stalks and accumulated the lowest stalk dry biomass content. The result of this study suggest that in attempt to improve NUE of sugarcane varieties should consider stalk height and stalk dry matter production as good parameters for the NUE trait. The two currently deemed high N-efficient varieties based on the ratio of sucrose to N accumulated in biomass, viz. N12 and N19, ranked fourth and sixth in terms of NUtE, respectively in this study.

The genetic variation with respect to the overall NUE was greater with LN application but declined with further N increment and ranged from 366 to 465, 96 to 143 and 49 to 67 for the respective LN, MN and HN treated plants. The finding of this study is in conformity with the notion that larger genetic variance in NUE is observed under N stressed conditions (Gallais & Hirel, 2004; Gallais & Coque, 2005).

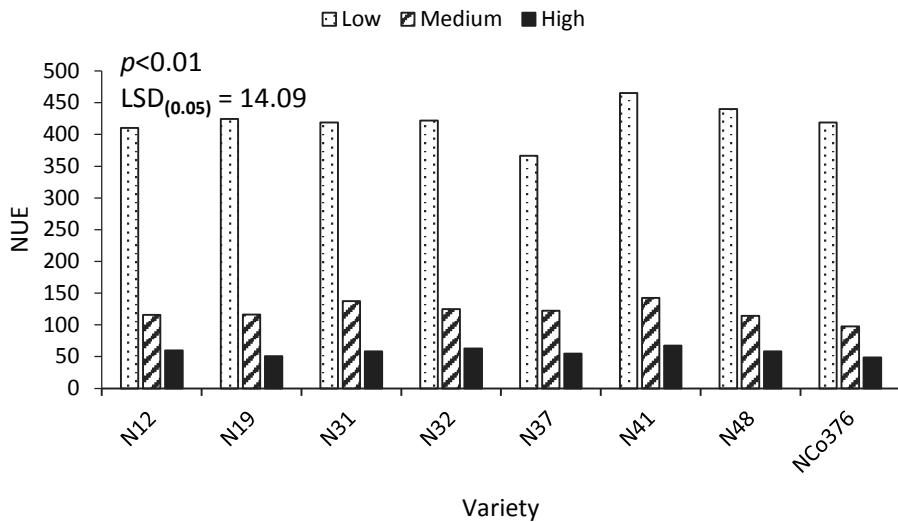


Figure 4.18. The effects of N level x variety interaction on the overall N-use efficiency of sugarcane varieties subjected to four N supply levels.

#### 4.4.6. Nitrogen (mg/g) concentration in shoot fractions

Although there were no significant effects of variety and N level x variety interactions with respect to brown biomass residue and green leaves, the main effects of N level were significant (Table 4.7). The N concentration in both brown biomass residue and green leaves increased linearly with increasing N supply. Supplying LN, MN and HN to plants increased N concentration in brown biomass residue of plants by 34.4%, 93.7% and 128.1%, respectively. Similarly, additions of N to respective LN, MN and HN treatment resulted in 33.3%, 63.2% and 91.1% of N concentration in green leaves (Table 4.7).

Nitrogen accumulation in stalk fraction increased linearly with increasing N supply (Table 4.7). Comparing with the NN treated plants, increasing N supply to LN, MN and HN increased stalk N concentration of plants by 2.0-, 6.5- and 8.6-fold, respectively. A significant N level x variety interaction was detected with respect to N accumulation in the stalk tissue (Figure 4.19). Across all the N levels, N37 accumulated significantly higher stalk N concentration in comparison with other varieties. At HN supply N41 was followed by N31, NC0376 and N12 whereas at MN supply the same variety accumulated stalk N concentrations comparable to that of N19 (Figure 4.19).

Nitrogen concentration in tops was significantly enhanced by increasing N application. In comparison with the NN treated plants, N application increased tops N concentration by 34%, 102% and 170% for plants treated with LN, MN and HN, respectively (Table 4.7). Of the eight varieties, N37 accumulated the highest N concentration in tops compared with other varieties when grown with HN supply (Figure 4.20). When supplied with MN, N37 together with N12 and N48 accumulated the highest N concentration in tops as contrasted to other varieties. Variety, N41 accumulated the lowest N concentration in tops as compared to other varieties when grown with MN and HN supply. Nitrogen concentration in tops was similar among varieties when treated with NN and LN supply (Figure 4.20).

