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NITROGEN USE EFFICIENCY OF THREE FIJIAN SUGARCANE (*Saccharum officinarum* L.) VARIETIES; MANA (LF60-3917), NAIDIRI (LF82-2122) AND QAMEA (LF94-694)

by

Daniel Deepak Kumar

A thesis submitted in fulfillment of the requirements for the degree of Master of Science in Biology

School of Biological and Chemical Sciences
Faculty of Science, Technology and Environment
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June, 2020

Declaration of Authenticity

Statement by Author

I, Daniel Deepak Kumar, declare that this thesis is my own work and that, to the best of my knowledge, it contains no material previously published, or substantially overlapping with material submitted for the award of any other degree at any institution, except where due acknowledgement is made in the text.

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Statement by Supervisor

The research in this thesis was performed under my supervision and to my knowledge is the sole work of Mr. Daniel Deepak Kumar.

Signature: ……………………………………… Date: 25/06/2020

Name: Dr. Tamara Osborne-Naikatini

Designation: Lecturer in Biology

This thesis is dedicated to the hardworking sugarcane farmers, laborers, cane truck drivers and everyone associated with the Fijian sugar industry who inspired this research, and to my beloved parents.

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Abstract

Sugarcane (*Saccharum officinarum* L.) production has great socioeconomic importance for Fiji. Due to declining soil fertility, large amounts of expensive fertilizers are being applied to commercial sugarcane cropping systems to sustain yield production. Nitrogen (N) is one of the most important mineral nutrients for plant growth and development, and hence is a major constituent of fertilizers. However, it has been widely reported that less than 50% of applied fertilizer N is taken up and used by sugarcane crops. The excess N remaining in the soil is lost via nitrate (NO_3^-) leaching, ammonia (NH_3) volatilization and denitrification including greenhouse gas (nitrous oxide, N_2O) emission. This can have serious consequences on the environment, such as contamination of surface and ground waters, eutrophication, loss of biodiversity, and global warming and climate change related risks. The high environmental and economic cost of N fertilizers has prompted efforts worldwide to reduce N inputs by focusing on improving N management in the sugarcane production system and increasing nitrogen use efficiency (NUE) of sugarcane.

Thus, a pioneer study in the Fijian context was conducted to determine NUE of three commercially grown local sugarcane varieties. Mana (LF60-3917) is the dominant variety, which constitutes almost 70% of the total sugarcane production in Fiji. Naidiri (LF82-2122) and Qamea (LF94-694) are newer varieties developed by the breeding programs of the Sugar Research Institute of Fiji (SRIF). The results of pot and field experiments revealed that Qamea had a significantly higher NUE in terms of both biomass production and sucrose yield than Mana and Naidiri at low N supply. Consequently, total above-ground biomass production and sucrose yield was significantly higher in Qamea compared with the other two varieties under low N condition. At high N supply, Qamea exhibited a significantly higher NUE in terms of sucrose yield than Mana and Naidiri but NUE in terms of biomass production did not differ significantly between the varieties. Mana and Naidiri were highly responsive to N levels hence showed significantly increased above-ground biomass and sucrose accumulation under high N treatment. However, no yield benefit from increased N nutrition was observed in Qamea.

This study provides first evidence that there is considerable genetic variation for NUE between commercially grown Fijian sugarcane varieties which is critical for sustainable sugarcane production in the future. It was clearly evident from the results obtained under greenhouse and field conditions that Mana and Naidiri perform better with increased N supply, whereas Qamea is ideally adapted to produce maximum above-ground biomass and sucrose yield under both N conditions. This is a significant finding with implications for the Fijian sugar industry. It highlights the need to consider variety-specific N fertilizer recommendations for improving N management that would mitigate the devastating environmental impacts of N losses from sugarcane production systems. Fijian sugarcane varieties have not been selected for NUE however, the findings of this study indicate that potential exists for breeding new varieties with improved NUE from existing varieties such as Qamea that can produce high cane and sugar yield under low N supply without a decline in performance under high N supply. This is increasingly important for maximizing sugarcane yield on low fertility soils, minimizing N fertilizer input and saving fertilizer costs that will ensure both economic and environmental sustainability of the Fijian sugar industry.

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Chapter 1

Introduction

Sugarcane (*Saccharum officinarum* L.) has been the dominant crop in Fiji since it was introduced in the 1870s and has had great socioeconomic importance (Singh *et al.*, 2012; Naidu *et al.*, 2017). However, sugarcane production has gradually declined in recent years. This could be attributed to several factors, of which infertile and highly weathered soils is perhaps the most important (Goundar *et al.*, 2014). Thus, large amounts of expensive fertilizers are being applied to commercial sugarcane cropping systems to sustain yield production.

Nitrogen (N) is quantitatively the most important mineral nutrient acquired by sugarcane for growth and development, and hence is a major constituent of fertilizers (Robinson *et al.*, 2013). A number of physiological processes are associated with N including tillering, biomass accumulation, increase in leaf area and chlorophyll synthesis (Das, 1936; Lofton and Tubaña, 2015). N is also a major component of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPCase), which are the two major enzymes involved in C_4 photosynthesis (Kumara and Bandara, 2001). Consequently, N deficiency hinders photosynthesis in sugarcane, resulting in reduced cane and sugar yield. Conversely, over-application of N is also reported to negatively affect sugarcane development and yield. According to Borges *et al.* (2016), excess N could cause increased vegetative growth, which increases lodging, causes self-shading and makes plants more susceptible to insects and pathogens. Moreover, high N application may cause decrease in sucrose concentration in sugarcane juice (Das, 1936; Robinson *et al.*, 2013). Nitrogen is directly associated with hydration of the tissue and increases the content of reducing sugars, which are both inversely related to sucrose content in the millable stalk (Das, 1936). Therefore, the right amount of N must be applied for optimum cane and sugar yield.

Application of large quantities of N fertilizers poses a serious threat to the environment. It is estimated that up to 65% of N applied to sugarcane cropping systems is lost via various pathways such as leaching, runoff, volatilization and denitrification (Meyer *et al.*, 2007). Nitrate (NO_3^-) can easily leach down soil layers due to its high mobility in the soil, leading to pollution of groundwater (Thorburn *et al.*, 2001). Water containing high nitrate concentrations is not suitable for human consumption. N contaminated runoff water from sugarcane production systems pollutes adjacent waterways (Jeong *et al.*, 2014). Elevated levels of dissolved inorganic N (DIN) in aquatic and marine ecosystems can contribute to algal bloom, eutrophication, habitat degradation and loss of biodiversity (Webster *et al.*, 2012; Kandulu *et al.*, 2018). Ammonia (NH_3) volatilization is an issue of air quality since it is an active precursor of airborne particulate matters, which are responsible for respiratory problems and some other human health issues (Dattamudi *et al.*, 2016). Denitrification causes increased emission of nitrous oxide (N_2O), a powerful greenhouse gas (GHG) with a global warming potential (GWP) 298 times greater than carbon dioxide (CO_2) (Borges *et al.*, 2019). These potentially severe consequences of excessive N fertilizer application on the environment has prompted efforts worldwide to reduce N inputs in agricultural production systems.

Consequently, a greater focus is being placed on implementing strategies for improving N management practices together with genetic improvement of crops. The International Plant Nutrition Institute has developed the 4R nutrient stewardship concept for fertilizer use in agriculture, which promotes the application of the right source (or product), at the right rate, right time, and right place (Bruulsema *et al.*, 2009). Studies on various crops have shown that there are significant differences in N response between varieties. Inefficient capture and poor conversion of the supplied N by some varieties is a major contributor to the inadvertent loss of N fertilizers from cropping systems (Hajari *et al.*, 2015). Therefore, in order to improve N management, the effectiveness with which N is used by individual varieties needs to be determined.

Nitrogen use efficiency (NUE) is a complex plant trait which is influenced by several physiological processes including the acquisition of N from the soil, utilization of the captured N for yield production, storage of excess N and subsequent remobilization from source to sink tissues during low N supply, and signaling and regulatory pathways controlling plant N status and growth (Glass, 2003; Masclaux-Daubresse *et al.*, 2010; Robinson *et al.*, 2013). There are several definitions for NUE, however it generally refers to yield (grain, biomass or sucrose) production per unit of N supplied (Moll *et al.*, 1982) or simply the ratio of output and input (Masclaux-Daubresse *et al.*, 2010). According to Robinson *et al.* (2007), NUE describes the combined efficiencies of N uptake from soil and internal N utilization by the plant. Nitrogen uptake efficiency (NUpE) is N accumulation in plant per unit of N supplied and nitrogen utilization efficiency (NUtE), also referred to as internal nitrogen use efficiency (iNUE) is yield (grain, biomass or sucrose) production per unit of N accumulated in plant (Moll *et al.*, 1982; Good *et al.*, 2004; Hajari *et al.*, 2015, 2017). Therefore, the overall NUE is expressed as the product of its two primary components, NUpE and NUtE.

NUE assessments for cultivar improvement in cereal crops, especially maize (*Zea mays* L.), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) are well documented (Hirel *et al.*, 2007), which have led to similar studies in sugarcane. Hydroponics, *in vitro*, pot, and/or field experiments have been employed to evaluate NUE of sugarcane varieties in many sugarcane producing countries such as South Africa (Schumann *et al.*, 1998; Weigel *et al.*, 2010; Hajari *et al.*, 2015, 2017; Snyman *et al.*, 2015), Australia (Robinson *et al.*, 2007, 2008, 2009), USA (Gascho *et al.*, 1986; Zhao *et al.*, 2014), India (Suman *et al.*, 2008), Pakistan (Saleem *et al.*, 2012), Argentina (Acreche, 2017), China (Yang *et al.*, 2019) and Japan (Dinh *et al.*, 2017, 2019). It is generally agreed from the literature to date that NUE in sugarcane is genotype dependent.

Sugarcane varieties with high NUE are identified by the ability to produce significantly higher biomass or sugar yields than others, under the same N supply and experimental conditions. The results of NUE assessments in sugarcane are highly useful in developing variety-specific N recommendations (Meyer *et al.*, 2007) and selection of varieties with high NUE for breeding programs (Robinson *et al.*, 2007). Additionally, the genes associated with high NUE in sugarcane can be identified and could be used to increase

NUE in other varieties via transgenesis (Whan *et al.*, 2010; Snyman *et al.*, 2015; Khan *et al.*, 2019). These strategies would reduce N fertilizer inputs and production costs, and mitigate the devastating environmental impacts of N losses from sugarcane production systems.

Fijian sugarcane varieties, however have not yet been evaluated for NUE. This is the basis of the present study. Mana (LF60-3917), which constitutes almost 70% of the total sugarcane production, is the dominant commercial variety in Fiji (Singh *et al.*, 2012; Naidu *et al.*, 2017). Naidiri (LF82-2122) and Qamea (LF94-694) are relatively newer varieties developed by the breeding programs of the Sugar Research Institute of Fiji (SRIF) and were released for commercial production in 1999 and 2014, respectively. Naidiri is the result of a cross between Mana (female parent) and MQ33-371 (male parent), while Qamea was produced from a cross between Mana and LF58-6023. Naidiri and Qamea are both reported to produce high cane and sugar yield (Naidu *et al.*, 2017). Therefore, these three commercially grown Fijian sugarcane varieties were chosen for this study.

Aim

The aim of this study was to determine nitrogen use efficiency (NUE) of three commercially grown Fijian sugarcane varieties under low and high nitrogen (N) conditions.

