Provision of nitrogen as ammonium rather than nitrate increases silicon uptake in sugarcane

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Abstract. Silicon (Si) is important in mitigating abiotic and biotic plant stresses, yet many agricultural soils, such as those of the rainfed production areas of the South African sugar industry, are deficient in plant-available Si, making Si supplementation necessary. However, Si uptake by sugarcane (Saccharum spp. hybrids) is limited even where silicate amendments improve soil Si status. Rhizosphere pH, which can affect Si uptake, can be manipulated using different N-form fertilizers. We tested whether (i) fertilization with NH$_4^+$ (rhizosphere acidification) increased Si uptake compared with NO$_3^−$ (rhizosphere alkalinization); and (ii) uptake differed between an N-efficient, more acid-tolerant cultivar (N12) and an N-inefficient, less acid-tolerant cultivar (N14). Two pot trials with low-Si soil were fertilized with calcium silicate (Ca$_2$SiO$_4$) slag, plus N from ammonium sulphate [(NH$_4$)$_2$SO$_4$], ammonium thiosulphate [(NH$_4$)$_2$S$_2$O$_3$] and calcium nitrate [Ca(NO$_3$)$_2$] (Trial 1) or N from (NH$_4$)$_2$S$_2$O$_3$ and Ca(NO$_3$)$_2$ only (Trial 2). Nitrate treatments significantly increased soil pH and soil Si compared with NH$_4^+$ treatments. However, NH$_4^+$ treatments significantly increased leaf and stalk Si content compared with NO$_3^−$, reflected in a significant negative relationship between soil pH and leaf Si. Acid-extracted soil Si was negatively related to leaf and stalk Si, likely due to adsorption of silicic acid to soil surfaces under higher pH of the NO$_3^−$ treatment and its reduced availability for plant uptake. We conclude that NH$_4^+$ increased Si uptake into leaf and stalk, and propose that reduced rhizosphere pH solubilized Si from Ca$_2$SiO$_4$ and increased silicic acid availability for plant uptake. By contrast, NO$_3^−$ may have reduced Si uptake due to adsorption of Si to soil surfaces at higher pH. Our results indicate that ammoniacal fertilizers, such as (NH$_4$)$_2$SO$_4$ and urea, have potential for promoting dissolution of applied Ca$_2$SiO$_4$ and subsequent uptake of Si by sugarcane.

Keywords: Ammonium; calcium silicate; nitrate; nitrogen; pH; rhizosphere; silicon uptake; sugarcane.

Introduction

Silicon (Si) is the second most abundant element in the Earth’s crust after oxygen, where it may constitute 28% of the soil mass as silicate minerals and water-soluble monosilicic acid (H$_4$SiO$_4$) (Epstein 2001). However, in the humid tropics and subtropics high rainfall and temperatures have subjected soils to intensive chemical weathering over the millennia, leading to a loss of soluble Si (desilication) through leaching or erosion, and the development of soils with low base saturation and that are high in aluminium and iron sesquioxides (McKeague and Cline 1963c; Savant et al. 1997a; Epstein 2001). Furthermore, crops such as

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rice (Oryza sativa) and sugarcane (Saccharum spp. hybrids), which are Si accumulators (>1.0 % shoot Si) (Ma et al. 2001; Gunter et al. 2012), are capable of removing up to 470 and 500 kg Si ha⁻¹ annum⁻¹, respectively, on productive, Si-rich soils (Ross et al. 1974; Savant et al. 1997b, 1999). On weathered low-Si soils, intensive farming of such crops can therefore aggravate the depletion of plant-available reserves of Si (i.e. silicic acid) and result in crops deficient in this nutrient (Savant et al. 1997a; Berthelsen et al. 2001b; Gunter et al. 2012).

Increasing evidence of the role of Si in plant adaptations that mitigate a range of abiotic and biotic stressors (for recent reviews, see Liang et al. 2007; Epstein 2009; Romero et al. 2011; Marafon and Endres 2013) has highlighted the need to provide certain crops with supplemental Si, to moderate yield losses where plant-available soil Si is low (<10 mg L⁻¹; 0.01 M CaCl₂ extraction) (Berthelsen et al. 2001a). This is particularly important where there is likely to be some form of plant stress (Winslow et al. 1997; Berthelsen et al. 2001b; Ma et al. 2001). In sub-Saharan Africa, including Madagascar, widespread yield reductions and disease susceptibility in rice have been ascribed to Si deficiencies, particularly in acidic, weathered Oxisol and Ultisol soils of highland and humid agro-ecological zones (Winslow 1992; Tsujiimoto et al. 2014). In South Africa, comparable soil and environmental conditions are associated with leaf Si deficiencies in sugarcane in the rainfed (non-irrigated) areas of production (Meyer et al. 1998; Meyer and Keeping 2001; Van der Laan and Miles 2010; Miles et al. 2014). Both pot and field experiments have shown that the correction of such deficiencies through Si provision in the form of calcium silicate, blast furnace slags and cement, could significantly increase leaf Si content up to 16 g kg⁻¹ dry matter and cane yield up to 53 % above unamended controls and lime treatments applied at the same rate (Du Preez 1970; Meyer and Keeping 2001). The silicate treatments decreased exchangeable Al and Mn, and increased pH to an extent similar to that of lime; hence the increased yields in the silicate treatments were due not only to elimination of toxic amounts of Al and Mn, but also to provision of sufficient Si to optimize yields (Du Preez 1970; Meyer and Keeping 2001). Similar results with sugarcane have been obtained elsewhere in the world in desilicated soils (for reviews, see: Savant et al. 1999; Alvarez and Datnoff 2001; Berthelsen et al. 2001b).