#### 4.4.5. Nitrogen (%) allocation to shoot fractions

Variation in N allocation to shoot fractions was investigated in this study as this trait could be significant in improving NUE of sugarcane. Although there were no significant effects of variety and N level x variety interaction with respect to N allocation to brown biomass residue, tops and green leaves, the main effect of N supply was significant (Table 7; Figure 4.21A – D). Significant differences for both main effect of N level and variety were only detected with respect to N allocation to the stalk tissue. Nitrogen allocation to brown leaves was 16.1%, 14.7%, 12.2% and 11.4% the respective NN, LN, MN and HN treated plants. Reduced N allocation to brown residue biomass at LN, MN and HN supply could be partly due to increased N availability which influenced green leaf area and leaf duration. On the contrary, rapid leaf senescence associated with N deficiency at NN supply could have resulted in poor remobilization of N to developing organs.

The NN and LN treated plants allocated lower 15.3% and 22.1% N, respectively to the stalk component as compared to 39.7% and 41.1% allocated for MN and HN treated plants, respectively (Table 4.7; Figure 4.21A – D). Among the varieties, N37 allocated the highest N to the tissue relative to other varieties. Similar findings where stalk N allocation ranging from 16 to 29% and 38 to 54% (under low and adequate N supply, respectively), were also reported by Robinson et al. (2009). The result suggests that under conditions of high N supply sugarcane inefficiently distribute greater proportion of excess N to the stalk rather than leaves (Stevenson et al., 1992) and the reverse is true for plants grown under low N supply. The

greater apportioning of N to stalk than leaves could be attributed to reduced N demand for metabolic use (Kingston, 2014).

Nitrogen allocated to tops fraction of NN supplied plants decreased by 6.8%, 20% and 15% in comparison with plants grown at LN, MN and HN respectively (Table 4.7). Application of NN and LN to plants resulted in more than 40% of N allocation to green leaves. Increasing N rate to plants from LN to respective MN and HN decreased N the amount of N allocated to green leaves by 29.7% and 34% (Table 4.7; Figure 4.21A – D). Stevenson et al. (1992) also showed that application of additional N to sugarcane during the cropping season failed to prevent a reduction in leaf N content. The findings of this study suggest that greater N allocation to leaves of sugarcane may be linked to dry biomass productivity but not NUE under low N input conditions. Therefore N allocation patterns cannot be considered a good parameter for improving NUE of sugarcane.

Table 4.7. Nitrogen concentration (mg/g) and percentage (%) N allocation patterns to brown leaf, stalk, tops and green leaf of sugarcane varieties subjected to four N supply regimes. Mean values in columns followed by dissimilar letters are significantly different at  $*p \leq 0.05$ ,  $**p \leq 0.01$  and  $***p \leq 0.001$ . NS = not significant.

Treatment	Brown leaf		Stalk		Tops		Green leaf	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%
<b>N level</b>								
No N	3.2d	16.1a	3.1d	15.3c	5.0d	24.9a	8.7d	43.7a
Low	4.3c	14.7a	6.5c	22.1b	6.7c	23.2b	11.6c	40.0b
Medium	6.2b	12.2b	20.2b	39.7a	10.1b	19.9c	14.2b	28.1c
High	7.3a	11.4b	26.8a	41.1a	13.5a	21.1c	16.7a	26.4d
LSD <sub>(0.05)</sub>	0.4	1.5	1.8	2.4	0.4	1.7	0.4	1.7
<b>Variety</b>								
N12	5.0a	13.8a	14.2b	30.0b	9.0ab	22.5a	12.5a	33.7a
N19	5.1a	14.0a	14.3b	29.0b	9.2ab	22.8a	13.1a	34.2a
N31	5.0a	12.6a	13.3b	27.6b	8.9ab	23.3a	12.8a	36.5a
N32	5.1a	13.6a	13.0b	28.4b	8.8abc	23.2a	12.2a	34.7a
N37	4.8a	11.7a	18.0a	33.7a	10.1a	22.1a	13.2a	32.5a
N41	5.1a	14.6a	12.4b	28.7b	7.5c	21.4a	12.1a	35.3a
N48	5.1a	13.2a	13.1b	28.4b	9.1ab	23.2a	13.2a	35.2a
NCo376	6.3a	15.2a	15.0b	30.6ab	8.1bc	19.9	13.4a	34.3a
LSD <sub>(0.05)</sub>	NS	NS	2.6	3.3	0.6	NS	NS	NS
<b>2-Way ANOVA</b>								
<b>F-statistics</b>								
N level	43.8***	15.7***	293.9***	238.7***	146.6***	13.0***	187.8***	193.6***
Variety	1.5NS	2.0NS	3.7***	2.6**	3.1**	1.8NS	1.9NS	1.8NS
N level x variety	0.8NS	1.2NS	1.8*	1.2NS	1.8*	0.9NS	1.6NS	0.8NS