Objectives

The objectives of this study were:

- To evaluate the effect of N supply on above- and below-ground biomass production, tissue N accumulation, NUpE, NUtE and overall NUE of the varieties after four months of growth in a pot trial under greenhouse conditions.
- To evaluate the effect of N supply on above-ground biomass production, cane yield, tissue N accumulation, sugar yield, NUpE, NUtE and overall NUE of the

varieties in terms of both biomass production and sucrose yield under field conditions at maturity.

- To identify varieties that are adapted to low or high N supply, or that can perform equally well under both N conditions.
- To determine if the results obtained under controlled greenhouse conditions could be replicated under field conditions.

Chapter 2

Nitrogen use efficiency of three Fijian sugarcane (*Saccharum officinarum* L.) varieties in a pot trial under greenhouse conditions

2.1 Introduction

Nitrogen (N) is one of the most important mineral nutrients for plant growth and development. Due to declining soil fertility, large quantities of N fertilizers are being applied to commercial agricultural systems to increase yield production. However, less than 50% of applied fertilizer N is taken up and used by crops (Raun and Johnson, 1999; Tilman *et al.*, 2002; Thorburn *et al.*, 2005). According to Meyer *et al.* (2007), up to 65% of N applied to sugarcane (*Saccharum officinarum* L.) cropping systems is lost via nitrate (NO_3^-) leaching (Thorburn *et al.*, 2001), ammonia (NH_3) volatilization (Dattamudi *et al.*, 2016) and denitrification including greenhouse gas (nitrous oxide, N_2O) emission (C. D. Borges et al., 2019), that can have serious consequences on the environment. The high environmental and economic cost of N fertilizer has prompted efforts worldwide to reduce N inputs by focusing on improving N management in the sugarcane production system and increasing nitrogen use efficiency (NUE) of sugarcane.

Plant NUE is a trait involving the interaction of numerous physiological processes including absorption, translocation, assimilation and remobilization of N, and signaling and regulatory pathways controlling plant N status and growth (Glass, 2003; Masclaux-Daubresse *et al.*, 2010; Robinson *et al.*, 2013). NUE is expressed as the product of nitrogen uptake efficiency (NUpE) and nitrogen utilization efficiency (NUtE) (Moll *et al.*, 1982; Good *et al.*, 2004; Moose and Below, 2009). NUpE is the ability of the plant to acquire the supplied N and NUtE (also referred to as internal nitrogen use efficiency, iNUE) is the ability of the plant to assimilate and remobilize the captured N for yield production (Moll *et al.*, 1982; Good *et al.*, 2004; Hajari *et al.*, 2015, 2017).

While NUE assessments of sugarcane have been conducted in many sugarcane producing countries, most of these studies have only focused on iNUE or the overall NUE of the varieties. Robinson *et al.* (2007) who were the first to investigate iNUE in Australian sugarcane genotypes, recognized the need for future research on NUE of sugarcane to also consider NUpE for a better understanding of how the genotypes acquire N from the soil and how the two efficiencies contribute to overall NUE. Subsequently, Hajari *et al.* (2017) became the first to determine both NUpE and NUtE and the contribution of the two subcomponents to overall NUE of two four-month-old pot-grown South African sugarcane genotypes. Similarly, Acreche (2017) reported on the contributions of NUpE and NUtE to NUE of sugarcane varieties grown under field conditions in Argentina.

Hydroponics, *in vitro*, pot, and/or field experiments have been employed to evaluate NUE of sugarcane varieties. NUE of sugarcane based on biomass and plant N content in a pot experiment has been reported in a few studies (Robinson *et al.*, 2007; Snyman *et al.*, 2015; Hajari *et al.*, 2017). Pot trials have a number of advantages over field experiments. Firstly, pot trials can be conducted under controlled conditions, whereas field conditions are highly variable which makes it difficult to control N supply in the field (Snyman *et al.*, 2015; Hajari *et al.*, 2015, 2017). Secondly, pot experiments are highly useful in assessing both above- and below-ground tissues, however studies in the field are mostly restricted to above-ground biomass with below-ground biomass being difficult to evaluate due to technical limitations (Robinson *et al.*, 2009; Hajari *et al.*, 2017). Thirdly, pot trials are useful for pilot studies to detect differences between varieties for NUE traits prior to extensive field studies (Schumann *et al.*, 1998; Robinson *et al.*, 2007; Snyman *et al.*, 2015; Hajari *et al.*, 2017). Finally, field trials are time consuming (12 - 18 months) and labor intensive, while pot experiments allow rapid (3 - 4 months) screening of sugarcane varieties.

Thus, in the present study, a pot experiment was conducted under greenhouse conditions to determine NUE of three commercially grown Fijian sugarcane varieties under low and high N supply. The objectives of the study were to evaluate the effect of N supply on above- and below-ground biomass production, tissue N accumulation, NUpE, NUtE and overall NUE of the varieties after four months of growth, and to identify varieties that are adapted to low or high N supply, or that can perform equally well under both N conditions.

2.2 Materials and Methods

2.2.1 Site description

The pot experiment was conducted under controlled greenhouse conditions at Toko, Tavua, Fiji (17°29′ S, 177°52′ E, 7 m above mean sea level) from December 2016 to April 2017. The pots were maintained under ambient light with temperatures ranging from 22 to 32 °C.

2.2.2 Plant material

Three commercially grown Fijian sugarcane varieties, Mana (LF60-3917), Naidiri (LF82-2122) and Qamea (LF94-694) were used in this study. Mana is the dominant variety, which constitutes almost 70% of the total sugarcane production in Fiji. Naidiri, produced from a cross between Mana (female parent) and MQ33-371 (male parent), and Qamea, produced from a cross between Mana (female parent) and LF58-6023 (male parent), are newer varieties developed by the breeding programs of the Sugar Research Institute of Fiji (SRIF). Seven to eight month old stalks of the three varieties were obtained from SRIF nursery at Drasa, Lautoka, Fiji. Plants were established from disease-free single nodal stem cuttings (“setts”) in seedling trays with sterile N-free planting medium. Four weeks old plantlets were each transplanted into a 20 L plastic pot (30 cm diameter) with free drainage, filled with washed coarse river sand (washed with dilute hydrochloric acid followed by distilled water several times to remove N and other nutrients), prior to commencing N treatments.

2.2.3 Experimental design and treatments

Plants were subjected to two levels of N supply; limiting (0.4 mM N) and non-limiting (10 mM N), subsequently referred to as low and high N supply, respectively. Inorganic N as ammonium nitrate (NH_4NO_3) was supplied to plants in both N treatments, in a nutrient solution (2 mM $MgSO_4$, 1 mM $CaSO_4$, 5 mM K_2SO_4, 0.457 mM KH_2PO_4, 42.5 µM K_2HPO_4, 100 µM FeEDTA, 10 µM $MnSO_4$, 10 µM H_3BO_3, 1 µM $CuSO_4$, 2.5 µM $ZnSO_4$, 0.35 µM Na_2MoO_4; pH 5.6). $CaSO_4$ was added to the low N nutrient solution to obtain the same osmolarity as of the high N nutrient solution. Nutrient solution alternated daily with tap water, was added until liquid was dripping from pots. This regime was based on a similar study with Australian sugarcane genotypes (Robinson *et al.*, 2007). During N treatment period, all tillers that emerged were immediately removed. Weeds and insects were controlled or prevented using recommended products.

A completely randomized design with three replications was used for this experiment.

2.2.4 Sampling and measurements

Plants were harvested after four months growth under the two N regimes and subsequently separated into above- and below-ground tissue. Agronomic and physiological parameters were measured.

2.2.4.1 Biomass

Fresh weights (FW) of all above- and below-ground tissue were recorded. The samples were then oven-dried at 60 °C, until a constant weight was reached and dry weight (DW) was determined.

2.2.4.2 N content

Dried plant parts were ground to a fine powder using a Wiley mill, sieved through a 2 mm mesh sieve and homogenized. Representative subsamples were taken for total N analysis, which was undertaken by Fiji Agricultural Chemistry Laboratory at Koronivia Research

Station in Nausori, Fiji. Shoot and root N content (% w/w) was determined by Kjeldahl digestion method described in Daly and Wainiqolo (1993), derived from the methods of Daly *et al.* (1984) and Blakemore *et al.* (1987). Total N accumulated in plant biomass was calculated as the product of N content (% w/w) and the respective biomass (Eqn. 2.1).

$$\text{N content in biomass (g N}_\text{b}) = \text{N content (\% w/w)} \times \text{biomass (g DW)} \tag{2.1}$$

2.2.4.3 Nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and nitrogen use efficiency (NUE)

NUpE, NUtE and NUE were calculated using formulae stated by Hajari *et al.* (2017), based on the definitions of Moll *et al.* (1982), Good *et al.* (2004) and Hawkesford (2012). Nitrogen uptake efficiency (NUpE) was computed as the ratio of N accumulated in biomass and N supplied (Eqn. 2.2). Nitrogen utilization efficiency (NUtE) was computed as the ratio of biomass and N accumulated in biomass (Eqn. 2.3). Overall nitrogen use efficiency (NUE) was calculated as the product of NUpE and NUtE which equates to the ratio of biomass and N supplied (Eqn. 2.4).

$$\text{NUpE}\left(\text{g N}_\text{b}\ \text{g N}_\text{s}{}^{-1}\right) = \frac{\text{N content in biomass (g N}_\text{b})}{\text{N supplied (g N}_\text{s})} \tag{2.2}$$

$$\text{NUtE}\left(\text{g DW g N}_\text{b}{}^{-1}\right) = \frac{\text{biomass (g DW)}}{\text{N content in biomass (g N}_\text{b})} \tag{2.3}$$

$$\begin{aligned}\text{NUE}\left(\text{g DW g N}_\text{s}{}^{-1}\right) &= \text{NUpE}\left(\text{g N}_\text{b}\ \text{g N}_\text{s}{}^{-1}\right) \times \text{NUtE}\left(\text{g DW g N}_\text{b}{}^{-1}\right)\\ &= \frac{\text{biomass (g DW)}}{\text{N supplied (g N}_\text{s})}\end{aligned} \tag{2.4}$$

2.2.5 Statistical analysis

Data were tested for normality using the Shapiro-Wilk test and homogeneity of sample variances using the Bartlett test. Normally distributed data with homogenous sample variances were subsequently subjected to a two-way analysis of variance (ANOVA) to determine the (relative) influences of the explanatory factors. Significant differences between treatment means were determined using Tukey's Honestly Significant Difference (HSD) pairwise tests. The level of significance (α) for these tests was set at $p < 0.05$. Where relevant, correlations between variables were analyzed using Pearson's product moment correlation coefficient at the same α level. All statistical analyses were performed using the R software package (version 3.6.1).