Average leaf Si content of sugarcane grown in coastal and inland non-irrigated areas of the South African sugar industry has, since first recorded in 2001, seldom exceeded the industry threshold (Miles and Rhodes 2013) of 7.5 g kg⁻¹ dry matter; in contrast, that of cane in the northern irrigated areas, where soils are less acid (pH > 6.5), ranged from 10 to 25 g kg⁻¹ (Van der Laan and Miles 2010). Furthermore, and notwithstanding the results of the experiments described by Du Preez (1970) and Meyer and Keeping (2001), more recent field trials with Si materials applied to low-Si soils (<10 mg L⁻¹ Si; 0.01 M CaCl₂) in the rainfed regions have failed to produce leaf Si values above the 7.5 g kg⁻¹ threshold, despite the greatly improved Si status of the soil and the strong liming capacity of the materials used (Keeping et al. 2013; Rhodes et al. 2013). Keeping et al. (2013) listed a number of possible reasons for this, in particular reduced Si solubility at higher pH due to increased adsorption of silicic acid to iron and aluminium sesquioxides (Beckwith and Reeve 1963; Jones and Handreck 1963; McKeague and Cline 1963b; Tavakkoli et al. 2011). On the other hand, Korndörfer et al. (2005) have argued that increases in pH in weathered soil promote the release of colloid-adsorbed Si to the soil solution and the transformation of polysilicic acid into monosilicic acid. In support of this, Oliveira et al. (2007) found that an increase in rhizosphere pH through fertilization with nitrogen (N) in the nitrate (NO₃⁻) rather than ammonium (NH₄⁺) form increased soil Si solubility and uptake in rice. However, in a 5-year field rotational cropping experiment including sugarcane, corn (Zea mays) and kikuyu grass (Pennisetum clandestinum), Khalid et al. (1978) found that relative amounts of applied Si recovered by plants decreased with increasing soil pH, while that extracted from the soil increased with increasing pH. The influence of soil pH on Si uptake is therefore contentious and further investigation is clearly necessary.

As part of a continuing effort to maximize Si uptake from applied calcium silicate (Ca₂SiO₄) sources under the rainfed growing conditions of sugarcane in South Africa, we reasoned that, based on these earlier findings, manipulation of fertilizer N-form may assist in solubilizing Si from applied Ca₂SiO₄. Although Ca₂SiO₄ is barely soluble in water at neutral pH, it will dissociate in a more acidic (soil) solution (Ma and Takahashi 2002). Hence, we predicted that reduced rhizosphere pH, resulting either from bulk acidification of soil due to nitrification of NH₄⁺ supplied as ammonium sulphate [(NH₄)₂SO₄] (Reaction A below) or from H⁺ ion extrusion from the root to balance charges following NH₄⁺ uptake (Reaction B below) (Thomson et al. 1993; Marschner 1995; Hinsinger et al. 2003), was the most likely mechanism whereby Si could be solubilized from Ca₂SiO₄, with both processes making silicic acid available in the immediate root environment.

Reaction A (bulk soil acidification):

\[ \text{NH}_4^+ + 2\text{O}_2 \rightarrow 2\text{H}^+ + \text{NO}_3^- + \text{H}_2\text{O} \]  \hspace{1cm} (1)

Reaction B (from H⁺ ion extrusion):

\[ 2\text{H}^+ + \text{Ca}_2\text{SiO}_4 \rightarrow \text{H}_6\text{SiO}_4 + 2\text{Ca}^{2+} \]  \hspace{1cm} (2)
Reaction B (H⁺ extrusion from root):

\[
\left(\text{NH}_4\right)_2\text{SO}_4 \rightarrow 2\text{NH}_4^+ + \text{SO}_4^{2-} \quad (3)
\]

\[2\text{NH}_4^+ \text{ produced by (3) is taken up by roots; } 2\text{H}^+ \text{ is exuded from the roots to balance charges and reacts with the product of (3) as follows:}\]

\[2\text{H}^+ + \text{SO}_4^{2-} \rightarrow \text{H}_2\text{SO}_4 \quad (4)
\]

\[2\text{H}_2\text{SO}_4 + \text{Ca}_2\text{SiO}_4 \rightarrow \text{H}_6\text{SiO}_4 + \text{CaSO}_4 \quad (5)
\]

Importantly, the decreased adsorption of silicic acid onto clay particles at low pH (see above) would allow more of the solubilized Si to remain in the soil solution and be available for plant uptake.

To investigate the influence of N-form on soil pH and Si uptake, we conducted two trials using potted sugarcane plants. Both trials tested the primary hypothesis that Si uptake and cane yield following fertilization with Ca₂SiO₄ would be differentially affected by simultaneous treatment of plants with either \(\text{NH}_4^+\) (rhizosphere acidification) or \(\text{NO}_3^-\) (rhizosphere alkalinization) as N-form treatments. In addition, the second trial tested the hypothesis that Si uptake and cane yield responses to Ca₂SiO₄ application under these N-form treatments would differ between an N-efficient, more acid-tolerant cultivar and an N-inefficient, less acid-tolerant cultivar. Specifically, we predicted that an N-efficient, more acid-tolerant cultivar may yield better than its counter-part due to its lower demand for N (Meyer et al. 2007) and greater tolerance of the more acidic soil than its counter-part due to its lower demand for N (Meyer et al. 2007).