% Fraction = (each fraction N concentration ÷ total shoot N concentration) x 100

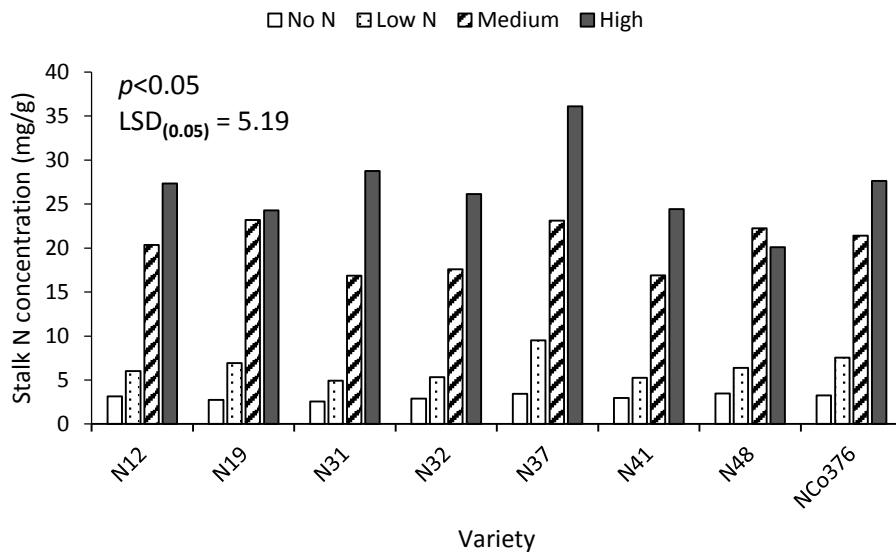


Figure 4.19. The interactive effects of N level x variety on stalk N concentration of eight sugarcane varieties subjected to four N supply levels.

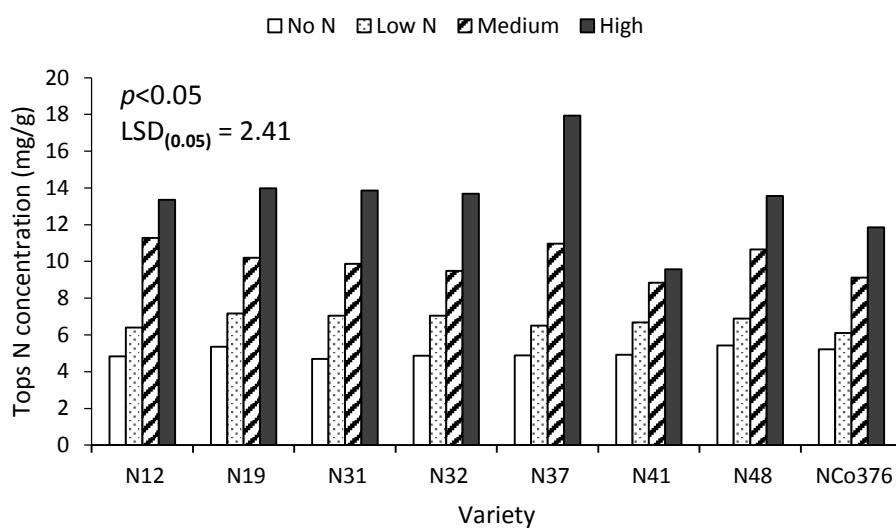


Figure 4.20. The interactive effects of N level x variety on tops N concentration of eight sugarcane varieties subjected to four N supply levels.

