Nitrogen uptake and nitrate-nitrogen accumulation in forage kale grown under varying amounts of water and nitrogen fertiliser

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Abstract

Grazing of forage kale can potentially cause ground-water pollution through nitrate leaching from excreted urine, particularly in wet conditions. It can also cause nitrate–nitrogen (NO_3–N) poisoning of grazing ruminants when crops take up excess soil N. Thus, N supply should be matched with crop requirements to achieve an optimum combination of yield and N content. An experiment was conducted on a stony Balmoral silt loam soil in Canterbury, investigating relationships between dry matter (DM) yield, N uptake and NO_3–N accumulation under four N application rates (0, 75, 150, 300 kg/ha), with and without irrigation. The final DM yield for the fully irrigated treatments showed a positive response to N application, ranging from 10 t DM/ha to 26 t DM/ha for the treatments receiving 0 and 300 kg N/ha, respectively. Similarly, for the non-irrigated (rain-fed) treatments, DM yield increased from 5 t DM/ha to 11 t DM/ha for the same N treatments. Total N uptake also increased with N application rate, from 88 to 350 kg N/ha for the treatments receiving 0 and 300 kg N/ha, respectively. Weighted whole plant tissue NO_3–N concentration increased from 0.04 mg/g for the 0 kg N/ha crops to 0.65 mg/g and 4.63 mg/g when 300 kg N/ha was applied for irrigated and rain-fed treatments, respectively. Furthermore, NO_3–N was higher in the stems than in the leaf lamina and petiole and increased from the upper to lower portions of the stems. In this study, the results support N application rates of 150 kg N/ha for rain-fed and 300 kg N/ha for irrigated crops, to produce optimum DM yield to feed non-pregnant animals.

Additional keywords: *Brassica oleracea* var. *acephala* L., Balmoral stony silt loam, feed utilisation, potential evapotranspiration, shallow soil

Introduction

Forage kale (*Brassica oleracea* var. *acephala* L.) crops are an important winter feed on pastoral farms in the temperate regions of Australia and New Zealand (Barry, 1978; Pembleton *et al.*, 2015) and are grown under a wide range of soil fertility situations (Wilson *et al.*, 2006). In most of these soils, additional nitrogen (N) fertiliser may be required to support full yield potential. Forage kale crops have large nutrient requirements; with an estimated 18 t DM/ha kale crop taking up about 360 kg N, 450 kg potassium (K) and 50 kg phosphorus (P)/ha (Wilson *et al*., 2006). Furthermore, forage kale crops take up large amounts of

N from the soil and will respond to additional fertilisers (Wilson and Maley, 2006; Chakwizira *et al.*, 2015a) if conditions are favourable for uptake. Under high N fertiliser inputs yield is enhanced but excess amounts of N can lead to accumulation of anti-nutritional compounds, such as nitrate–N (NO_3–N) (Fletcher and Chakwizira, 2012a), potentially leading to animal health issues and/or environmental pollution. There is an increased risk of NO_3–N poisoning of grazing ruminant animals (Nichol, 2007) when N uptake is high and the excess is stored as NO_3–N in plant tissues. Cash *et al.* (2006) have published guidelines for NO_3–N concentration for animal feeds highlighting levels <0.35 mg/g as generally safe for all conditions and livestock types and those >2.26 mg/g as lethal, causing acute symptoms and death. These figures are more conservative than those published elsewhere (Nichol, 2007), but useful as the effects of NO_3–N vary among individual animals, condition and age of livestock, and other feeds in the diet.

Nitrate–N concentration varies with plant parts (Fletcher and Chakwizira, 2012a), being higher in the stems, petioles and midrib than in the leaf lamina. Forage intake of kale crops has been shown to vary with plant part (Barry *et al.*, 1984), being lower in the leaf lamina and basal stems than in the middle and upper part of the stem when offered to sheep. The consequence is that the sheep preferentially graze plant parts that have potentially higher NO_3–N concentration and therefore are exposed to a higher risk of nitrate toxicity. It is unclear how additional fertiliser N may cause a differential partitioning of NO_3–N in the plant organs. Forage kale stems contribute to more than 60% of the total DM yield (Chakwizira *et al.*, 2010; 2015a) and livestock tend to reject the lower part of the stems under 'normal' winter stocking rates of 16–20 cows/ha (Judson and Edwards, 2008). At high stocking rates, 70–80% of the DM is utilised (Judson and Edwards, 2008) but higher utilisation may be achieved and enhanced by extending grazing (de Ruiter and Malcolm, 2015). Consequently, this means livestock are forced to graze lower parts of the stems that have potentially higher NO_3–N concentration.