**Methods**

**Trial establishment**

The two trials were conducted in a shadehouse with clear polycarbonate roofing and walls of 40% green shade cloth, over two successive seasons (December 2009 and 2010). Pot trials were preferred to field trials as they afford superior control over extraneous conditions (and especially variability in soil composition) when basic principles, such as those studied here, are under investigation.

Sugarcane transplants were produced from single budded setts, cut from mature stalks in field-grown plots of commercial sugarcane of the same age and from the same field. For both trials, 1-month-old transplants were planted into 3.0 L plant pots (one seedling per pot) placed on individual drip trays. Each pot contained 3.5 kg (dry weight) of soil sieved through a 1.0-mm mesh. The required amount of soil was collected from the top 15-cm soil layer at the same locality (Gratton Farm, Eshowe, South Africa, 31°28’29”E; 28°51’21”S; 473 m abls) and from the same section of fallow field during November of each year. The soil type was a Glenrosa form, Inceptisol (Soil Survey Staff 2003), which in KwaZulu-Natal (South Africa) typically consists of grey loamy sands, moderately to strongly acid, with a general paucity of plant foods (Beater 1970). The soil was chosen as it is typical of the weathered, comparatively high acid saturation (i.e. high soluble Al) and low-Si soils of the rainfed regions of the South African sugar industry, as described earlier. The characteristics of the soil, based on samples taken from the field in September 2009, are given in Table 1. Liming is recommended for soils in the industry with an acid saturation index > 20%, except for cv. N12 (used in this study; see below), where a 40% threshold is applied (Schoeder et al. 1995).

Calcium silicate slag (Calmasil®, a stainless steel slag with 10.3% Si; supplied by PDB Holdings, Pty Ltd, Middelburg, South Africa) was supplied to all pots as a Si fertilizer by thoroughly incorporating it into the soil on a pot-by-pot basis at 12 g pot⁻¹ (=3429 mg Si kg⁻¹), which was equivalent to a product rate of 7.4 tons ha⁻¹ and a Si rate of 757 kg ha⁻¹. Trials 1 and 2 were planted 3 and 6 days, respectively, after Ca₂SiO₄ incorporation, followed by application of N treatments (see below) in aqueous solution at 200 mL pot⁻¹. Soil analyses (of five samples from the soil collected) by the South African Sugarcane Research Institute were performed to determine the availability of Si in the soil solution.

**Table 1. Characteristics of soil from Gratton Farm (Eshowe, South Africa) used in Trials 1 and 2 (mean values from five field samples, standard errors in parentheses).**

| P   | K   | Ca  | Mg  | Si | pH (water) | ASI (%) | OM (%) | Clay (%) | Sand (%) | Silt (%) |
|-----|-----|-----|-----|----|------------|---------|--------|----------|----------|----------|
| 59.2 (5.7) | 69.0 (2.3) | 109.8 (12.8) | 36.7 (3.2) | 8.4 (0.7) | 5.0 (0.04) | 26.4 (3.3) | 1.3 (0.1) | 8.8 (0.6) | 83.4 (0.4) | 7.4 (0.6) |
Institute’s (SASRI) Fertilizer Advisory Service (FAS) indicated that potassium \( (K) \) was required at 150 kg ha\(^{-1} \) as well as N at 120 kg ha\(^{-1} \). However, previous research (Fageria 2005) and our own experience have shown that fertilizer rates in pot trials need to be substantially increased above their field equivalents to ensure that leaf nutrient thresholds are attained. Therefore, K was applied to all pots in both trials as potassium chloride (KCl) at 300 kg K ha\(^{-1} \) (0.94 g KCl pot\(^{-1} \)). Nitrogen rates were similarly increased above those recommended for the field (see below). No other nutrient supplements were required.

Plants were watered daily to every third day with 100–500 mL at a time, depending on growth stage and ambient temperatures. Overflow of leachate from drip trays was avoided to prevent nutrient loss. Pots were spaced 1 m apart to allow easy access and to ensure plants had good light exposure.

**Treatments and design**

In Trial 1, three N treatments were balanced to provide 300 kg N ha\(^{-1} \) to all pots as follows: T1: \((\text{NH}_4\text{)}_2\text{SO}_4 (2.38 \text{ g pot}^{-1})\); T2: ammonium thiosulphate \([(\text{NH}_4\text{)}_2\text{S}_2\text{O}_3 (2.67 \text{ g pot}^{-1}) + \text{dicyandiamide (DCD; 15 mg pot}^{-1} \); T3: calcium nitrate \([\text{Ca(NO}_3)_2] (4.25 \text{ g pot}^{-1})\). Thiosulphate and DCD were used for T2 as they are both nitrification inhibitors and prevented the bacterial conversion of NH\(_4\) to NO\(_3\), while Treatment T1 was included to control for any potential effects of DCD on Si uptake. All pots were planted to the commercial cultivar N11 (Anon. 1986). Comparisons of T1 and T2 with T3 tested the primary hypothesis that Si uptake would be affected by different N-form treatments.