2.3 Results and Discussion

2.3.1 Biomass and N accumulation under low and high N supply

Above- and below-ground biomass production (Fig. 2.1) and tissue N accumulation in plants were measured to determine how the three varieties differ in response to limiting (low) and non-limiting (high) N supply. After four months of growth at the two N supply rates, above-ground biomass production in Mana (LF60-3917) and Naidiri (LF82-2122) was significantly reduced with low N supply however, no significant difference was observed in Qamea (LF94-694). Below-ground biomass production of Qamea with low N supply (21.48 g DW) was significantly greater than that with high N supply (14.31 g DW), while that of Mana and Naidiri remained the same at both N supply rates. Varietal differences were significant at low N supply, with Qamea producing the highest above- and below-ground biomass (39.16 ± 0.74 g DW and 21.48 ± 0.85 g DW, respectively) but at high N supply, biomass accumulation between varieties did not differ significantly. This is consistent with the findings of Robinson *et al.* (2007) and Hajari *et al.* (2017) for below-ground biomass, in similar pot trials under controlled glasshouse conditions with Australian and South African sugarcane varieties, respectively. However, for above-ground biomass, the opposite trend was reported by Hajari *et al.* (2017). Shoot to root ratio (Fig. 2.1) was significantly lower for all varieties at low N supply and was similar between varieties at both N supply levels. The significantly higher below-ground biomass produced in Qamea under low N supply compared to high N supply and in comparison with the other two varieties, could indicate the ability of Qamea to respond efficiently to low N availability. This is in agreement with previous studies under low N conditions

(Snyman *et al.*, 2015; Hajari *et al.*, 2017) and is likely an advantage in N capture (Thorburn *et al.*, 2003; Smith *et al.*, 2005; Robinson *et al.*, 2009). As a result, the above-ground biomass production in Qamea was unaffected at the two N supply rates and was significantly greater than that in Mana and Naidiri. These results suggest that, at low N supply, varietal differences in above-ground biomass production could be attributed to the differences in below-ground biomass production.

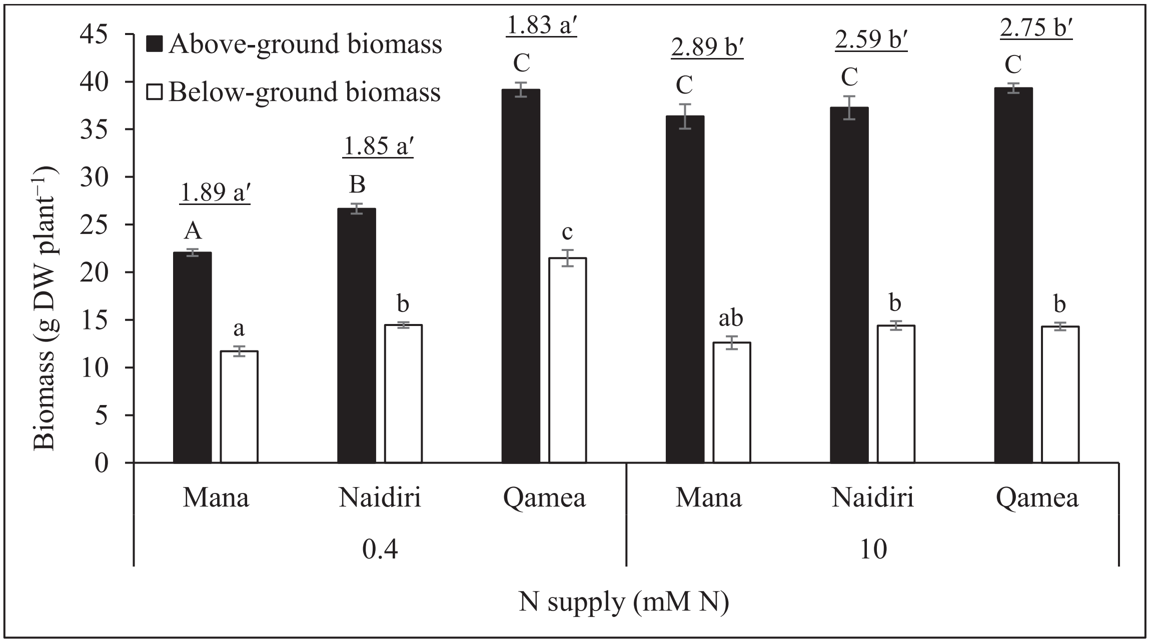


Fig. 2.1 The effect of N supply on above- and below-ground biomass production in three Fijian sugarcane varieties after four months in a pot trial under greenhouse conditions. Shoot:root ratios are given above each bar. Different uppercase (A-C) and lowercase letters (a-c) denote significant differences for above- and below-ground biomass, respectively, and letters with symbol (a′-b′) denote significant differences for shoot:root ratio (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$). Vertical bars represent ± S.E. of the mean (3 plants).

Total N accumulation in above- and below-ground tissue (Fig. 2.2) demonstrated similar trends to biomass production responses under the two N supply rates. This agrees with the results previously reported (Robinson *et al.*, 2007). While total N accumulated in shoots of Mana and Naidiri was significantly higher at high N compared with low N supplied plants, Qamea showed no significant difference. In contrast, total N accumulated in roots of Qamea was significantly greater at low N supply but remained the same at both N supply rates in Mana and Naidiri. As with biomass production, significant varietal differences were found only at low N supply, with Qamea having the highest above- and below-ground N content (0.339 ± 0.008 g N and 0.129 ± 0.005 g N, respectively). This provides further evidence that increased allocation to below-ground biomass in Qamea at low N supply is of significant advantage for N capture and storage from the soil.

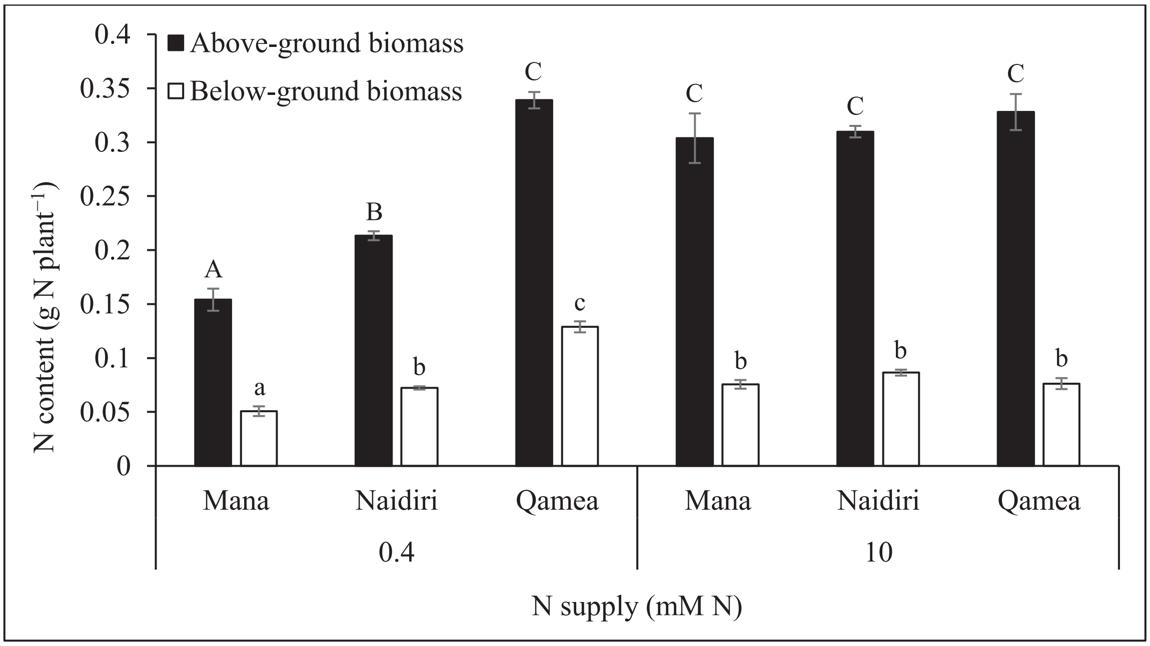


Fig. 2.2 The effect of N supply on total N accumulation in above- and below-ground biomass in three Fijian sugarcane varieties after four months in a pot trial under greenhouse conditions. Different uppercase (A-C) and lowercase letters (a-c) denote significant differences for total N accumulation in above- and below-ground biomass, respectively (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$). Vertical bars represent ± S.E. of the mean (3 plants).

2.3.2 Determination of NUpE, NUtE and NUE

Nitrogen uptake efficiency and nitrogen utilization efficiency (also referred to as internal nitrogen use efficiency), the subcomponents of NUE, were calculated (Table 2.1) to determine how much the two efficiencies contribute to overall NUE and how much variation for specific traits exists between the selected varieties at the two N supply levels. The results showed that all varieties had significantly higher NUpE, NUtE (except Qamea) and NUE at low N supply compared with high N supply. Within N treatments, NUpE, NUtE and NUE of the varieties varied significantly at low N supply but there were no differences between varieties at high N supply. These findings are in accordance with those of Hajari *et al.* (2015) under in vitro conditions and Hajari *et al.* (2017) in a similar pot trial. Qamea had the highest NUpE (0.6958 ± 0.0086 g N_b g N_s^{-1}) and NUE (90.19 ± 2.35 g DW g N_s^{-1}) but ranked the lowest in terms of NUtE (129.67 ± 3.87 g DW g N_b^{-1}). Conversely, Mana had the highest NUtE (166.67 ± 12.36 g DW g N_b^{-1}) but it had the lowest NUpE (0.3046 ± 0.0219 g N_b g N_s^{-1}) and NUE (50.22 ± 0.76 g DW g N_s^{-1}). Naidiri ranked in between Qamea and Mana for the two NUE traits and overall NUE. These results indicate that at low N supply, NUpE seems to contribute more strongly to overall NUE and that there is considerable variation for the two traits between the studied varieties. However, it is difficult to elucidate from the results if the same is true with high N supply since no significant differences were observed within this N treatment. A likely reason for this could be that the N supply was too high (Hajari *et al.*, 2015, 2017) compared to the N demands of the varieties which is evident from the significantly lower (more than 10-fold) N uptake efficiencies. This could be supported by the fact that N uptake in plants is generally controlled by negative feedback mechanisms and consequently any further uptake is inhibited once N demand is satisfied (Glass, 2003; Robinson *et al.*, 2009; Gastal *et al.*, 2015). Another interesting observation was that, while N utilization efficiency of Mana varied significantly between the two N supply rates, NUtE of Qamea and Naidiri showed no significant difference. This could suggest that the internal N use in Qamea and Naidiri is independent of N supply thus of the two NUE traits, N uptake could possibly have a greater influence on the overall NUE of these varieties under different N supply levels. The present study reaffirms the importance of evaluating both N uptake and internal N use efficiencies when assessing sugarcane varieties for N use efficiency, as highlighted by other researchers (Robinson *et al.*, 2007, 2009; Hajari *et al.*, 2015, 2017; Snyman *et al.*, 2015).

Table 2.1. The effect of N supply on N uptake efficiency (NUpE), N utilization efficiency (NUtE) and N use efficiency (NUE) in three Fijian sugarcane varieties after four months in a pot trial under greenhouse conditions.

N supply (mM N)	Variety	NUpE ($g\ N_b\ g\ N_s^{-1}$)	NUtE ($g\ DW\ g\ N_b^{-1}$)	NUE ($g\ DW\ g\ N_s^{-1}$)
0.4 (low N)	Mana	0.3046 ± 0.0219^{b}	166.67 ± 12.36^{b}	50.22 ± 0.76^{b}
	Naidiri	0.4248 ± 0.0058^{c}	143.98 ± 0.47^{ab}	61.16 ± 0.75^{c}
	Qamea	0.6958 ± 0.0086^{d}	129.67 ± 3.87^{a}	90.19 ± 2.35^{d}
10 (high N)	Mana	0.0226 ± 0.0016^{a}	129.58 ± 3.93^{a}	2.91 ± 0.12^{a}
	Naidiri	0.0236 ± 0.0002^{a}	130.42 ± 3.92^{a}	3.07 ± 0.09^{a}
	Qamea	0.0240 ± 0.0013^{a}	133.27 ± 5.77^{a}	3.19 ± 0.02^{a}

Data represent the mean of three plants ± S.E. Means with the same letters (a-d) within a column are not significantly different (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$).