In New Zealand, historical cases of fatal NO_3–N poisoning have been reported (Brakenridge, 1956; Bolan and Kemp, 2003), with fatalities as high as 10% of the herd in some instances. Bolan and Kemp (2003) also reported a high incidence of abortion and dead calves and lambs at birth, after both pregnant cows and ewes, respectively, were fed high NO_3–N pastures and forage brassica crops. It is therefore important to manage N supply and hence the accumulation of NO_3–N in forage crops that are fed in winter to pregnant stock.

Nitrate–N concentrations have also been shown to vary with N supply (Pelletier *et al.*, 1976; Fletcher and Chakwizira, 2012a) and drought stress (Brakenridge, 1956). In deep soils (~1.6 m soil depth), Fletcher and Chakwizira, (2012a) showed that the rates of N and time of application were important determinants of NO_3–N concentration in a range of forage brassica crop species, including forage kale. Averaged across all species, plant NO_3–N content was greater in crops that received the double optimum rate of N than either the early or late N treatments. Furthermore, the late N treatment (3.7 mg/g) accumulated almost double the plant NO_3–N content of the early N treatment (2.0 mg/g).

The risk of nitrate leaching is high on soils with low water-holding capacity

(WHC) and if winter grazing coincides with periods of high rainfall (Malcolm *et al*., 2016). Management of crops to match N supply and crop N demand is a logical approach; however, N application rates will depend on background soil fertility, soil moisture and yield potential (Wilson *et al*., 2006). There are quantitative data on N uptake and partitioning (Wilson and Maley, 2006), and NO_3–N accumulation, for kale crops grown under variable N rates, particularly for the deep soils (Fletcher and Chakwizira, 2012a; Chakwizira *et al.*, 2015b). However, there is little research on the combined effects of water and N on N uptake and NO_3–N accumulation in forage kale crops grown on shallow and stony soils in New Zealand, where most of the forage kale crops are grown for winter grazing. The objective of this experiment was to determine responses to irrigation and N fertiliser, and their interactions on N uptake, tissue N concentration (N_{conc}) and NO_3–N accumulation in forage kale crops grown in shallow soils.

Materials and Methods

Experimental details

The experiment was conducted at the Lincoln University Ashley Dene Research and Development Station (ADRDS) (43°38'45.5"S 172°20'34.4"E, 30 m a.s.l.). The site was situated on a shallow Balmoral/ Lismore stony silt loam (Mottled Argillic Pallic Soil, Udic Ustochrept) (Webb and Bennett, 1986; Webb and Burgham, 1997), with a shallow topsoil (0.2 m in depth) over gravel. The soil has a WHC of about 90 mm/m of depth, recalculated from Sim *et al*. (2012). The site was previously in lucerne (*Medicago sativa* L.) from 2008 to 2011 followed by kale from 2011 to 2013.

The climate at ADRDS is temperate, with mild to cool winters and warm summers. Mean annual rainfall is 600 mm, distributed evenly throughout the year (Figure 1). Weather data from a temporary weather station at the experimental site and long-term data (1970–2010) from the Broadfields meteorological station (NIWA, 2016) at Lincoln (~10 km from the site) are shown in Figure 1. The cumulative evapotranspiration (ET) for the growing season was 644 mm and the total rainfall was 638 mm. However, the cumulative ET for the main growth period (sowing to 20 March) was 565 mm, compared with total rainfall of 387 mm (see Figure 1) (i.e. ~180 mm gap as irrigation). Furthermore, the total amount of rainfall for the end of season (after 20 March 2014) was 251 mm (~40% of the season total) compared with a cumulative ET of 81 mm. This meant there were no irrigation events after 20 March 2014.