Based on the results of Trial 1, which showed no effects of DCD on Si uptake in T2 (see Results), DCD was excluded from Trial 2 and N rates were adjusted to 210 kg ha\(^{-1} \) for all N treatments. Plants were watered daily to every third day with 100–500 mL at a time, depending on growth stage and ambient temperatures. Overflow of leachate from drip trays was avoided to prevent nutrient loss. Pots were spaced 1 m apart to allow easy access and to ensure plants had good light exposure.

**Data collection and analysis**

**Soil analysis.** Trial 1 was harvested at 18 weeks and Trial 2 at 20 weeks. Trial duration was limited to this period to avoid root binding and water stress in the relatively small pots; use of larger pots was constrained by soil transport costs and logistics. Soil samples were taken individually from half the total number of pots (i.e. replicates) in each trial to produce six samples per treatment for Trial 1 and five samples per treatment for Trial 2. Samples were submitted to the SASRI FAS for pH and Si determination. In Trial 1, soil Si was extracted using 0.02 N sulphuric acid \( (\text{H}_2\text{SO}_4) \) (Kanamugire et al. 2006) only, while in Trial 2, 0.02 N \( \text{H}_2\text{SO}_4 \) and 0.01 M calcium chloride \( (\text{CaCl}_2) \) were used. The latter extraction method is now under general international use for determining readily available Si in soil solution, with satisfactory correlations with plant uptake; stronger acid extractants often show much weaker relationships with plant uptake as they also solubilize Si that is more strongly adsorbed onto sesquioxides and not readily available (Berthelsen et al. 2001b; Berthelsen and Korndörfer 2005; Sauer et al. 2006). Silicon concentrations were determined photometrically using the ammonium molybdate method described by Fox et al. (1969) for pH. pH was measured in water.

**Leaf, stalk and yield analysis.** Leaf samples (third fully unfurled or ‘top visible dewlap’ leaf) were taken at trial harvest from the major tillers in each pot; leaf blades were stripped from the midrib and the blades dried, ground and submitted as separate samples per pot for Si and N analysis by the SASRI FAS. Thereafter, all tillers were removed at the base, stripped of all leaf material and the stalks chopped into pieces ~1 cm long. The leaves from each pot were combined into bundles, while the chopped stalk pieces from each pot were combined into separate paper packets. All the material was dried to constant weight at an oven at 60 °C and the dry mass of leaf and stalk material determined individually from half the total number of pots (i.e. replicates) in each trial to produce six samples per treatment for Trial 1 and five samples per treatment for Trial 2. Samples were submitted to the SASRI FAS for pH and Si determination. In Trial 1, soil Si was extracted using 0.02 N sulphuric acid \( (\text{H}_2\text{SO}_4) \) (Kanamugire et al. 2006) only, while in Trial 2, 0.02 N \( \text{H}_2\text{SO}_4 \) and 0.01 M calcium chloride \( (\text{CaCl}_2) \) were used. The latter extraction method is now under general international use for determining readily available Si in soil solution, with satisfactory correlations with plant uptake; stronger acid extractants often show much weaker relationships with plant uptake as they also solubilize Si that is more strongly adsorbed onto sesquioxides and not readily available (Berthelsen et al. 2001b; Berthelsen and Korndörfer 2005; Sauer et al. 2006). Silicon concentrations were determined photometrically using the ammonium molybdate method described by Fox et al. (1969). pH was measured in water.

**Statistical analysis.** All data were tested for univariate normality (Anderson–Darling or Shapiro–Wilk tests) and homogeneity of variance (Bartlett’s test) prior to analysis of variance (ANOVA). Where conditions for parametric analysis were not met, log\(_{10}\) transformations were applied prior to ANOVA. Rather than back-transforming means, raw means (and their standard errors) for treatments were calculated for the purposes of presentation. Planned
comparisons of means were performed using Fisher’s protected least-significant difference (LSD) test. Linear regression analysis was performed using individual pot values across all treatments within each trial to examine relationships between soil pH, soil Si, leaf Si and stalk Si concentrations. GenStat 14th edn and SigmaPlot 12.5 were used for analyses.

**Results**

**Soil analysis**

The NH4 ions treatments lowered pH significantly compared with the NO3 treatment in both trials (Table 2). In Trial 1, soil Si extracted with 0.02 N H2SO4 was significantly lower in Treatment T2 than in T1 and T3. While soil Si in T1 was also lower than that of T3, the two treatments did not differ significantly (Table 2). In Trial 2, Si extracted using H2SO4 was significantly less in Treatment T1 than in T2 (Table 2), while values between T1 and T2 did not differ significantly using 0.01 M CaCl2 as an extractant (Table 2). It is notable that both soil Si and pH were appreciably higher in Trial 2 treatments compared with their equivalents in Trial 1 (Table 2; Fig. 1).

There was a significant positive relationship between soil pHwater and H2SO4-extractable soil Si in both trials (Fig. 1A and B), but no relationship between pH and CaCl2-extractable soil Si (Fig. 2A).

Cultivar did not affect soil Si content (both extractants), but pH was significantly higher for cv. N14 than for cv. N12 (Table 2). There were no significant N treatment × cultivar interactions for pH or soil Si.