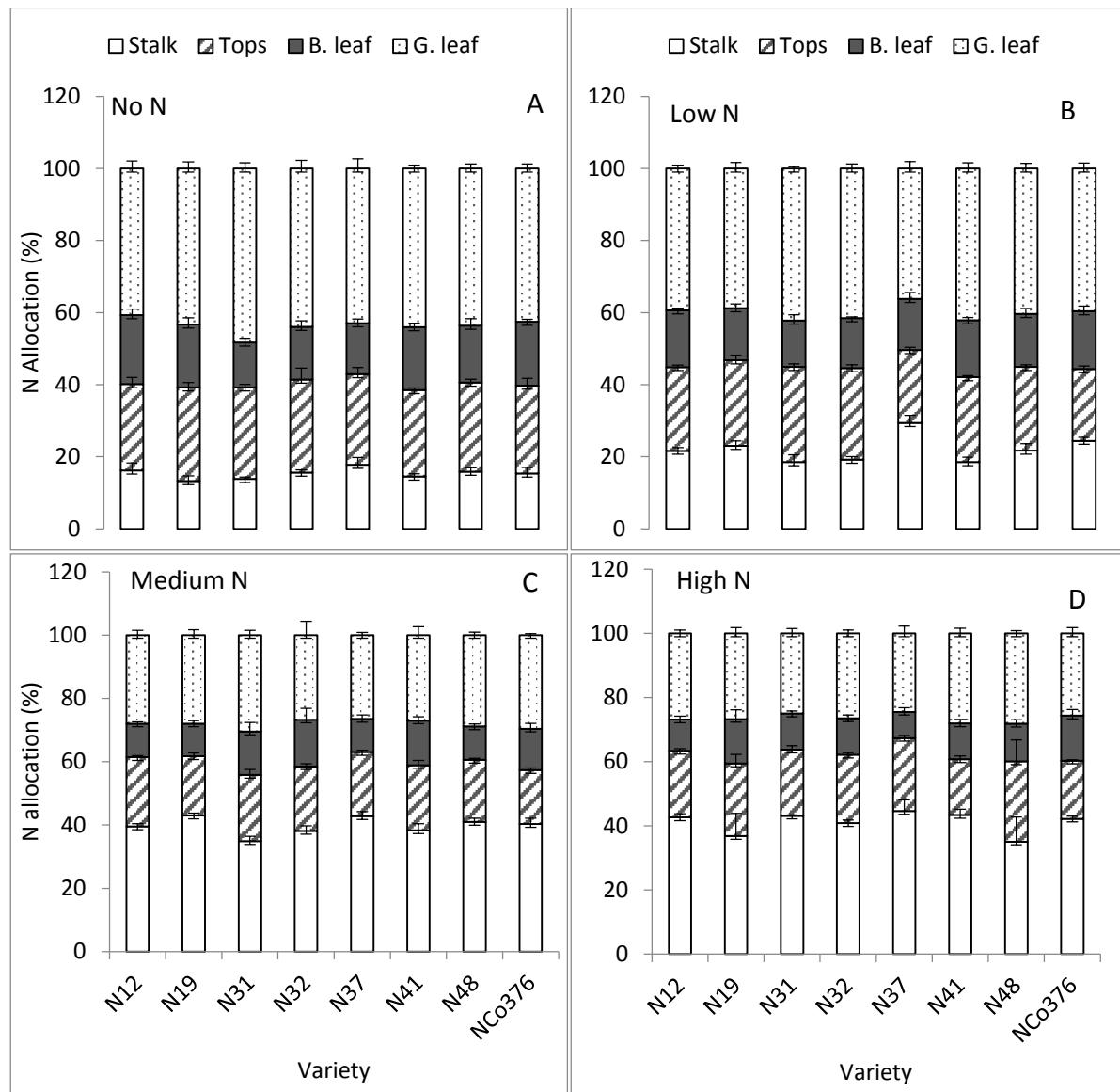


Figure 4.21. Proportion of N concentration allocated to B. leaf, stalk, tops and G. leaf of 180 day old sugarcane varieties grown with (A) no, (B) low, (C) medium and (D) high N supply. Values are representing means of five replicates with bars indicating standard error.