Random soil samples for basal soil fertility to a depth of 0.15 m were taken on 30 July 2013 and the average soil test results were: pH 5.8, Olsen P 16 mg/kg, K 160 mg/kg, Ca 1000 mg/kg, Mg 45 mg/kg, Na 25 mg/kg, sulphate-S 5 mg/kg soil and available mineral N 78 kg/ha. The concentrations of soil nutrients were determined as 'MAF quick-test units' (Mountier *et al*., 1966) and were converted into mg/kg dry soil using the following conversion factors: P, x1.1; Ca, x125; K, x20; Mg, Na, x5 (Chapman and Bannister, 1994). Basal fertiliser comprising 250 kg/ha triple superphosphate (20.5% P and 1% S) and 10 kg/ha borate 46 (15% boron) was broadcast and incorporated into the soil before sowing. Soil mineral N (nitrate and ammonium combined) tests were taken from individual plots before the application of the N fertiliser treatments to a depth of

0.3 m (shallow because of limited depth of soil) or shallower if limited by stone layer. Nitrogen fertiliser treatments were applied as urea (46% N) on three dates (30, 57 and 91 days after sowing (DAS)) with 20%, 40% and 40% of the total N per treatment (0, 75, 150, and 300 kg N/ha) applied on each date, respectively.

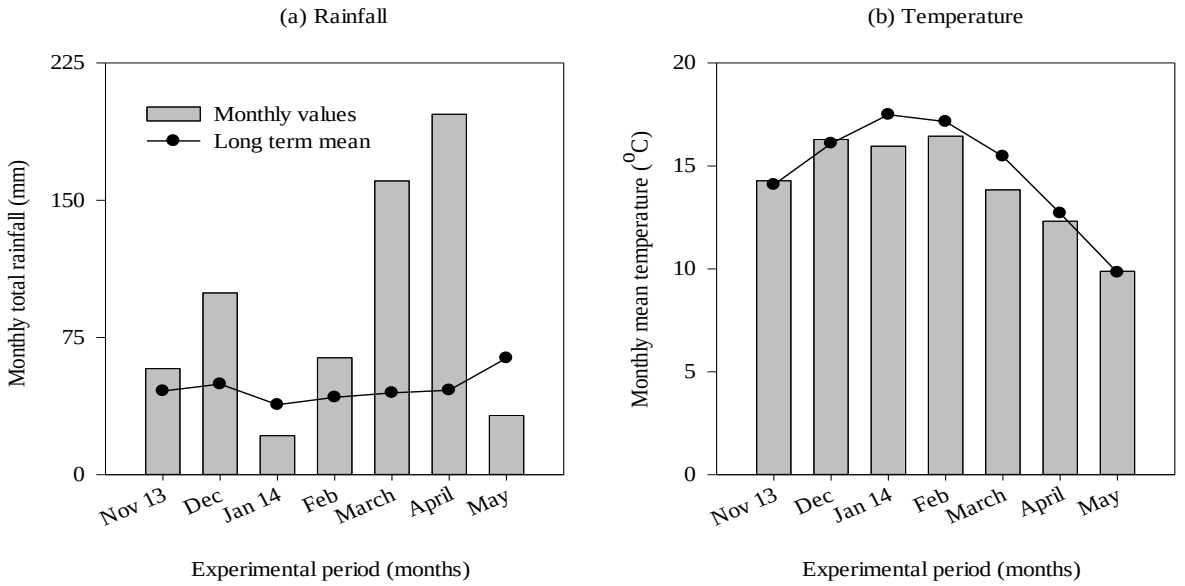

Figure 1: Monthly (a) total rainfall and (b) average temperature at Ashley Dene Research and Development Station (ADRDS), Canterbury, New Zealand. Long-term data are from 1970 to 2010 (NIWA, 2016).