**Leaf, stalk and yield analysis**

Leaf and stalk Si content increased significantly in the NH4+ treatments compared with the NO3 treatments in both trials, with no effect from the inclusion of a nitrification inhibitor (Table 3). There was no effect of cultivar on leaf or stalk Si content (Table 3). A significant negative relationship occurred between leaf Si content and soil pH at harvest in Trial 2 (Fig. 1D), but not in Trial 1 (Fig. 1C) where the relationship between the two variables was weakly negative. There was also a significant negative relationship between leaf Si content and H2SO4-extractable soil Si at harvest in Trial 2 (Fig. 3B), but not in Trial 1 (Fig. 3A) where the relationship was weakly negative; there was no significant relationship between leaf Si content and CaCl2-extractable soil Si in Trial 2 (Fig. 2B).

In Trial 2, stalk Si content was significantly negatively related to H2SO4-extractable soil Si (Fig. 4). Leaf and stalk Si content were significantly positively correlated (r = 0.78, P < 0.001, N = 20, Pearson’s correlation).

We found no effect of the N treatments on leaf N content in Trial 1 (Table 3). However, leaf N was significantly greater in T2 than in T1 in Trial 2 (Table 3); furthermore, stalk N content was significantly increased in T2 in this trial (Table 3).

Although cultivar did not affect leaf N content in Trial 2, stalk N in this trial was significantly greater in cv. N12 than in cv. N14 (Table 3). There were no significant interactions between N treatment and cultivar for leaf Si and leaf N, or stalk Si and stalk N in Trial 2 (Table 3).

Neither the N treatments nor cultivar treatments affected dry leaf or stalk mass at harvest (Table 4). There were no significant interactions between N treatment and cultivar for both yield parameters (Table 4).

**Discussion**

Our results support the hypothesis that reduced soil pH, resulting either from bulk acidification of soil due to nitrification of NH4 supplied as (NH4)2SO4 or (NH4)2S2O3 or from H+ ions exuded from the root to balance charges after NH4 uptake, increased the solubilization of Si from Ca2SiO4. As our treatments included a nitrification inhibitor (DCD or (NH4)2S2O3), it is likely that the balancing of cation charges through H+ exudation into the rhizosphere (Equations (3) – (5)) was the dominant mechanism for reducing rhizosphere pH under our experimental...
conditions. Under field conditions, both mechanisms could play a role, with their relative contributions depending on soil characteristics (e.g. pH buffering capacity) and cultivar (Haynes 1990; Hinsinger et al. 2003). This is the first published study to demonstrate that application of N in the form of NH$_4^+$ and an associated reduction in rhizosphere pH increased plant uptake of Si from an applied source.

Our results indicate that sugarcane cultivars can influence soil pH and that cv. N12 (the most acid- and Al-tolerant...
of South African sugarcane cultivars; Schroeder et al. 1995) may have a greater capacity to acidify the rhizosphere via $H^+$ exudation than cv. N14. Increased dissociation of $Ca_2SiO_4$ under more acidic soil conditions, as in Reaction B, would increase the availability of silicic acid for plant uptake, especially when this occurs in the immediate vicinity of the roots, as in Equations (3)–(5). Our study found no support for the alternative hypothesis that rhizosphere alkalinization, as a result of fertilization with $NO_3^−$, increased Si uptake into leaves or stalk. Our results concur with those of Leusch and Buchenauer (1989), who reported significantly increased Si uptake by wheat from applied silicate slag when the plants were fertilized with N as $NH_4^+$ rather than $NO_3^-$. There was also no evidence for a differential effect (i.e. no significant interaction) of N-form on Si uptake between cultivars, and cultivars showed no significant differences in leaf or stalk Si content. Hence, despite the lower soil pH associated with cv. N12, we cannot conclude that this N-efficient, more acid-tolerant cultivar (Schroeder et al. 1995; Schumann et al. 1998) is likely to take up more Si under low soil pH conditions than an N-inefficient, less acid-tolerant cultivar (cv. N14; Schumann et al. 1998).

However, in line with its greater N-efficiency, cv. N12 exhibited a higher stalk N content, although this did not manifest itself as increased yield in this cultivar compared with cv. N14. As the $NO_3^-$ treatment significantly increased leaf and stalk N content in Trial 2, it might be argued that plant Si content was reduced under the $NO_3^-$ treatment due to a dilution effect resulting from enhanced growth.

Table 3. Leaf and stalk Si and N content in Trials 1 and 2 at harvest (18 weeks for Trial 1 and 20 weeks for Trial 2). Values are means ± standard error. $T_0$ = N-form treatment. N = 12 (Trial 1) and 10 (Trial 2). Probability (P) values are from ANOVA; NS, not significant. Means within the same column followed by the same letter are not significantly different (Fisher’s protected LSD, P < 0.05).