2.3.3 Relationship between NUE and biomass production

To investigate the relationship between NUE and biomass production, varieties were compared under varied N supply. The results of this study showed that there is considerable variation between the studied varieties for biomass production responses, which could be attributed to varietal differences observed in NUE. When grown with low N supply, Qamea was found to have the highest NUE, and consequently produced the highest above-ground biomass. Similarly, Mana had the lowest NUE, which resulted in the lowest above-ground biomass production. Under high N supply, no significant differences were determined for NUE, and thus all varieties displayed a similar response in terms of above-ground biomass production.

The underlying physiological mechanisms causing above-ground biomass production responses in relation to NUE could be explained by examining the contributions of NUpE and NUtE of the varieties at the two N supply levels (Beatty *et al.*, 2010). Qamea which produced the highest below-ground biomass at low N supply, exhibited the highest NUpE and NUE. Hajari *et al.* (2017) also reported similar results in pots under low N supply. As discussed earlier, increased allocation to below-ground biomass in response to low N availability allows exploitation of a greater soil volume which significantly increases the ability of plants to take up N from the soil (Thorburn *et al.*, 2003; Smith *et al.*, 2005; Robinson *et al.*, 2009). The capacity of roots to store the acquired N which could be remobilized from source to sink tissues during low N supply, is another important trait (Robinson *et al.*, 2009, 2013; Hawkesford and Howarth, 2011). This was also clearly evident in the results, with Qamea displaying the highest N accumulation in below-ground biomass. These root-associated traits observed in Qamea, may explain its significantly higher NUpE at low N supply. In contrast, NUtE was the lowest in Qamea. Despite this, Qamea had the highest above-ground biomass production under low N supply. Mana which was found to have a significantly higher NUtE than Qamea, produced significantly lower above-ground biomass. This finding contradicts with previous studies (Robinson *et al.*, 2007; Hajari *et al.*, 2017), which demonstrated that genotypes with the highest NUtE produced the highest above-ground biomass. A possible explanation for this observation could be found by examining the differences in total N accumulation in above-ground biomass between the varieties. Qamea had significantly higher above-ground N content than Mana. This could be directly related to the differences in NUpE of the varieties. Since NUpE is a measure of N taken up into the crop per unit of N available in soil (Hawkesford and Howarth, 2011), N content in above-ground biomass could be expressed as the product of NUpE and N supplied (Eqn. 2.5).

$$\text{N content in biomass } (\text{g N}_\text{b}) = \text{NUpE } \left(\text{g N}_\text{b}\ \text{g N}_\text{s}{}^{-1}\right) \times \text{N supplied } (\text{g N}_\text{s}) \qquad (2.5)$$

Thus, at low N supply, a significantly higher NUpE greatly increases N uptake from the soil and results in a significantly higher N content in above-ground biomass in Qamea. NUtE is a measure of biomass produced per unit of N taken up into the crop (Hawkesford and Howarth, 2011). Therefore, above-ground biomass production could be expressed as the product of NUtE and N content in above-ground biomass (Eqn. 2.6).

$$\text{Biomass } (\text{g DW}) = \text{NUtE } \left(\text{g DW g N}_\text{b}{}^{-1}\right) \times \text{N content in biomass } (\text{g N}_\text{b}) \qquad (2.6)$$

A significantly higher N content in above-ground biomass in Qamea, ensures availability of higher amount of N internally for biomass production and hence yields a significantly higher above-ground biomass. There was a significant ($p < 0.00001$) positive correlation between NUpE and biomass production at low N supply (Fig. 2.3) but no significant correlation was observed between NUtE and biomass production. Based on this, the relative influence of NUpE appears greater than NUtE on the overall NUE of sugarcane varieties under limiting N conditions. However, as explained earlier, the same trend could not be determined for high N conditions since no significant differences were observed between varieties for NUpE, NUtE and NUE under high N supply. The possible contributions of both NUpE and NUtE to overall NUE of sugarcane under varied N supply have been investigated in only a few studies (Hajari *et al.*, 2015, 2017; Snyman *et al.*, 2015) and remain unclear to date. Nevertheless, studies on other crops have also shown that N uptake contributes more to overall NUE at low N supply (Kamprath *et al.*, 1982; Presterl *et al.*, 2002; Hirel and Lemaire, 2006).

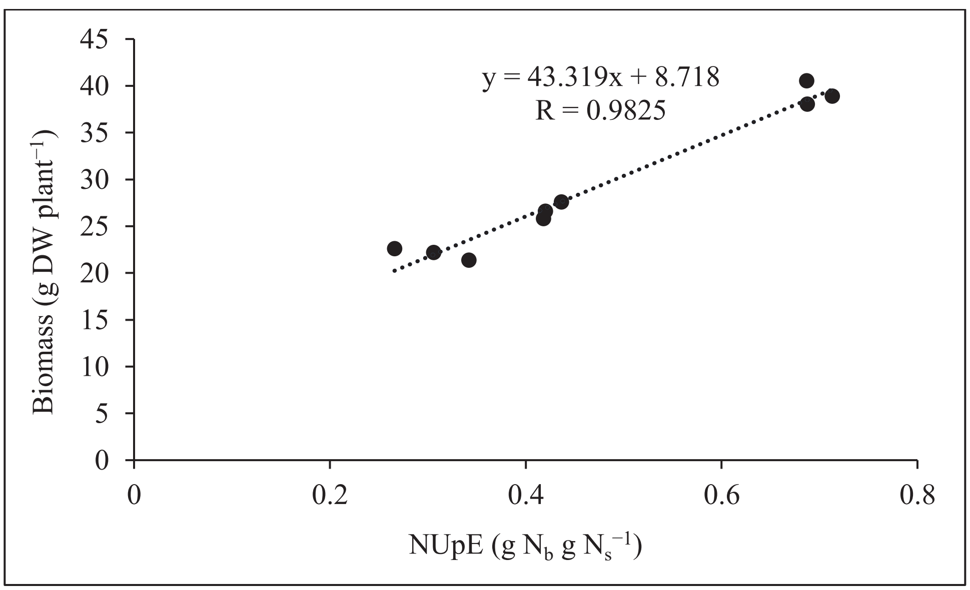


Fig. 2.3 The relationship between above-ground biomass production (g DW plant^{-1}) and nitrogen uptake efficiency (NUpE, g N_b g N_s^{-1}) in three Fijian sugarcane varieties after four months with low N supply in a pot trial under greenhouse conditions.

Comparing the above-ground biomass of varieties at low and high N supply revealed further differences between the varieties. Mana and Naidiri were both found to produce significantly higher above-ground biomass at high N supply compared to low N supply but there was no significant difference observed in Qamea. This is a crucial finding, since it indicates that Qamea is ideally adapted to produce maximum above-ground biomass at both N supply levels, whereas Mana and Naidiri perform better under high N conditions. Hirel *et al.* (2007) stated that the identification of genotypes that are adapted to low N or high N conditions, and those that can perform well irrespective of N supply, is a prerequisite for improving crop productivity under low N input. Fijian sugarcane varieties have not been selected for NUE however, the findings of the present study show that potential exists for breeding varieties with improved yield production under low N supply without a decline in performance under high N supply.

2.4 Conclusion

The present study evaluated nitrogen use efficiency of three Fijian sugarcane varieties with varied N supply, in a pot trial under controlled greenhouse conditions. At limiting (low) N supply, Qamea (LF94-694) had a significantly higher NUE than Mana (LF60-3917) and Naidiri (LF82-2122), which was also reflected in a higher above-ground biomass production compared with the other two varieties. It was determined that a significantly greater below-ground biomass resulted in an increased NUpE, and consequently a higher NUE in Qamea. This lends confidence to the notion that NUpE has a greater contribution to overall NUE under low N conditions. There were no significant differences found between varieties for any of the parameters measured at non-limiting (high) N supply hence possible contributions of NUpE and NUtE to overall NUE could not be elucidated at this N supply rate. It is clearly evident from the results that Mana and Naidiri are better adapted to high N conditions in terms of above-ground biomass production, whereas Qamea has the ability to perform equally well under both N conditions. Subsequently, a field experiment was conducted to determine if these findings could be replicated under field conditions, the results of which are reported in the following chapter.

Chapter 3

Nitrogen use efficiency of three Fijian sugarcane (*Saccharum officinarum* L.) varieties under field conditions

3.1 Introduction

The potential threat posed by extensive nitrogen (N) fertilizer application in sugarcane production systems to environmental and economic sustainability, has prompted intensive research worldwide on nitrogen use efficiency (NUE) of sugarcane. NUE is a plant trait influenced by a number of physiological processes such as N acquisition, utilization, storage and remobilization, and signaling and regulatory pathways governing plant N status (Glass, 2003; Masclaux-Daubresse *et al.*, 2010; Robinson *et al.*, 2013). It generally refers to the effectiveness with which N is used by plants to produce yield (Moll *et al.*, 1982) and is expressed as the combined efficiencies of N uptake from soil and internal N utilization by the plant (Robinson *et al.*, 2007).

NUE of sugarcane can be determined in terms of biomass production (Robinson *et al.*, 2007; Snyman *et al.*, 2015; Hajari *et al.*, 2017) or sucrose yield (Schumann *et al.*, 1998; Weigel et a., 2010) in response to supplied N. In sugarcane, mature stalk is the part of the crop which is of economic value. Therefore, the above-ground biomass is of a great importance in sugarcane research (Bonnett, 2014). Sucrose content, given its importance to production of sugar, is another important yield parameter in sugarcane (Bonnett, 2014). Studies on NUE of sugarcane have demonstrated considerable variation for both yield responses between sugarcane varieties.

Fijian sugarcane varieties, however had not been evaluated for NUE. Thus, a pot experiment was conducted under greenhouse conditions to determine NUE of three commercially grown Fijian sugarcane varieties after four months of growth at low and high N supply. The results of this study are reported in the previous chapter. While pot trials serve as adequate and useful tools for pilot studies to detect differences between varieties for NUE traits (Schumann *et al.*, 1998; Robinson *et al.*, 2007; Snyman *et al.*, 2015; Hajari *et al.*, 2017), there are significant differences between greenhouse and field conditions. Hence, there is a need to confirm the results of the pot trial under field conditions. Moreover, cane yield and sucrose content, the two main yield components, can be determined in mature plants (12 - 18 months old) in the field (Snyman *et al.*, 2015).

Thus, a field experiment was conducted to determine NUE of three commercially grown Fijian sugarcane varieties under low and high N supply. The objectives of the study were to evaluate the effect of N supply on above-ground biomass production, cane yield, tissue N accumulation, sugar yield, NUpE, NUtE and overall NUE of the varieties in terms of both biomass production and sucrose yield at maturity, to identify varieties that are adapted to low or high N supply, or that can perform equally well under both N conditions, and to determine if the results obtained under controlled greenhouse conditions could be replicated under field conditions.