#### 4.5. The effect of N on Brix content (%)

In comparison with the NN treated plants, the brix content of LN supplied plants increased by 2.5%. However, further N increment resulted in 4.3% and 18.0% decline in Brix content of MN and HN treated plants, respectively, when compared with the LN treated plants. The finding of this study substantiates the results from earlier studies showing that sugar content declines at excess N availability levels. For example, Schumann et al. (1998) reported lower sucrose concentration in potted ratoon sugarcane crop when grown at higher N supply levels. These authors have ascribed this decrease in sucrose concentration to lower sucrose purity at higher N application rates.

Wiedenfeld (1995) and Wiedenfeld & Enciso (2008) observed a significant decline in sugar content in response to increasing N application rate. Muchow et al. (1996) also reported that significantly higher sucrose concentration in fresh millable stalks was enhanced by low N fertility. The authors attributed this decrease in sucrose concentration to decrease in stalk dry matter content. The observed decrease in brix content in stalks following increased N fertilization can be ascribed to excessive storage of N, as amino acids (asparagine), in stalks as was reported by Keating et al. (1999).

Significant N level x variety interactions were detected with respect to brix content in stalk. Among the varieties, N41 and N37 recorded the highest brix content when treated with NN relative to NCo376 which achieved the lowest Brix content (Figure 4.22). When grown with LN, MN and HN supply, N41 and N48 recorded the highest brix content in comparison with other varieties. Variety N48 is well known to accumulate substantially high sucrose content in stalk (Ramburan, 2014). As was also found in the present study, the sucrose concentrations in N48 were also comparable to those accumulated in N41 under LN, MN and HN treatments. In contrast, N12 recorded the lowest Brix content under LN supply whilst lower brix content were also recorded by N19 when grown at MN and HN supply (Figure 4.22).

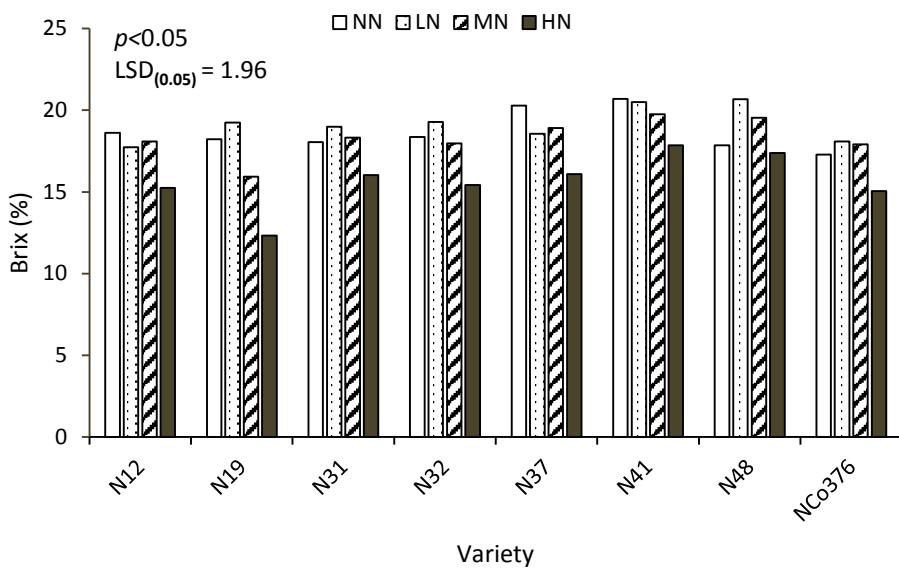


Figure 4.22: The effects of N level x variety interaction on brix (%) of eight sugarcane varieties subjected to four N supply levels

## 5. CONCLUSIONS AND RECOMMENDATIONS

The results obtained in this study have shown that stalk height of plants was increased by NN and LN treatments with crop age. Amongst the varieties, N41 was superior and consistently had the tallest stalks throughout the experiment. Stalk count was initially significantly enhanced by N application but all N treatments recorded statistically similar stalk counts at harvest. It can be concluded that application of LN was sufficient to attain maximum stalk height whereas MN and HN application levels were not desirable as they suppressed stalk elongation which lead to weak and shorter stalks. Shoot and whole-plant dry biomass yield of plants plateaued in response to LN treatment, as a result, further N increment to respective MN and HN treatments resulted in a decline of dry biomass by 8.7% and 13.4%. Of the eight varieties, N41 produced the highest shoot and whole-plant dry biomass across all N levels. The increased dry biomass production under LN supply is linked to increased stalk height which resulted greater stalk dry matter production and overall biomass production. Based on this finding it is concluded that the LN rate was the maximum N concentration required to attain corresponding maximum biomass yields.