| Treatment/statistic | Leaf Si (g kg$^{-1}$) | Leaf N (g kg$^{-1}$) | Stalk Si (g kg$^{-1}$) | Stalk N (g kg$^{-1}$) |
|---------------------|----------------------|---------------------|----------------------|----------------------|
| **Trial 1**         |                      |                     |                      |                      |
| T1 [(NH$_4$)$_2$SO$_4$] | 7.1 ± 0.4a          | 18.6 ± 0.7          | 11.1 ± 0.6a          | –                    |
| T2 [(NH$_4$)$_2$SiO$_3$ + DCD] | 7.3 ± 0.7a          | 19.6 ± 0.4          | 11.6 ± 0.9a          | –                    |
| T3 [Ca(NO$_3$)$_2$] | 5.4 ± 0.7b          | 19.1 ± 0.5          | 8.2 ± 0.7b           | –                    |
| P value             | <0.05                | 0.4                 | <0.001               | –                    |
| **Trial 2**         |                      |                     |                      |                      |
| T1 [(NH$_4$)$_2$SiO$_3$] | 10.8 ± 0.4          | 8.5 ± 0.6           | 6.4 ± 0.3            | 2.6 ± 0.4            |
| T2 [Ca(NO$_3$)$_2$] | 7.0 ± 0.5           | 11.2 ± 0.7          | 5.0 ± 0.3            | 5.3 ± 0.5            |
| P value             | <0.001               | <0.01               | 0.002                | <0.001               |
| Cultivar N12        | 8.4 ± 0.5           | 9.7 ± 0.7           | 5.5 ± 0.3            | 4.7 ± 0.5            |
| P value             | 0.2                  | 0.8                 | 0.3                  | <0.02                |
| P value: $T_0$ × cultivar | 0.6                  | 0.1                 | 0.9                  | 0.9                  |

Figure 3. Regression curves and equations for leaf Si concentration in Trial 1 (A) and Trial 2 (B) against 0.02 N $H_2SO_4$-extractable soil Si concentration at trial harvest (18 weeks for Trial 1 and 20 weeks for Trial 2). Data points are individual pot values.
under greater N uptake (e.g. Fallah 2012; Artigiani et al. 2014; Tsujimoto et al. 2014). However, no significant differences in plant dry mass were recorded between N treatments, indicating that the higher Si levels under the NH₄⁺ treatment were not a byproduct of a reduced dilution effect in this treatment.

The lack of a yield difference between N-form treatments, notwithstanding the difference in leaf and stalk tissue Si content, may be due to the absence of imposed stress. There is increasing evidence that Si amendment—due to its action in plant stress alleviation—has significant effects on yield only in the presence of biotic and/or abiotic stress (reviews by Ma 2004; Epstein 2009; Wu et al. 2013; Haynes 2014; Zhu and Gong 2014). Thus, in mature field-grown sugarcane subject to crop stress (e.g. water deficit, pests or disease), the economic benefits of Si amendment and enhanced uptake are more likely to be expressed (Meyer and Keeping 2005).

Our findings agree with those of Oliveira et al. (2007) for upland rice to the extent that these authors found that application of N at a higher ratio of NH₄⁺ to NO₃⁻ progressively reduced soil pH and was associated with lower 0.5 M CaCl₂-extractable soil Si. However, our results contrast with theirs for plant uptake of Si in relation to soil pH (see Fig. 1), which in Oliveira et al.’s (2007) study increased significantly with increasing rhizosphere pH. Notwithstanding this relationship, their Fig. 6 indicates a positive relationship between the NH₄⁺ : NO₃⁻ ratio and the shoot Si content, which is contradictory to the positive relationship they found between Si uptake and soil pH (their Fig. 5) (since the pH decreased with increasing NH₄⁺ provision).

Oliveira et al. (2007), Korndörfer et al. (2005) and de Camargo et al. (2007) argued that the concentration of available soil Si (whether native Si or added as an amendment) decreases with increasing soil acidity (Korndörfer et al. 2005). However, numerous studies have found that the solubility of silicic acid in the soil solution decreases with increasing pH up to a value of 9.8 (the pK₁ for dissociation of silicic acid, H₄SiO₄, to the silicate anion, H₃SiO₄⁻ (Haynes 2014)), due to the preferential adsorption of H₃SiO₄⁻ onto iron and Al sesquioxides (Beckwith and Reeve 1963, 1964; Jones and Handreck 1963; McKeeage and Cline 1963a, b; Hingston and Raupach 1967; Kato and Owa 1996; Tavakkoli et al. 2011; Makabe-Sasaki et al. 2013). In mature soils, sesquioxides form the most important general source of rapidly acid-soluble Si (Beckwith and Reeve 1964); consequently, highly weathered soils, such as that used in the present study, can rapidly remove ‘added’ Si from the soil solution through adsorption reactions, making it unavailable for plant uptake (Jones and Handreck 1965; Tavakkoli et al. 2011).

Notwithstanding the greater Si uptake in the NH₄⁺ treatments, significantly less soil Si was extracted using 0.02 N H₂SO₄ from the NH₄⁺ treatments than from the NO₃⁻ treatments (especially in Trial 2; Table 2), and there was a significant positive association between acid-extractable Si and soil pH (Fig. 1). A likely explanation for this paradox is that under higher soil pH conditions of the NO₃⁻ treatment, greater amounts of applied Si were present as the silicate anion and adsorbed on soil surfaces (i.e. removed from soil solution) than under the NH₄⁺ treatment, making this fraction unavailable for plant uptake. However, this adsorbed fraction may be detectable at

### Table 4.

Dry leaf and stalk mass for Trials 1 and 2 at harvest (18 weeks for Trial 1 and 20 weeks for Trial 2). Values are means ± standard error. *P* = N-form treatment. **N** = 12 (Trial 1) and 10 (Trial 2). Probability (P) values are from ANOVA.