Experimental design

The experiment was a randomised complete block design, replicated three times. It consisted of eight treatments: a factorial combination of two rates of irrigation (rain-fed (control) and full irrigation replacement of potential ET) and four rates of N (0, 75, 150 and 300 kg/ha). Irrigation was applied twice weekly (maximum = 50 mm/week if no rain) to replace ET. Each plot had its own trickle irrigation supply, with emitters spaced 150 mm × 150 mm apart.

Cultivation involved deep ploughing followed by power harrowing. The cultivar 'Gruner', a giant kale (Westwood *et al.*, 2014) was drilled on 21 October 2013 at 4 kg/ha in 150 mm rows into a cultivated seedbed using a Taege drill with an Oyjoord cone seeder. Plot size was 4 m × 10 m. Seeds were pelleted with 'Superstrike® Brassica' (a.i. thiamethoxam, iprodiane and thirum; PGG Wrightson, Christchurch, New Zealand) to guard against springtails (*Bourletiella* spp.) and fungal infections (Salmon and Dumbleton, 2006). Agrichemicals were applied as required to control weeds, pests and diseases.

Measurements

Dry matter (DM) harvests were taken at four-weekly intervals from 17 December 2013 to the final harvest on 21 May 2014. This involved cutting and removing all plants within a 0.5 m^2 quadrat to approximately 10 mm height in each plot. Different areas of the plots were cut at each harvest. The number of plants and total fresh mass per quadrat were determined in the field and a representative five-plant

subsample was retained to determine total DM yield. Dry mass was determined after drying at 60°C to a constant weight. Total N_{conc} was determined by the Dumas combustion using a LECO CNS-200 analyser (LECO Corporation, St Joseph, MI) at The New Zealand Institute for Plant & Food Research laboratory, at Lincoln. Total N uptake was then calculated as the product of crop DM yield and the N_{conc}.

At the final harvest, two additional plants per plot were harvested for NO_3–N analyses on the same day as the final DM harvest. These plants were separated into stem, leaf lamina and petiole (which included the midrib of the leaf), hereafter referred to as the petiole. Each stem was divided into three equal lengths, denoted as upper, middle and lower stem. Samples were crushed, immediately frozen at about -18°C, then freeze-dried and then finely ground to pass through a 1-mm screen and analysed using the ion-chromatographic (IC) method (Lachat Instruments, Loveland, CO, USA) (Kalbasi and Tabatabai, 1985). Results are expressed in mg NO_3–N/g DM (Wright and Davison, 1964). Whole plant tissue NO_3–N concentration for each of the treatments was calculated using NO_3–N concentration from each plant component weighted by the proportion of the component in the total DM.

Data analyses

Analysis of variance (GenStat v.17 (VSN International, Hemel Hampstead, UK)) was used to test the treatment effects. Significant interactions and main effects were separated using Fisher's protected least significant difference ($LSD_{\alpha=0.05}$) tests. Different scales have been used for the NO_3-N concentration of different portions plotted against the N rate (Figure 3), because the values for the stems are up to nine-fold higher than the leaf component values.

Results

The overall DM yield differed ($P < 0.01$) with both water and N supply throughout the growing season (Figure 2a). For the rain-fed N treatments, DM yield increased ($P < 0.01$) from 5.2 t DM/ha for the control N (0 kg N/ha) treatments to 11.3 t DM/ha for the treatments receiving 300 kg N/ha. Similarly, for the irrigated treatments, DM yield increased from 10.1 t DM/ha to 25.8 t DM/ha for the same N treatments.

The total amount of N taken up by the crops was higher ($P < 0.001$) for the irrigated treatments earlier in the season (up to end-February) (Figure 2b). However, from mid-March, N uptake increased rapidly for the rain-fed treatments and hence the similar ($P = 0.30$) total N uptake between the water treatments at the final harvest. Consequently, N uptake increased ($P < 0.001$) with N supply, irrespective of water treatments from a mean of 88.6 kg N/ha for the control N treatments to 350 kg N/ha for the 300 kg N/ha treatments at the final harvest.