Nitrogen application significantly influenced biomass and N allocation to shoot fractions. We therefore conclude that sugarcane plants respond to LN supply by allocating greater proportion of dry biomass to the stalk component whilst under MN and HN plants respond by allocating greater biomass to green leaves. It is further concluded that in response to LN supply, plants tended to allocate greater proportions of N to green leaves whilst greater proportion of N is allocated to the stalk component in response to MN and HN supply. With respect to leaf numbers, sugarcane plants supplied with LN in this study, maintained more green leaves than did those receiving with NN, MN and HN supply. It can therefore be concluded that in response to LN supply, sugarcane plants maintain a higher number of leaves per plant with a reduced total leaf area. This claim is substantiated by the fact that (GLA) increased significantly with increasing N rate. We conclude that increased N availability is responsible for greater GLA of sugarcane.

Leaf SPAD values increased with both plant age and increasing N supply. Among the varieties, NCo376 (lowest in NUE) followed by N48 and N41 (highest in NUE) recorded the highest leaf SPAD values at 174 DAT, indicating higher chlorophyll contents in leaves and

likely higher photosynthesis of those varieties. It can be concluded that higher leaf chlorophyll, as measured by higher SPAD values, could be used as an indication of high photosynthetic rate and but cannot be considered as a good parameter for NUE. Additionally, we conclude that the SPAD-502 could be used as a sensitive tool to monitor leaf N status of sugarcane.

Changes in root morphology have been liked to increased biomass accumulation and NUE of several plant taxa. Although the root dry biomass accumulation was suppressed by NN and LN supply in this study, the roots were significantly longer than those treated with MN and HN supply. It can be concluded that sugarcane plants adapt to N deficient conditions by adjusting their root morphology, particularly root length, for better nutrient uptake.

Similar to other crop plants, the overall NUE reported in this study was greater under low N conditions as compared with medium and high N supply. We therefore conclude that in sugarcane the highest NUE is observed under low N fertilisation. However, at higher N rates, luxury N consumption decreases NUE. Of all the studied varieties, N41 was superior over other varieties in terms of NUpE, NUtE and the overall NUE. It is speculated in this study that increased NUE in N41 was linked performance with respect to parameters such as stalk height, dry biomass allocation to stalk, maintenance of more but short green leaf numbers and dry matter production when supplied with LN. This finding suggests that, in future breeding programs aimed at selecting varieties for NUE, breeders should consider the above agronomic parameters as important tools for increasing NUE of sugarcane. Low NUE at MN and HN is mainly associated with greater biomass allocation to green leaves, reduced stalk dry matter production and most importantly luxury N consumption.

Based on these results, the method of screening sugarcane varieties developed in this study could be useful in future programs aimed at improving NUE and exploitation of sugarcane response to increasing N application. This is because site level observation has shown that there are relatively little N leaching losses from the trial setup. The results reported here are also in agreement with the field ranking of N41 and N48 (high NUE) and NCo376 (low NUE) based on the ratio of biomass to N content (Weigel et al., unpublished data). Furthermore, the method was very practical as compared with laborious, expensive and time

consuming field experimentations. However, the practicality of this method for long-term evaluations of sugarcane varieties NUE was not determined in this study.

For future studies it is recommended that studies should also work on:

- Studying the combined nitrogen and water stress effects on NUE efficiency of sugarcane.
- Identification and selection of genes contributing to increased tolerance of sugarcane to N deficiency.
- Investigating the role of N metabolic and carboxylation enzymes on NUE of sugarcane.
- Investigate the effect of N treatments on sucrose yield (g/plant or t/ha).

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