| Treatment/statistic | Leaf (g) | Stalk (g) |
|---------------------|----------|-----------|
| **Trial 1**         |          |           |
| T1 [(NH₄)₂SO₄]      | 4.6 ± 0.2| 17.3 ± 0.7|
| T2 [(NH₄)₂SO₄ + DCD]| 4.7 ± 0.3| 17.5 ± 1.1|
| T3 [Ca(NO₃)₂]       | 3.9 ± 0.2| 18.1 ± 0.6|
| P value             | 0.1      | 0.2       |
| **Trial 2**         |          |           |
| T1 [(NH₄)₂SO₄]      | 31.0 ± 0.9| 21.8 ± 0.9|
| T2 [Ca(NO₃)₂]       | 33.2 ± 0.9| 21.2 ± 1.3|
| P value             | 0.1      | 0.7       |
| Cultivar N12        | 30.9 ± 1.0| 22.1 ± 1.2|
| Cultivar N14        | 33.3 ± 0.8| 20.9 ± 0.9|
| P value             | 0.06     | 0.4       |
| P value: *P* × cultivar | 0.7      | 0.5       |

Figure 4. Regression curve and equation for stalk Si concentration against 0.02 N H₂SO₄-extractable soil Si concentration in Trial 2 at harvest (20 weeks). Data points are individual pot values.
trial termination as significantly higher levels of Si in the NO$_3^-$ treatment when released into the solution by the relatively strong 0.02 N H$_2$SO$_4$ extraction. Soil Si values under the NH$_4^+$ treatment were likely further reduced by greater plant uptake compared with the NO$_3^-$ treatment, leaving less for extraction from the soil at trial termination.

Both processes may explain the significant negative association between acid-extractable soil Si at harvest and leaf Si content (Fig. 3B). The acid extraction apparently gave an unrealistically high measure of the plant-available Si in the NO$_3^-$ treatment during plant growth, a property of stronger acid extractants that has been well-documented (Berthelsen and Korndörfer 2005; Sauer et al. 2006; Haynes 2014). Extraction with 0.01 M CaCl$_2$, on the other hand, yielded no significant difference in soil Si between NH$_4^+$ and NO$_3^-$ treatments, and no significant associations between soil Si and pH (Fig. 2A) or soil Si and leaf Si (Fig. 2B). This result is consistent with the above explanation, as 0.01 M CaCl$_2$, being a dilute, neutral salt solution, would have extracted mostly soluble Si and less of the Si adsorbed to soil surfaces. The weakly negative association between CaCl$_2$-extracted soil Si and leaf Si (Fig. 2B), rather than an expected positive association based on previous studies (e.g. Berthelsen et al. 2001b; de Camargo et al. 2007; Haynes et al. 2013; Miles et al. 2014), nonetheless indicates that this method solubilized more Si in the NO$_3^-$ treatment than was readily available for plant uptake.

While several other studies have reported significant positive correlations between soluble soil Si and pH in slag-amended soils (Korndörfer et al. 2005; de Camargo et al. 2007; Oliveira et al. 2007; Haynes et al. 2013, Haynes et al. 2013) and Haynes (2014) made the critical point that this relationship is probably coincidental rather than causative, as the continued dissolution of the slag material would inevitably raise soil pH while supplying extractable Si. Hence, an increase in soil pH per se may not enhance native or applied Si solubility, or significantly improve its uptake. For example, de Camargo et al. (2007) found that liming an acid, low-Si soil, had no effect on CaCl$_2$-extractable soil Si and produced only a slight increase in plant Si compared with wollastonite and Ca-Mg silicate. In concurrence with our results, Khalid et al. (1978) and Tavakkoli et al. (2011), working in a sugarcane–corn–kikuyu grass rotation experiment and in rice, respectively, found that plant uptake of applied Si decreased with increasing soil pH, even though soil Si extracted with either phosphate solution (Khalid et al. 1978) or 0.01 M calcium chloride and 0.5 M ammonium acetate (Tavakkoli et al. 2011) increased with increasing soil pH. When these authors used water as an extractant, soluble Si decreased with increasing pH, which they ascribed to adsorption on soil surfaces (Khalid et al. 1978; Tavakkoli et al. 2011). However, the reaction of silicic acid with Al—the solubility of which is greatly increased at low pH values (<5.5)—to form insoluble hydroxysilicates is probably of particular importance in reducing Si solubility in acid soils (see Keeping et al. 2013; Haynes 2014, and references cited therein).

As both of our trials were planted only a few days after application of the Calmasil slag, it is likely that the slag was reacting (dissociating) during the entire period of the trials. The longer duration of Trial 2 (20 weeks) might therefore explain the much higher final pH and higher extractable Si in this trial compared with Trial 1 (18 weeks), and why N-NH$_4^+$ promoted its uptake to a greater degree in Trial 2 (Table 3, Fig. 2). Kato and Owa (1996, 1997) found that the increase in pH and Ca concentration of paddy soils following the addition of strongly alkaline Si-rich slags could suppress further dissolution of the slags as well as the soil Si concentration, the latter due to increased adsorption of Si onto the solid phase at higher pH. However, neutralization of soil pH (due to plant root and microbial respiration) increased Si dissolution from both the slags and the soil solid phase. Addition of N as NH$_4^+$ in the present study (as well as acid extraction for soil Si determination) likely achieved a similar result, suggesting that cultural practices, including addition of organic matter and associated increased microbial respiration, can be used to promote the dissolution of slags and improve crop Si uptake (Kato and Owa 1997; Ma and Takahashi 2002). Furthermore, a gradual increase in soil acidity due to nitrification of NH$_4^+$ fertilizers (urea is the most widely used N fertilizer in the South African sugar industry) and organic N is likely to release adsorbed Si from applied sources and make it available for plant uptake.