The weighted whole plant tissue NO_3–N concentration also increased with both N and water supply (Figure 3). For the rain-fed treatments, the whole plant tissue NO_3–N concentration increased from 0.04 mg/g for the 0 kg N/ha treatments to 4.63 mg/g when 300 kg N/ha was applied. Similarly, for the fully irrigated treatments, the whole plant tissue NO_3–N concentration increased from 0.03 mg/g for the 0 kg N/ha treatments to 0.65 mg/g when 300 kg N/ha was applied. Furthermore, the whole plant tissue NO_3–N concentration differed with plant part, being higher ($P < 0.05$) in the stems than in the leaf lamina and the petiole under both water treatments (Figure 3a). The whole plant tissue NO_3–N concentration also varied with the position on the stem, increasing down the stem.

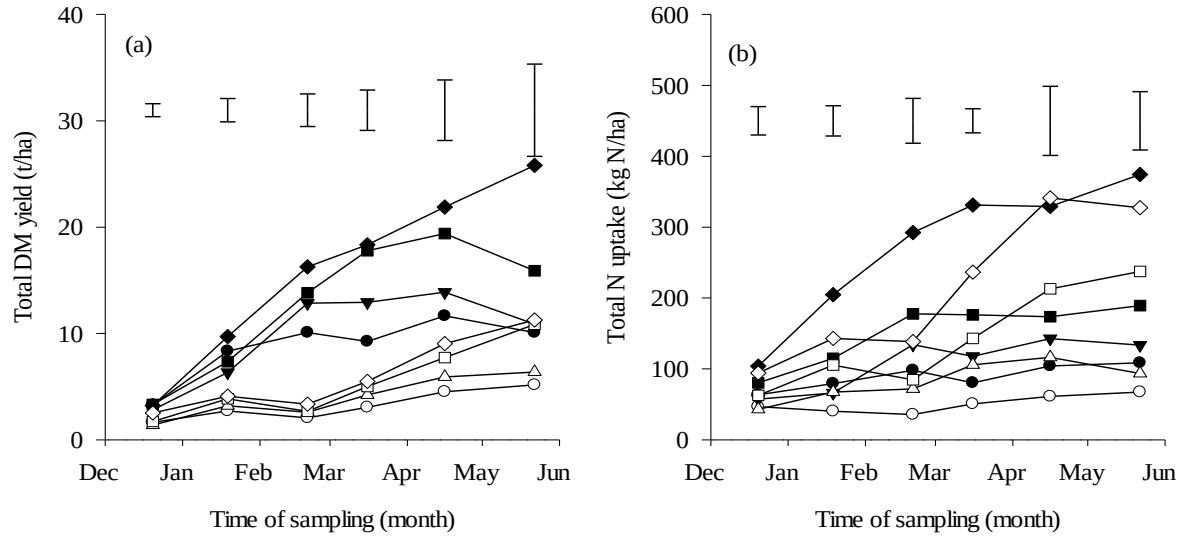

Figure 2: Mean total (a) dry matter yield (t DM/ha) and (b) nitrogen uptake (kg N/ha) for forage kale crops ('Gruner') grown under rain-fed (open symbols) and irrigated (solid symbols) conditions and different nitrogen rates (○●, 0; △▲, 75; □■,150 and ◇◆, 300 kg N/ha) at Ashley Dene Research and Development Station (ADRDS), Canterbury, New Zealand in 2013–14 season (October 2013 to May 2014). Vertical bars represent the least significant differences ($LSD_{0.05}$; d.f. = 149).