3.2 Materials and Methods

3.2.1 Site description

The field experiment was carried out from December 2016 to June 2018 on a commercial sugarcane farm at Toko, Tavua, Fiji (17°29′ S, 177°52′ E, 7 m above mean sea level). According to Leslie (2012), the soil at this site is a Typic Haplustalfs (Soil Survey Staff, 2003). The chemical characterization of the soil is presented in Table 3.1. Total rainfall from planting to harvest was 3937 mm and average temperatures ranged from 16 to 33 °C.

Table 3.1. The chemical characterization of the soil in the top one meter of the field at planting.

pH	Electrical Conductivity (mS/cm)	Total Carbon (%)	Total Nitrogen (%)	Olsen Available Phosphorus (mg/kg)	Exchangeable Bases (me/100g)			
					Ca	Mg	K	Na
6.5	0.08	1.1	0.15	8	18.72	12.13	0.38	0.06

3.2.2 Plant material

Three commercially grown Fijian sugarcane varieties, Mana (LF60-3917), Naidiri (LF82-2122) and Qamea (LF94-694) were used in this study (see Section 2.2.2 for a description of these varieties). Seven to eight month old stalks of the three varieties were obtained from SRIF nursery at Drasa, Lautoka, Fiji for seedcane. Plant crop was established from disease-free stem cuttings ("setts").

3.2.3 Experimental design and treatments

Sugarcane was planted manually by placing three-budded setts in furrows. Plots consisted of five 10 m long rows, 1.6 m apart, planted at a density of 15 buds per linear meter of furrow. Nitrogen was applied as urea (46% N) at two rates; (1) no N (N_0: 0 kg N ha^{-1}), and (2) recommended N (N_{120}: 120 kg N ha^{-1}), subsequently referred to as low and high N, respectively. This N regime was based on a similar study with Australian sugarcane genotypes (Robinson *et al.*, 2009). The high N rate was applied in a split application with 40% of the fertilizer applied as basal dressing in the furrows at the time of planting and the remaining 60% topdressed at 90 days after planting (DAP). Phosphorus in the form of single superphosphate (18% P_2O_5) and potassium as muriate of potash (60% K_2O) were applied in single applications at the recommended rates of 40 kg P ha^{-1} (as basal dressing at planting) and 100 kg K ha^{-1} (as topdressing at 90 DAP), respectively. Weeds and insects were controlled or prevented using recommended products.

The experimental design was a split-plot with three replications. The main plot was divided into the two N treatments and the three varieties were assigned in the subplots.

3.2.4 Sampling and measurements

3.2.4.1 Soil sampling and analyses

Soil from the experimental field was sampled before planting for chemical characterization. Nine subsamples per plot were collected from a depth of 0-1 m, at random positions approximately 0.25 m from the planting rows, using an 8 cm (diameter) soil auger. These subsamples were homogenized to obtain a representative composite sample for the entire field.

The samples were oven-dried at 40 °C, ground and sieved through a 2 mm mesh sieve, and subsamples were sent for chemical analyses to the Fiji Agricultural Chemistry Laboratory at Koronivia Research Station in Nausori, Fiji; chemical analyses followed procedures described in Daly and Wainiqolo (1993). The method for determination of total organic carbon was an adaptation of the Walkley and Black (1934) oxidation procedure. Total N was determined by a semi-micro Kjeldahl method (Blakemore *et al.*, 1987) and mineral N by the distillation method of Bremner and Keeney (1965). Plant-available phosphorus in soil was extracted based on the phosphorus extraction method of Olsen (1954) and determined colorimetrically using the Murphy and Riley (1962) method as described by Blakemore *et al.* (1987). The extraction method for exchangeable bases was adapted from the method described by Blakemore *et al.* (1987). The chemical composition of the soil in the top one meter at the beginning of the experiment is shown in Table 3.1.

3.2.4.2 Plant sampling

Plant samples were taken at ripening, in June 2018 (540 DAP), which coincided with the sugarcane harvesting season (June - December) in Fiji. An area of 16 m^2 was selected at random in the center of each plot and the total number of stalks in this area was counted to determine the stalk population density. The entire above-ground tissue (stalk, leaves and top) of all plants within one linear meter was harvested manually at randomly selected positions along the three central rows of each plot. The samples were immediately transported to the laboratory to determine agronomic and physiological parameters.

3.2.4.3 Biomass and cane yield

Subsamples of nine plants taken from each sample were oven-dried at 60 °C, until a constant weight was reached and dry weight (DW) of total above-ground tissue was determined. Cane yield (CY) on a fresh-weight basis was quantified (as payment to growers is based on this) following Acreche *et al.* (2015), as the product of average millable stalk (cane) weight and the determined stalk population density (Eqn. 3.1).

$$\text{Cane yield (Mg ha}^{-1}\text{)} = \text{Average millable stalk weight (Mg stalk}^{-1}\text{)} \times \text{Stalk population density (stalks ha}^{-1}\text{)} \quad (3.1)$$

3.2.4.4 N content

Total N analysis was undertaken by Fiji Agricultural Chemistry Laboratory at Koronivia Research Station in Nausori, Fiji (see Section 2.2.4.2 for a description of the method). Total above-ground N accumulation at maturity was calculated as the product of N content (% w/w) and above-ground dry biomass (Eqn. 3.2).

$$\text{N content in biomass (kg N}_\text{b}\text{ ha}^{-1}\text{)} = \text{N content (\% w/w)} \times \text{biomass (kg DW ha}^{-1}\text{)} \quad (3.2)$$

3.2.4.5 Sucrose content and sugar yield

A subsample of three millable stalks from each sample was chosen randomly. Sugarcane juice was extracted and immediately kept at 4 °C to reduce chemical changes (Qudsieh *et al.*, 2001; Pereira *et al.*, 2017). Subsequently, the juice samples were centrifuged (14 000 × g, 4 °C, 10 minutes) and the supernatants were stored at – 20 °C until assayed (Pereira *et al.*, 2017). Sucrose content was determined by high-performance liquid chromatography (HPLC) (Hunt *et al.*, 1977; Qudsieh *et al.*, 2001; Pereira *et al.*, 2017) using a Waters 515 HPLC pump equipped with a Shimadzu HIC-6A oven, a Dr. Maisch GmbH NH_2 column (250 mm × 4.6 mm, 5 μm) and a Waters 2410 refractive index (RI) detector. Separation employed reverse phase isocratic elution with acetonitrile and ultrapure water (85:15 v/v) used as mobile phase at a flow rate of 1.0 mL/min. The solvent mixture was degassed for 30 minutes using a sonicator, prior to the analysis. Sucrose solutions (0.2 - 1.2 % w/v) were used as calibration standards. Sugarcane juice samples were thawed in a water bath with ice and filtered through sterile Millipore filters with hydrophilic nylon membrane (0.45 μm pore size, 33 mm diameter), using a syringe. The samples were then diluted with ultrapure water at a ratio of 1:25. The injection volume was 20 μL. Oven temperature was set at 40 °C and the total run time of the analysis was 15 minutes. All analyses were performed in triplicates. Sugar yield (SY) on a fresh weight basis was quantified following Acreche *et al.* (2015), as the product of sucrose content (% w/w) and cane yield (Eqn. 3.3).

$$\text{Sugar yield } (\text{Mg ha}^{-1}) = \text{Sucrose content } (\%\ \text{w/w}) \times \text{cane yield } (\text{Mg ha}^{-1}) \quad (3.3)$$

3.2.4.6 Nitrogen uptake efficiency (NUpE), nitrogen utilization efficiency (NUtE) and nitrogen use efficiency (NUE)

NUpE, NUtE and NUE in terms of biomass production were calculated using formulae stated by Acreche (2017). Nitrogen uptake efficiency (NUpE) was computed as the ratio of N accumulated in above-ground biomass and N available in soil during the crop cycle (Eqn. 3.4). Nitrogen utilization efficiency (NUtE) was computed as the ratio of above-ground biomass and N accumulated in above-ground biomass (Eqn. 3.5). Overall nitrogen use efficiency (NUE) was calculated as the product of NUpE and NUtE which equates to the ratio of above-ground biomass and N available in soil during the crop cycle (Eqn. 3.6).

$$\text{NUpE } \left(\text{kg N}_\text{b}\ \text{kg N}_\text{s}{}^{-1}\right) = \frac{\text{N content in biomass } (\text{kg N}_\text{b}\ \text{ha}^{-1})}{\text{N available in soil during the crop cycle } (\text{kg N}_\text{s}\ \text{ha}^{-1})} \quad (3.4)$$

$$\text{NUtE } \left(\text{kg DW kg N}_\text{b}{}^{-1}\right) = \frac{\text{biomass } (\text{kg DW ha}^{-1})}{\text{N content in biomass } (\text{kg N}_\text{b}\ \text{ha}^{-1})} \quad (3.5)$$

$$\begin{aligned}\text{NUE } \left(\text{kg DW kg N}_\text{s}{}^{-1}\right) &= \text{NUpE } \left(\text{kg N}_\text{b}\ \text{kg N}_\text{s}{}^{-1}\right) \times \text{NUtE } \left(\text{kg DW kg N}_\text{b}{}^{-1}\right) \\ &= \frac{\text{biomass } (\text{kg DW ha}^{-1})}{\text{N available in soil during the crop cycle } (\text{kg N}_\text{s}\ \text{ha}^{-1})} \quad (3.6)\end{aligned}$$

The amount of N available in soil during the crop cycle was calculated as the sum of soil mineral N (N-NO_3 content) before planting, N applied as fertilizer and mineralized N (Eqn. 3.7). Mineralized N (88.2 kg N ha^{-1} yr^{-1}) was estimated at 2 % of the total soil N content in the root zone, based on Angus (2001). This is consistent with the values (71 and 78.2 kg N ha^{-1} yr^{-1}) reported by Angus (2001) and Acreche (2017), respectively, and is within the range of 60 - 120 kg N ha^{-1} yr^{-1} stated by Weigel *et al.* (2010). For the purpose of this experiment, the loss of N from this system was considered to be negligible as per Acreche (2017).

$$\text{N available in soil during the crop cycle } (\text{kg N}_\text{s}\text{ ha}^{-1}) = \text{soil mineral N } (\text{kg N ha}^{-1}) + \text{N fertilizer application rate } (\text{kg N ha}^{-1}) + \text{mineralized N } (\text{kg N ha}^{-1}) \quad (3.7)$$

NUtE and NUE were also calculated on the basis of sucrose yield by replacing biomass with sucrose content in Eqns. (3.5) and (3.6), respectively. Since NUpE is the ratio of N accumulated in plant tissue and N available in soil, it remains the same irrespective of whether NUE is calculated in terms of biomass production or sucrose yield. NUtE was computed as the ratio of sucrose content and N accumulated in above-ground biomass (Eqn. 3.8). NUE was calculated as the product of NUpE and NUtE which equates to the ratio of sucrose content and N available in soil during the crop cycle (Eqn. 3.9).

$$\text{NUtE } \left(\text{kg Suc kg N}_\text{b}{}^{-1}\right) = \frac{\text{sucrose content } (\text{kg Suc ha}^{-1})}{\text{N content in biomass } (\text{kg N}_\text{b}\text{ ha}^{-1})} \quad (3.8)$$

$$\text{NUE } \left(\text{kg Suc kg N}_\text{s}{}^{-1}\right) = \text{NUpE } \left(\text{kg N}_\text{b}\text{ kg N}_\text{s}{}^{-1}\right) \times \text{NUtE } \left(\text{kg Suc kg N}_\text{b}{}^{-1}\right) = \frac{\text{sucrose content } (\text{kg Suc ha}^{-1})}{\text{N available in soil during the crop cycle } (\text{kg N}_\text{s}\text{ ha}^{-1})} \quad (3.9)$$

3.2.5 Statistical analysis

Data were tested for normality using the Shapiro-Wilk test and homogeneity of sample variances using the Bartlett test. Normally distributed data with homogenous sample variances were subsequently subjected to a two-way analysis of variance (ANOVA) to determine the (relative) influences of the explanatory factors. Significant differences between treatment means were determined using Tukey's Honestly Significant Difference (HSD) pairwise tests. The level of significance (α) for these tests was set at $p < 0.05$. Where relevant, correlations between variables were analyzed using Pearson's product moment correlation coefficient at the same α level. All statistical analyses were performed using the R software package (version 3.6.1).