In addition to the potential use of NH$_4^+$ fertilizer in solubilizing applied Si, several studies have found that sugarcane accumulates NH$_4^+$ more efficiently as an N-source compared with NO$_3^-$ (de Armas et al. 1992; Robinson et al. 2011; Hajari et al. 2014). There is also evidence that NH$_4^+$ can significantly improve the use of other nutrients, especially P, by reducing rhizosphere pH and thereby increasing the solubility of P compounds, and also stimulating root growth (Jing et al. 2010, 2012). Together with the environmental and cost disadvantages of NO$_3^-$ arising from its high mobility and loss from the soil, an argument can be made for reducing NO$_3^-$ content in soils in favour of NH$_4^+$ and organic N-forms (Robinson et al. 2011), which are likely also to improve Si uptake.

An important distinction needs to be drawn between the enhancement of plant-available Si in association with raised soil pH following slag amendment of low-Si soils, and the abundance of native Si in higher pH, less-weathered soils, such as those that predominate in the northern irrigated regions of the South African sugar
industry, where leaf Si levels are satisfactory (Van der Laan and Miles 2010). Miles et al. (2014) emphasize that this latter relationship between abundant available Si and higher pH is due to the occurrence of clays dominated by Si-rich minerals such as feldspars, vermiculites and smectites, which provide relatively high levels of soluble Si. In lower pH soils, such as those in the rainfed production areas, weathering processes have resulted in clay fractions dominated by low-Si minerals such as kaolinites and sesquioxides, while the high levels of exchangeable Al in such soils are also strongly associated with reduced acid- and CaCl₂-extractable Si (Miles et al. 2014).

While weathering processes play a fundamental role in the development of desilicated soils, there is recent recognition of the effect of agriculture on terrestrial Si cycling, in particular with regard to biogenic silica (BSi; stored in biomass and soils as amorphous Si or plant phytoliths (SiO₂-nH₂O)). Struyf et al. (2010) and Vandevenne et al. (2012) showed that conversion of natural ecosystems to agriculture over centuries has disrupted cycling of BSi and created a new loop in the global Si cycle, wherein Si is exported from landscapes during agricultural harvest instead of being replenished through litterfall. Removal of Si through long-term cropping will ultimately deplete soil reserves of BSi, impacting both crop production and fluxes of Si from terrestrial environments into freshwater and oceanic systems (Struyf et al. 2010; Keller et al. 2012; Vandevenne et al. 2012). As SiO₂·nH₂O solubility increases markedly with increasing pH, Haynes and Zhou (2014) argued that in the short term an increase in soil pH may increase Si availability in agricultural soils, but in the longer term it will reduce the available BSi-derived Si. In acidic soils where pH correction is necessary (e.g. through lime or slag application), replenishment of BSi through retention of crop residues may therefore be as important in preserving supplies of plant-available Si as the provision of Si in the form of silicate slags.

Conclusions
There still appears to be some controversy surrounding the effects of soil pH on Si solubility, whether from native or applied Si. Our finding that Si uptake was significantly enhanced under lower pH caused by fertilization with NH₄⁺, notwithstanding greater amounts of acid-extractable Si under higher pH produced by NO₃⁻ fertilization, is worthy of further study at the soil chemistry level. We argue that the most likely explanation for our results is the reduction of rhizosphere pH from H⁺ extrusion to balance charges in response to NH₄⁺ uptake, followed by greater solubilization (dissociation) of Ca₂SiO₄ in the more acidic root environment and production of plant-available silicic acid. In line with basic studies in the 1960s (Beckwith and Reeve 1963, 1964; Jones and Handreck 1963; McKeague and Cline 1963c), lower soil pH would also have reduced adsorption of Si to clay particles, especially Fe and Al sesquioxides, further increasing Si in soil solution. Results reported by other authors, such as Oliveira et al. (2007), which imply a direct positive effect of pH on Si solubility and uptake, may have misinterpreted the inevitable effect that addition of basic Si-rich slags has on soil pH while still raising soil Si concentration and therefore plant uptake (Haynes et al. 2013; Haynes 2014). Abundant native Si in the clays of higher-pH, less-weathered soils may also have contributed to the tenet that Si is more soluble in higher pH soils.

From an applied viewpoint, the use of ammoniacal N fertilizers to improve dissolution of applied slag and release readily available Si is worthy of field study. In addition to the provision of silicate materials to weathered Si-depleted soils, we advocate the preservation of the biogenic pool of Si as far as possible through the retention of crop residues and incorporation of organic matter.

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Contributions by the Authors
The original concept for the study arose through discussion between R.S.R. and M.G.K. Calculations for treatments were performed by R.S.R., while the study itself was managed by M.G.K. Trial design and statistical analysis were performed by C.S. Debate over the findings and their interpretation were conducted between M.G.K., R.S.R. and N.M. The manuscript was written and revised by M.G.K. with criticisms and input from all other authors.

Conflicts of Interest Statement
None declared.

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