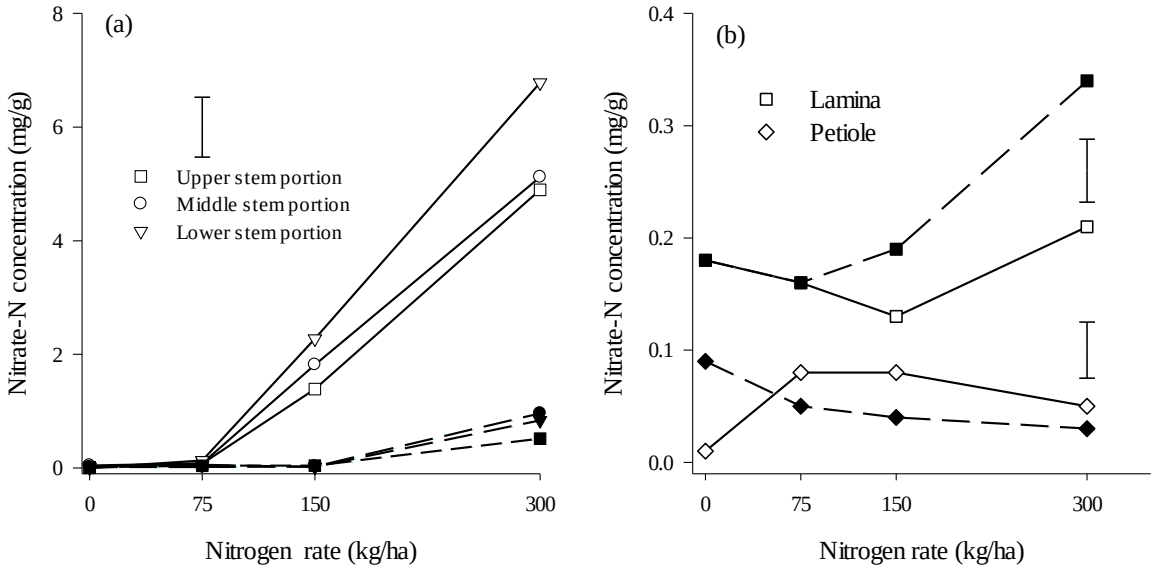

Figure 3: Nitrate–nitrogen (NO_3–N) concentration of different portions of the: (a) stem (lower, mid, upper thirds) and (b) leaf (lamina, petiole) components of forage kale ('Gruner') crops grown under varying amounts of irrigation: rain-fed (solid lines, open symbols) and fully irrigated (broken lines, closed symbols) under different rates of nitrogen (N) fertiliser in shallow soils at Ashley Dene Research and Development Station (ADRDS) in the 2013–2014 season. Vertical bars represent the least significant differences ($LSD_{0.05}$; d.f. = 78).

For the rain-fed treatments, tissue NO_3–N concentration differed with N application for all the components except the leaf lamina, from a mean of ≤0.08 mg/g for treatments receiving ≤75 kg N/ha to more than a 17-fold increase for the stems when ≥150 kg N/ha was applied. Furthermore, tissue NO_3–N concentration for the stems in the rain-fed treatments increased three-fold (Figure 3a) when 300 kg N/ha was applied compared with the 150 kg N/ha treatments.

The relationship between tissue NO_3–N concentrations for the fully irrigated treatments was consistently below the 0.35 mg/g threshold (Figure 4b), except for the highest N rate. However, for the rain-fed treatments, ≥150 kg N/ha treatments had tissue NO_3–N concentrations above the 0.35 mg/g threshold and therefore may pose animal welfare risks when feeding pregnant animals.

There was an interaction ($P < 0.001$) between water and N supply on N_{conc} (Figure 4a). The N_{conc} increased ($P < 0.001$) with N supply more rapidly under rain-fed than irrigated conditions; with regression slopes of 0.006 and 0.003 respectively. Both tissue NO_3–N concentrations and N_{conc} increased rapidly with N rate under rain-fed conditions, hence the quadratic relationship between these variables under rain-fed conditions (Figure 4b).