3.3 Results and Discussion

3.3.1 Biomass and cane yield

Total above-ground dry biomass and millable stalk dry weight (Fig. 3.1) were measured to determine how the three varieties differ in response to low (no N fertilizer applied) and high (120 kg ha^{-1} N applied as urea) N supply. After eighteen months growth under field conditions, total above-ground biomass production and stalk dry weight in Mana (LF60-3917) and Naidiri (LF82-2122) were significantly higher with high N supply however, no significant differences were observed in Qamea (LF94-694) between the two N supply rates. Varietal differences were significant only at low N supply, with the highest for both parameters recorded in Qamea. Cane yield (CY) determined on a fresh weight basis (Table 3.2), displayed the same trends and ranged from 70.34 ± 7.07 to 130.24 ± 6.14 Mg ha^{-1}. Comparable results for cane yield of plant crop, ranging between 44.57 and 114.80 Mg ha^{-1}, were obtained by Acreche *et al.* (2015) with thirteen sugarcane varieties grown without fertilizer application in Argentina. The significantly higher total above-ground biomass and cane yield produced in Qamea in comparison with the other two varieties under low N supply, indicates the ability of Qamea to respond efficiently to low N availability. These findings are consistent with the above-ground biomass results of the pot trial reported in the previous chapter. Unlike in the pot experiment, below-ground biomass production in the field could not be assessed due to technical limitations. However, given the similarities in above-ground biomass results between the two experiments, the significant differences in total above-ground biomass production between varieties at low N supply in the field could most likely be attributed to differences in below-ground biomass production, as demonstrated in the pot study. This was also confirmed by Robinson *et al.* (2009) in a similar field trial with Australian sugarcane genotypes, which showed that the genotype with significantly greater below-ground biomass compared to all other genotypes, performed better at low N supply.

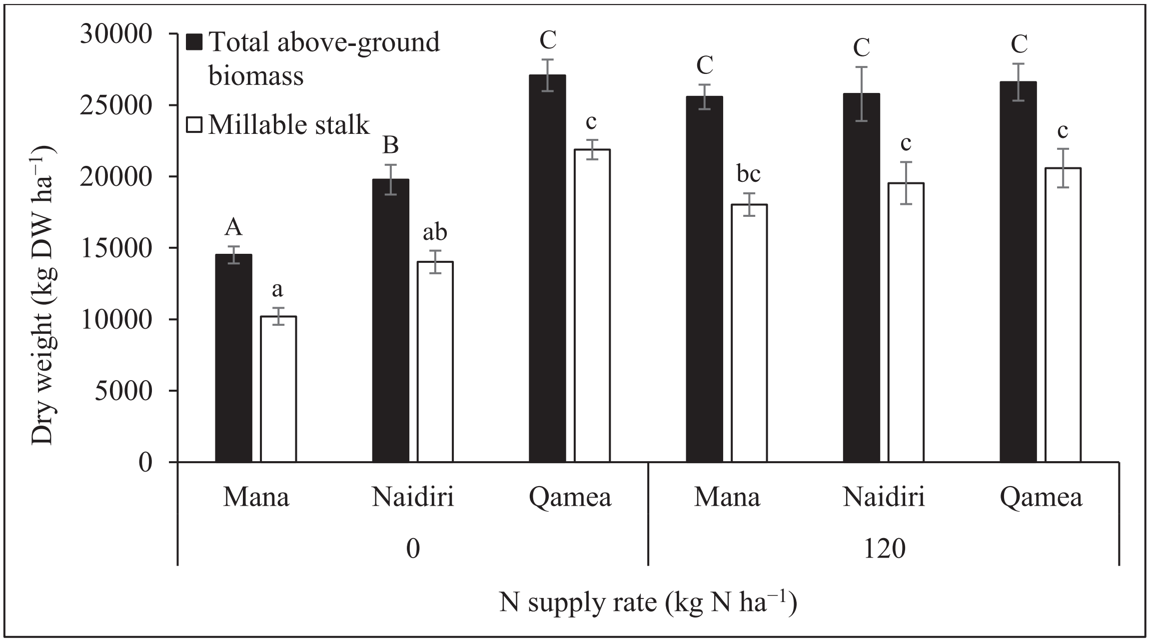


Fig. 3.1 The effect of N supply on total above-ground biomass and millable stalk production in three Fijian sugarcane varieties at maturity under field conditions. Different uppercase (A-C) and lowercase letters (a-c) denote significant differences for total above-ground biomass and millable stalk dry weight, respectively (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$). Vertical bars represent ± S.E. of the mean (9 plants).

Table 3.2. The effect of N supply rate on the cane yield (CY) and sugar yield (SY) in three Fijian sugarcane varieties at maturity under field conditions.

N supply rate (kg N ha^{-1})	Variety	Cane yield (Mg ha^{-1})	Sugar yield (Mg ha^{-1})
0 (low N)	Mana	70.34 ± 7.07^{a}	7.12 ± 0.22^{a}
	Naidiri	96.21 ± 8.57^{ab}	9.00 ± 0.30^{a}
	Qamea	128.86 ± 8.02^{c}	18.95 ± 0.29^{c}
120 (high N)	Mana	125.33 ± 7.66^{bc}	12.43 ± 0.56^{b}
	Naidiri	127.97 ± 4.00^{c}	14.24 ± 0.43^{b}
	Qamea	130.24 ± 6.14^{c}	18.16 ± 0.66^{c}

Data represent the mean of nine plants (cane yield) and three plants (sugar yield) ± S.E. Means with the same letters (a-c) within a column are not significantly different (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$).

3.3.2 N accumulation

Total N accumulation in above-ground biomass (Fig. 3.2) displayed mostly similar patterns to biomass production responses under the two N supply rates. Total N content in Mana and Naidiri was significantly lower in the low N treatment than high N treatment. The same was reported by Robinson *et al.* (2008) for Australian sugarcane genotypes. However, Qamea showed no significant difference between the two N treatments. This provides further evidence that Qamea is well adapted to meet its N demand irrespective of N supply. Qamea and Mana were found to have the highest and the lowest N content, respectively, at both N supply rates. As with biomass production, N accumulation results also agree with the earlier pot study (Chapter 2).

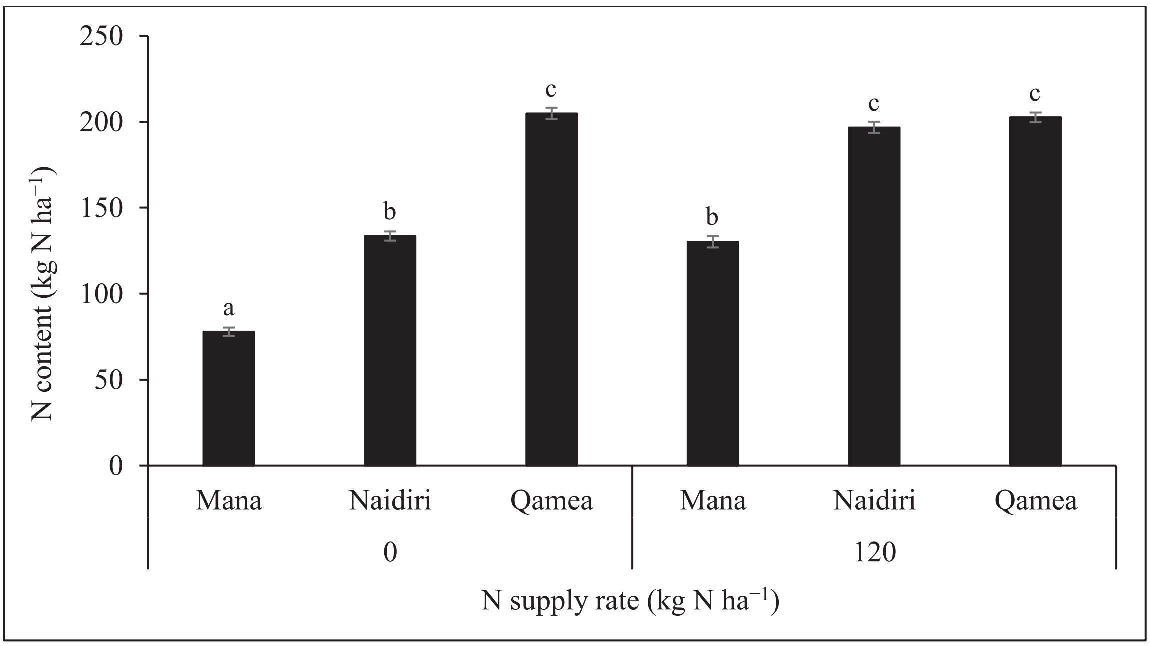


Fig. 3.2 The effect of N supply on total N accumulation in total above-ground biomass in three Fijian sugarcane varieties at maturity under field conditions. Different letters (a-c) denote significant differences (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$). Vertical bars represent ± S.E. of the mean (9 plants).

3.3.3 Sucrose content and sugar yield

Sucrose content on a fresh weight basis (Fig. 3.3) was determined at maturity to elucidate yield responses of the three varieties to varied N conditions. Mana and Naidiri were highly responsive to N levels and hence showed significantly increased sucrose accumulation under high N treatment. Qamea was unaffected by N supply and produced the highest sucrose yield (relative to the other two varieties) at both N supply levels. These findings are in agreement with those of Schumann *et al.* (1998) and Weigel *et al.* (2010), who demonstrated that certain South African sugarcane varieties produced higher sucrose yields at low N levels and benefitted very little from higher N nutrition while others had superior yields at high N fertility due to increased total N accumulation in shoots and greater stalk mass. Sugar yield (SY) closely followed the trends in cane yield as presented in Table 3.2 for comparison. It ranged from 7.12 ± 0.22 to 18.95 ± 0.29 Mg ha^{-1}. Acreche *et al.* (2015) reported sugar yield ranging between 3.38 and 10.53 Mg ha^{-1} in sugarcane plant crop in Argentina while Thompson (1988) and Weigel *et al.* (2010) both recorded up to 21 Mg ha^{-1} in South African varieties.