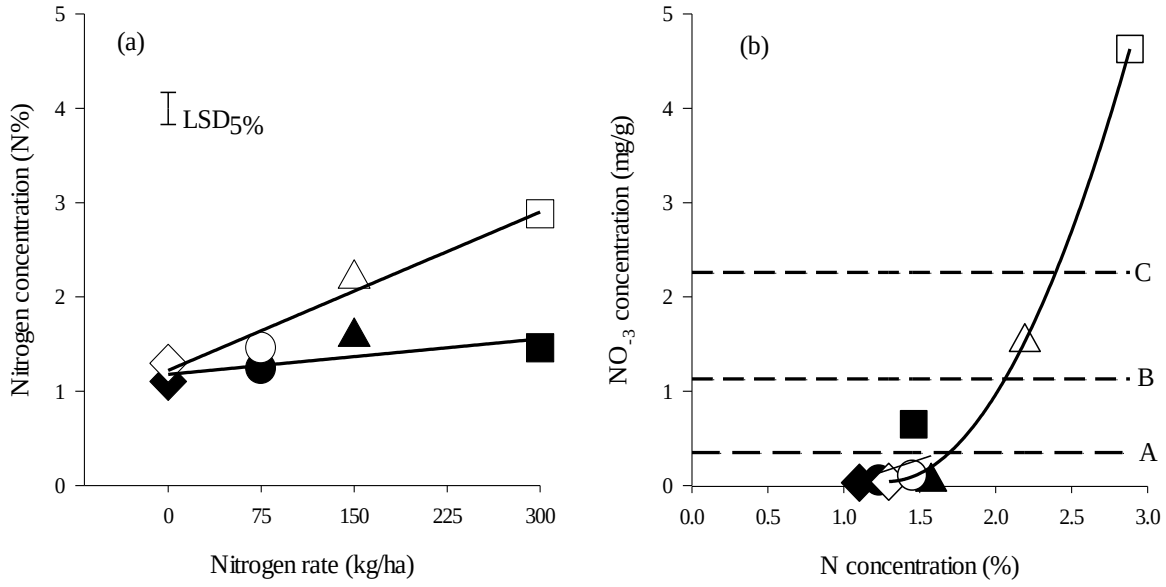

Figure 4: The nitrogen concentration (N_{conc}) for forage kale ('Gruner') grown under different nitrogen (N) rate: (○●, 0; △▲, 75; □■,150 and ◇◆, 300 kg N/ha); (a) and (b) relationship between average weighted whole-plant nitrate–nitrogen (NO_3–N) concentration and N_{conc} for irrigated (solid symbols) and rain-fed (open symbols) crops. The dotted lines are the threshold N_{conc} for animal feed (Cash *et al.*, 2006): A (≤0.35 mg/g, safe for all conditions and livestock classes), B (0.35–1.13 mg/g, safe for non-pregnant livestock) and C (1.13–2.26 mg/g, not recommended for feeding any livestock classes). The regression for (a) irrigated ($Y=0.001x + 1.18$; $R^2=0.59$) and rain-fed ($Y=0.006x + 1.22$; $R^2=0.98$) treatments.

Discussion

The increase in DM yield with both irrigation and N supply is consistent with those reported previously: 5–13 t DM/ha for rain-fed production (Wilson *et al*., 2006; Chakwizira *et al.*, 2009; 2013) and 12–27 t DM/ha for irrigated crops (Wilson *et al*., 2006; Brown *et al.*, 2007; Chakwizira *et al*., 2013; 2015a). Low DM yield for N and water-stressed forage kale crops was attributed to limited leaf expansion and hence reduced radiation interception (Chakwizira *et al*., 2013).

The non-response of total N uptake to irrigation was inconsistent with previous reports for forage kale crops grown under deep soils with high WHC, where water availability had strong effects on N uptake (Chakwizira *et al*., 2013). This could be attributed to the high rainfall during March and April (Figure 1) when growth rates recovered in the rain-fed treatments and any surplus soil N was taken up by the crops (Figure 2). In shallow soils, the timing of autumn rain can have important consequences for N loading effects on potential N leaching during winter grazing. A suggested mechanism was that during and immediately after late rainfall (Figure 1) and subsequent late growth, albeit slower due to decreasing temperatures and radiation, the rain-fed treatments continued to take up residual mineral N in the soil. Similar responses to later rainfall have been reported for forage kale by Chakwizira *et al*. (2013). However, the overall DM yield for the rain-fed crops was lower than the irrigated, but they took up similar amounts of N (Figure 2). This resulted in a raised pool of N stored as NO_3–N (Figure 3). Chakwizira *et al*. (2015b) showed that there was a link between the concentration of N as NO_3–N and the N_{conc} in the plant tissues (Figure 4), meaning there is an apparent lag in the rate of metabolism of N in tissues.

The weighted whole plant tissue NO_3–N concentration (≥1.52 mg/g) for crops grown at ≥150 kg N/ha for the rain-fed crops was higher than the recommended threshold (≥0.35 mg/g) for feeding any livestock class (Cash *et al*., 2006). However, for the irrigated crops, all N rates except the 300 kg N/ha were safe for all conditions and livestock classes (Figure 4b), with the higher N rate safe for non-lactating livestock. The rapid increase in tissue NO_3–N concentration for the plots receiving ≥150 kg N/ha in rain-fed crops and 300 kg N/ha for the irrigated treatments (Figure 3; 4) coincided with the N rates at which either maximum DM yield was achieved (irrigated crops) or DM yield ceased to respond to N supply (rain-fed treatments) (Figure 2). These N rates also coincide with the point at which any increase in tissue N_{conc} (about 1.5% N; Figure 4b) resulted in a rapid increase in NO_3–N concentration in both irrigation treatments, indicating excess N supply. This is consistent with historical (ap Griffith and Johnston, 1961) and recently (Chakwizira *et al*., 2015b) published reports. The whole-plant tissue N_{conc} decreased with increased DM yield (data not shown), which is consistent with the findings for forage kale (Fletcher and Chakwizira, 2012b; Chakwizira *et al*., 2015b).

The rapid tissue NO_3–N concentration increase for the summer water-stressed, rain-fed crops, following high rainfall from early autumn (Figure 1), is consistent with published experimental results for forage kale (Chakwizira *et al*., 2013) and review work (Wright and Davison, 1964) for a number of crops. This has been attributed to the fact that under severe water stress, crops may stop taking up N (Bolan, 1998;

Gonzalez-Dugo *et al.*, 2010), leading to a build-up of NO_3–N concentration in the soil. When water becomes available, plants tend to take up excessive amounts of NO_3–N. The differences between the water treatments could also be attributed to the DM yield differences, and hence the dilution of N_{conc} in the plant tissues, previously reported for forage kale (Fletcher and Chakwizira, 2012b; Chakwizira *et al*., 2015b).

The increase in tissue NO_3–N concentration along the stem length, from the upper part to the lower part of the stem (Figure 3) means that the current push for high feed utilisation (Judson and Edwards, 2008) may force the animals to graze most of the lower part of the stem, thereby inadvertently exposing them to the higher NO_3–N concentrations in the plant. Therefore, there could be a need to find a balance between acceptable feed utilisation and exposure of animals to crops with an increased risk of nitrate poisoning. As the stems and petioles make up more than 60% of the forage kale DM (Chakwizira *et al*., 2010; Fletcher and Chakwizira, 2012a; Westwood and Mulcock, 2012), high NO_3–N concentrations in the stem and combined petiole and midrib may increase the total plant NO_3–N concentration above acceptable thresholds for feeding pregnant animals. This is a particular issue for kale crops, as they are commonly fed to pregnant dairy cows during winter and at higher stocking rates (de Ruiter and Malcolm, 2015). Caution in N fertiliser management should be exercised, especially when crops are grown under water stress.

Conclusions

The DM yield, N uptake and whole-plant tissue NO_3–N concentration increased with N supply. At the final harvest, whole plant tissue NO_3–N concentration was higher in the rain-fed than the irrigated crops, and in the stems than the petiole and leaf lamina. The tissue NO_3–N concentration was highest at the bottom of the kale stem and decreased towards the top. The current push for high feed utilisation in forage kale crops must take account of NO_3–N distribution in the plant tissues, as hard grazing of forage kale, including the lower parts of stems, may inadvertently expose the animals to the higher tissue NO_3–N concentrations. Care in N fertiliser management should also be exercised, particularly when crops are grown under water stress. We recommend N application rates based on pre-season soil tests for both mineral N and available mineralisable N. Under the climate and soil conditions of this study, the N applications rates of 150 kg N/ha for rain-fed crops and 300 kg N/ha for irrigated crops, were adequate for maximum DM production and for feeding non-pregnant animals.

Acknowledgements

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