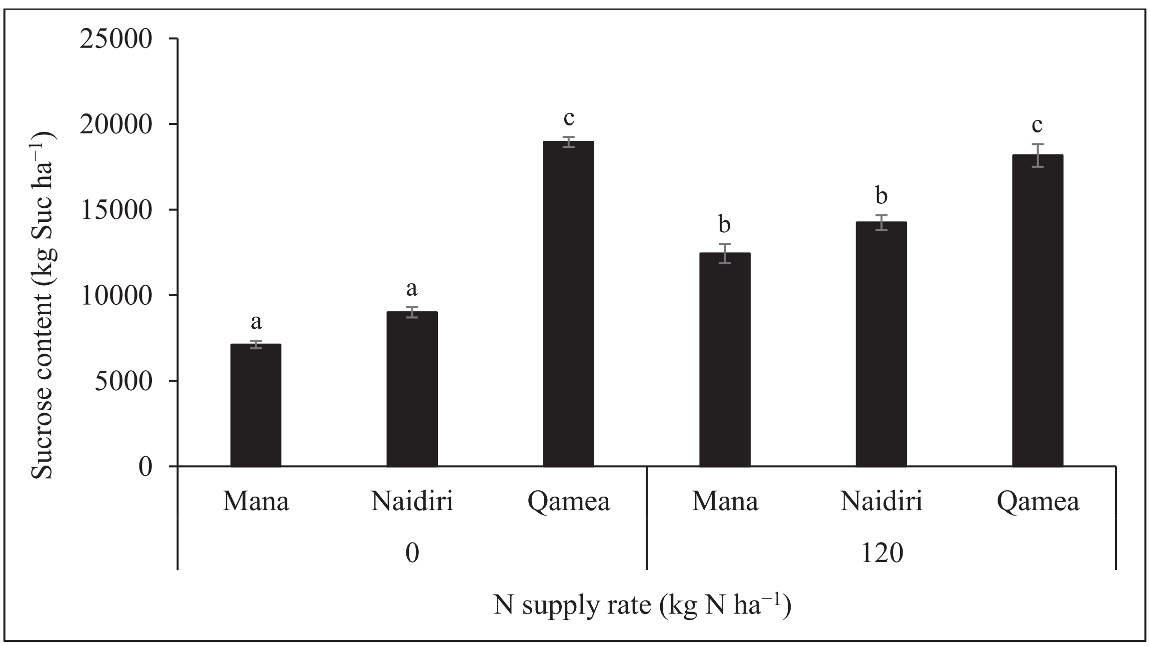


Fig. 3.3 The effect of N supply on sucrose content in three Fijian sugarcane varieties at maturity under field conditions. Different letters (a-c) denote significant differences (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$). Vertical bars represent ± S.E. of the mean (3 plants).

3.3.4 Determination of NUpE, NUtE and NUE

Nitrogen use efficiency and its subcomponents, nitrogen uptake efficiency and nitrogen utilization efficiency (also referred to as internal nitrogen use efficiency), were calculated in terms of both biomass production and sucrose yield (Table 3.3) to determine any variation between the varieties for specific traits at the two N supply levels.

Table 3.3. The effect of N supply on N uptake efficiency (NUpE), N utilization efficiency (NUtE) and N use efficiency (NUE) in three Fijian sugarcane varieties at maturity under field conditions.

N supply rate (kg N ha^{-1})	Variety	NUpE (kg N_b kg N_s^{-1})	In terms of biomass production		In terms of sucrose yield	
			NUtE (kg DW kg N_b^{-1})	NUE (kg DW kg N_s^{-1})	NUtE (kg Suc kg N_b^{-1})	NUE (kg Suc kg N_s^{-1})
0 (low N)	Mana	0.350 ± 0.011^{a}	132.12 ± 8.76^{b}	45.92 ± 2.66^{a}	91.43 ± 2.84^{b}	32.01 ± 0.99^{a}
	Naidiri	0.601 ± 0.012^{b}	104.73 ± 4.91^{a}	63.05 ± 3.56^{b}	67.39 ± 2.24^{a}	40.48 ± 1.35^{b}
	Qamea	0.922 ± 0.015^{c}	106.98 ± 3.60^{a}	98.42 ± 3.06^{c}	92.51 ± 1.43^{b}	85.25 ± 1.32^{d}
120 (high N)	Mana	0.380 ± 0.010^{a}	138.32 ± 4.18^{b}	52.68 ± 2.31^{ab}	95.47 ± 4.30^{b}	36.31 ± 1.63^{ab}
	Naidiri	0.575 ± 0.010^{b}	99.05 ± 6.90^{a}	57.08 ± 4.30^{b}	72.42 ± 2.17^{a}	41.61 ± 1.25^{b}
	Qamea	0.592 ± 0.008^{b}	102.09 ± 7.40^{a}	60.14 ± 3.95^{b}	89.70 ± 3.27^{b}	53.06 ± 1.94^{c}

Data represent the mean of nine plants (NUpE, and NUtE and NUE in terms of biomass production) and three plants (NUtE and NUE in terms of sucrose yield) ± S.E. Means with the same letters (a-d) within a column are not significantly different (ANOVA with Tukey HSD pairwise comparisons, $p < 0.05$).

3.3.4.1 NUpE

NUpE, the ratio of N accumulated in total above-ground biomass and N available in soil, remains the same whether NUE is calculated on the basis of biomass production or sucrose yield. Contrary to the findings of Acreche (2017), all varieties varied significantly for NUpE at low N supply, with Qamea and Mana exhibiting the highest and the lowest NUpE, respectively. At high N supply, no significant difference was observed between Qamea and Naidiri and both were found to have significantly higher NUpE than Mana. The same trend was observed in total N accumulation. NUpE of Qamea was significantly higher under low N compared with high N condition, while that of Mana and Naidiri remained the same under both N conditions. This could explain why total N content in Qamea remains the same regardless of N supply, whereas total N content in Mana and Naidiri significantly increases with high N supply (see Section 2.3.3 for a discussion on the relationship between NUpE and N content in above-ground biomass).

3.3.4.2 NUtE and NUE in terms of biomass production

NUtE was significantly higher in Mana than the other two varieties under both N treatments. This was opposite to the trend observed for NUpE. Internal nitrogen use efficiency of Australian genotypes were found to be generally higher at low N supply (Robinson *et al.*, 2008) however, the rate of N supply had no effect on NUtE of any of the varieties in this study. NUE of the varieties varied significantly at low N supply but there were no differences between varieties at high N supply. Qamea had the highest NUE (98.42 ± 3.06 kg DW kg N_s^{-1}) and Mana had the lowest (45.92 ± 2.66 kg DW kg N_s^{-1}) under low N supply. NUE of Qamea was significantly higher in the low N treatment than high N treatment but there was no significant effect of N supply on NUE of Mana and Naidiri. This could be attributed to the significantly higher NUpE in Qamea with low N supply and indicates that NUpE contributes more strongly than NUtE to overall NUE in terms of biomass production, under low N conditions. However, neither of the two NUE traits seems to have significantly greater contribution to overall NUE under high N conditions. Despite significantly higher NUpE in Qamea and Naidiri compared with Mana, and conversely, a significantly higher NUtE in Mana than in Naidiri and Qamea, no significant difference was observed in NUE between the varieties at high N supply.

These findings are in accordance with the results obtained in the pot experiment (Chapter 2).

3.3.4.3 NUtE and NUE in terms of sucrose yield

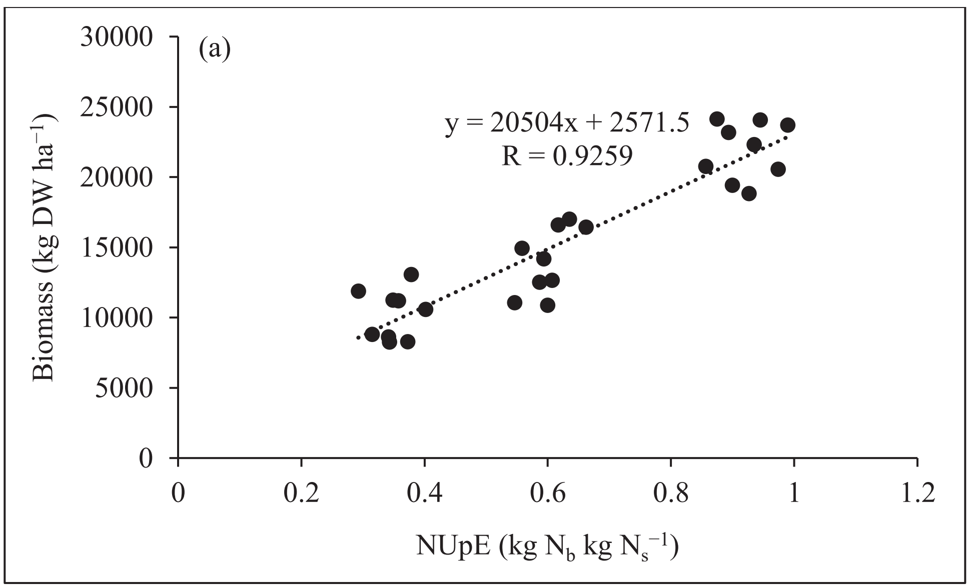
Mana and Qamea displayed significantly higher NUtE than Naidiri in both N treatments. There was no effect of N supply on NUtE of any of the varieties. Varietal differences in NUE were also significant at both N supply levels. Qamea exhibited the highest NUE under the two N conditions. NUE of Mana and Naidiri remained the same between N treatments however, Qamea showed a significantly higher NUE at low N supply compared with high N supply. As with NUE in terms of biomass production, this could be attributed to the significantly higher NUpE in Qamea with low N supply. Another clear indication that NUpE contributes more significantly than NUtE to overall NUE under low N conditions, is the significantly higher NUE exhibited by Naidiri compared with Mana, which had a significantly higher NUtE than Naidiri. The effect of NUpE overshadowed the effect of NUtE on the overall NUE of Naidiri under low N supply (for a fuller discussion on the relative effects of NUpE and NUtE on overall NUE, see Section 2.3.3). As was observed with NUE in terms of biomass production, neither of the two subcomponents had a significantly greater contribution to overall NUE in terms of sucrose yield, under high N supply. Naidiri had a significantly higher NUpE compared with Mana, and conversely, Mana had a significantly higher NUtE than Naidiri, but no significant difference was observed in NUE between the two varieties at high N supply.

3.3.5 Relationship between NUE, biomass production and sucrose yield

The relationship between NUE, biomass production and sucrose yield was investigated by comparing the varieties under varied N supply. The results of the present study showed that there is considerable variation between the studied varieties for biomass production and sucrose yield responses, which could be attributed to varietal differences observed in NUE. When grown with low N supply, Qamea was found to have the highest NUE in terms of both biomass production and sucrose yield, and consequently exhibited the highest above-ground biomass and sucrose content. Similarly, Mana had the lowest NUE, which resulted in the lowest above-ground biomass production and sucrose content. At high N supply, no significant differences were determined for NUE in terms of biomass production, and thus all varieties showed similar response to above-ground biomass production. However, in terms of sucrose yield, Qamea had a significantly higher NUE than the other two varieties and this was also reflected in the sucrose content of the varieties.

Above-ground biomass production responses in relation to the NUE of the varieties, were mostly consistent under both greenhouse and field conditions. Varietal differences in biomass production were significant only at low N supply. The highest above-ground biomass was observed in Qamea, which also displayed the highest NUpE. The opposite was true for Mana. In contrast, NUtE was significantly higher in Mana compared with Qamea. This finding contradicts with previous studies (Robinson *et al.*, 2007; Hajari *et al.*, 2017), which showed that genotypes with the highest NUtE produced the highest above-ground biomass. A possible reason for this observation has been explained in the previous chapter (see Section 2.3.3). It was suggested that above-ground biomass production under low N conditions greatly depended on total N accumulation in biomass, which is directly related to NUpE of the varieties. This was also depicted in the current study, with Qamea exhibiting a significantly higher N content than Mana. Consequently, there was a significant ($p < 0.00001$) positive correlation between NUpE and biomass production at low N supply (Fig. 3.4a) but no significant correlation was observed between NUtE and biomass production. However, under high N condition, there was no significant correlation between either of the two NUE traits with biomass production. Despite significantly higher NUpE in Qamea and Naidiri compared with Mana, and conversely, a significantly higher NUtE in Mana than in Naidiri and Qamea, NUE did not differ significantly between the varieties. As a result, no significant difference was observed in biomass production between the three varieties in the high N treatment.

Sucrose yield responses generally followed similar patterns to biomass production under the two N supply rates. As with biomass production, varietal differences in sucrose content at low N application rate was found to be associated with differences in NUpE. There was a significant ($p < 0.0001$) positive correlation between NUpE and sucrose content at low N supply (Fig. 3.4b) but no significant correlation was observed between NUtE and sucrose content. This could be due to the same reason described earlier, for above-ground biomass production under low N condition. At high N application rate, there was no significant correlation between NUpE or NUtE with sucrose content. Although Naidiri had a significantly higher NUpE compared with Mana, and conversely, Mana had a significantly higher NUtE than Naidiri, no significant difference was observed in NUE between the varieties. Hence, there was no significant difference in sucrose content between the two varieties under high N condition.



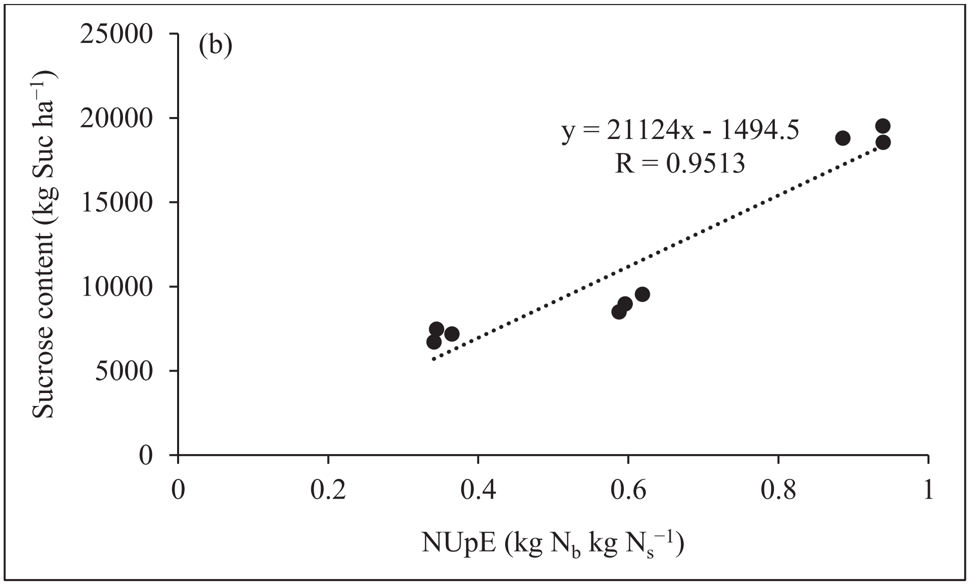


Fig. 3.4 The relationship between nitrogen uptake efficiency (NUpE, kg N_b kg N_s^{-1}) and (a) total above-ground biomass production (kg DW ha^{-1}) and (b) sucrose content (kg Suc ha^{-1}) in three Fijian sugarcane varieties at maturity with low N supply under field conditions.

Comparing the above-ground biomass production and sucrose yield under low and high N supply revealed significant differences in N response between the varieties. Mana and Naidiri were both found to produce significantly reduced above-ground biomass and sucrose yield with low N supply. Zhao *et al.* (2014) also reported suppressed shoot DW accumulation due to N deficiency in three sugarcane genotypes grown under low N application rates in Florida. However, total above-ground biomass production and sucrose content in Qamea at low N supply showed no significant differences from that observed under high N supply. This demonstrates adaptability of Qamea to N limitation. A further proof of the ability of Qamea to meet its N demand under varying N supply can be found by comparing N accumulation in above-ground biomass at the two N supply rates. While total N content in Mana and Naidiri was significantly lower in the low N treatment, Qamea showed no significant difference between the two N treatments. As discussed earlier, this could be due to increased allocation to below-ground biomass (Robinson *et al.*, 2009) in Qamea under low N condition, which was demonstrated in the pot trial (Chapter 2). Increased below-ground biomass allows exploitation of a greater soil volume which significantly increases N acquisition (Thorburn *et al.*, 2003; Smith *et al.*, 2005; Robinson *et al.*, 2009) and storage (Robinson *et al.*, 2009, 2013; Hawkesford and Howarth, 2011), and may well explain the significantly higher NUpE in Qamea with low N supply. Conversely, the fact that N uptake in plants is generally controlled by negative feedback mechanisms and consequently any further uptake is inhibited once N demand is satisfied (Glass, 2003; Robinson *et al.*, 2009; Gastal *et al.*, 2015), could be the possible reason for the significantly lower NUpE in Qamea with high N supply. These findings indicate that, while Mana and Naidiri perform better with increased N supply, Qamea is ideally adapted to produce maximum above-ground biomass and sucrose yield under both N conditions.

3.4 Conclusion

This study determined nitrogen use efficiency of three Fijian sugarcane varieties under field conditions with varied N supply. At low (no N fertilizer applied) N supply, Qamea (LF94-694) had a significantly higher NUE in terms of both biomass production and sucrose yield than Mana (LF60-3917) and Naidiri (LF82-2122), which resulted in a significantly higher above-ground biomass and sucrose content compared to the other two varieties. This was attributed to the significantly higher NUpE in Qamea with low N supply and indicates that NUpE has a greater contribution to overall NUE under low N conditions. At high (120 kg ha^{-1} N applied as urea) N supply, Qamea exhibited a significantly higher NUE in terms of sucrose yield than Mana and Naidiri however, NUE in terms of biomass production did not differ significantly between the varieties. Neither of the two subcomponents of NUE was found to have a significantly greater contribution to overall NUE under high N condition. It is clearly evident from the results of the present study and those of the earlier pot study (Chapter 2) that Mana and Naidiri are better adapted to high N conditions in terms of yield production, whereas Qamea has the ability to perform equally well under both N conditions. Further work is currently underway to determine NUE in three subsequent ratoon crops and elucidate any differences that may exist between plant and ratoon crops.

Chapter 4

Implications of nitrogen use efficiency of three Fijian sugarcane (*Saccharum officinarum* L.) varieties for the Fijian sugar industry

This study provided first evidence that there is considerable genetic variation for nitrogen use efficiency (NUE) between commercially grown Fijian sugarcane varieties which is critical for sustainable sugarcane production in the future. The results of pot and field experiments revealed that Qamea (LF94-694) had a significantly higher NUE in terms of both biomass production and sucrose yield than Mana (LF60-3917) and Naidiri (LF82-2122) under low N conditions. Consequently, total above-ground biomass production and sucrose yield was significantly higher in Qamea compared with the other two varieties at low N supply. At high N supply, Qamea exhibited a significantly higher NUE in terms of sucrose yield than Mana and Naidiri but NUE in terms of biomass production did not differ significantly between the varieties. Mana and Naidiri were highly responsive to N levels hence showed significantly increased above-ground biomass and sucrose accumulation under high N treatment. However, no yield benefit from increased N nutrition was observed in Qamea.

The underlying physiological mechanisms causing biomass production and sucrose yield responses to N supply in relation to NUE were explained by examining the contributions of NUpE and NUtE. It was demonstrated that total above-ground biomass production and sucrose content under low N conditions greatly depended on total N accumulation in biomass, which is directly related to NUpE of the varieties. Qamea had a significantly higher NUpE with low N compared to high N supply. This was attributed to increased allocation to below-ground biomass in Qamea under low N condition, which allows exploitation of a greater soil volume and significantly increases N acquisition and storage. As a result, while total N content in Mana and Naidiri was significantly decreased in the low N treatment, Qamea showed no significant difference between the two N treatments and the same was reflected in the above-ground biomass production and sucrose yield of the varieties. A significant positive correlation was found between both biomass production and sucrose yield with NUpE but no significant correlation was observed with NUtE at low N supply. This lends confidence to the notion that NUpE has a greater contribution than NUtE to overall NUE under low N conditions. However, under high N condition, there was no significant correlation between either of the two NUE traits with biomass production or sucrose yield. This study reaffirms the importance of evaluating both N uptake and internal N use efficiencies when assessing sugarcane varieties for N use efficiency, as highlighted by other researchers (Robinson *et al.*, 2007, 2009; Hajari *et al.*, 2015, 2017; Snyman *et al.*, 2015).

Hirel *et al.* (2007) stated that identification of genotypes that are adapted to low N or high N conditions, and those that can perform well irrespective of N supply, is a prerequisite for maintaining high crop productivity under low N fertilizer input. It was clearly evident from the results obtained under greenhouse and field conditions that Mana and Naidiri perform better with increased N supply, whereas Qamea is ideally adapted to produce maximum above-ground biomass and sucrose yield under both N conditions. This is a significant finding with implications for the Fijian sugar industry. It highlights the need to consider variety-specific N fertilizer recommendations (Meyer *et al.*, 2007) for improving N management that would mitigate the devastating environmental impacts of N losses from sugarcane production systems. Fijian sugarcane varieties have not been selected for NUE however, the findings of this study indicate that potential exists for breeding new varieties with improved NUE from existing varieties such as Qamea that can produce high cane and sugar yield under low N supply without a decline in performance under high N supply. This is increasingly important for maximizing sugarcane yield on low fertility soils, minimizing N fertilizer input and saving fertilizer costs that will ensure both economic and environmental sustainability of the Fijian sugar industry.

Further work is currently underway to determine NUE in three subsequent ratoon crops and elucidate any differences that may exist between plant and ratoon crops. However, the unavailability of published data on the rate of net N mineralization in Fijian sugarcane-growing soils and the rate of N losses via various pathways, are considerable limitations of these studies. As a result, for the purpose of this study, mineralized N was estimated at

2 % of the total soil N content in the root zone, based on Angus (2001) and the loss of N from the system was considered to be negligible as per Acreche (2017). Apart from addressing these limitations, avenues for future research could include:

- ✓ evaluation of NUE of other varieties in Fijian sugarcane germplasm and determination of variety-specific N requirements (Meyer *et al.*, 2007).
- ✓ identification of genes associated with increased NUpE in Qamea under low N conditions for NUE improvement in other varieties through transgenesis (Whan *et al.*, 2010; Snyman *et al.*, 2015; Khan *et al.*, 2019).
- ✓ isolation and characterization of nitrogen-fixing (diazotrophic) bacteria from the core root microbiome of sugarcane varieties and quantification of the contribution of biological nitrogen fixation (BNF) to the plant total N uptake (Boddey *et al.*, 1991; Asis *et al.*, 2002; Liu *et al.*, 2014; Yeoh *et al.*, 2016; Schultz *et al.*, 2017; Oliver and de Almeida Silva, 2018).

Since the results were generally consistent in pot and field experiments and varietal differences were significant mostly at low N supply, greenhouse pot trials under limiting N conditions is recommended for initial screening of sugarcane varieties for NUE before final confirmation is obtained under field conditions.

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