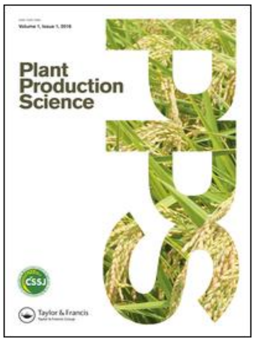

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An application of digital imagery analysis to understand the effect of N application on light interception, radiation use efficiency, and grain yield of maize under various agro-environments in Northern Mozambique

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ABSTRACT

Light-based analysis is a fundamental approach to quantify the effects of factors determining crop growth in a given environment. The objectives of this study are to confirm the applicability of a digital imagery technique to extract green leaf areas for estimating light interception (LI) of maize canopy and to understand the effect of fertilizer application on the LI and radiation use efficiency (RUE) of maize under various agro-environments in Northern Mozambique. A locally recommended variety, Matuba, was grown in a single season with three different N application rates (0, 30, and 80 kgN ha^{-1}) at one hot/dry low-elevation site, two hot/humid mid-elevation sites, and one cool/humid high-elevation site. Repeated measurements with quantum sensors revealed that the digital imagery is applicable to estimate the LI of maize except for leaf-senescing period close to maturity. The N application demonstrated profitable yield increases with agronomic nitrogen use efficiencies (kg grain yield per kg N input) of 20.6–35.3 kg kg^{-1} except for the low-elevation site with severe drought stress. In the mid-elevation sites, the yield increases were mostly explained by the improvement of RUE while the effect on LI was small because the vegetative growth was naturally vigorous under high temperatures irrespective of N inputs. At the high-elevation site, the N application improved its stagnant initial canopy development and increased both RUE and LI. The simple and inexpensive imagery technique should be useful to identify physiological basis of maize responses to fertilizer application and its interaction with regional environment even under poorly equipped regions in the tropics.

Abbreviations: AE_N: agronomic nitrogen use efficiency; CC: canopy coverage; CDC: canopy decline coefficient; CGC: canopy growth coefficient; CIR: cumulative intercepted radiation; HI: harvest index; LI: light interception; RUE: radiation use efficiency; SSA: Sub-Saharan Africa

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Introduction

Maize is the principal food crop in Mozambique and accounts for 21% of the total caloric consumption per capita (FAOSTAT database as of July 2015). Increased and sustainable production of maize is required to address the continuous population growth and rural poverty of the country. However, the maize yield remains extremely low with a national average of less than 1 t ha^{-1}. This value is far below the potential yield of maize and the lowest level among the Eastern and Southern African countries. One of the major constraints of maize yield is the low mineral fertilizer input. According to Benson et al. (2012), Mozambique used 51,400 metric tons of fertilizer in 2010, which corresponded to 11.4 kg per hectare of arable land area, whereas 90% of the fertilizer was used on large-scale farmlands of cash crops such as cotton, tobacco, and sugarcane. In the 2005–2008 survey, it was estimated that only 4–5% of smallholder farmers, the main producers of maize, used mineral fertilizer (Benson et al., 2012).

To improve maize yields with efficient fertilizer management, two agronomic aspects should be considered in field-based research. Firstly, it is important to accumulate empirical data on the agronomic responses of maize to fertilizer inputs in a given set of pedo-climatic conditions and cultivation management practices. Agronomic responses to fertilizer inputs are often evaluated by the agronomic nitrogen use efficiency (AE_N), which is defined as the increase in grain yield per kg of applied N fertilizer

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Table 1. Physical and chemical properties of soils (0–15-cm depth) at the four experimental locations.

	Bulk density	Texture (%)[a]				Total C content[b]	Total N content[b]	Available P[c]	CEC[d]
Location	g cm^{-3}	Clay	Silt	Sand	pH 1:2.5 (H_2O)	g kg^{-1}	g kg^{-1}	mg kg^{-1}	cmol kg^{-1}
Nampula	1.50	5.0	7.0	88.0	5.5	5.0	0.4	7.9	1.7
Gurue	1.43	9.3	11.0	79.7	5.7	5.3	0.4	39.3	4.2
Mutuali	1.48	15.5	20.1	64.3	5.4	11.1	0.6	49.7	7.7
Lichinga	1.26	28.1	22.3	49.5	4.8	13.2	1.0	39.0	6.7

[a] sieving and pipetting method.
[b] NC analyzer, Sumigraph NC-220F (SCAS, Japan).
[c] Bray No.2 method (Bray & Kurts, 1945).
[d] Ammonium acetate extract method at pH 7.0.

(Fageria & Baligar, 2005). An empirical data-set of AE_N can be utilized for economic analysis to determine whether fertilizer use is beneficial in growing maize at a given field condition. Heisey and Mwangi (1996) noted that greater AE_N should be required to implement further fertilizer use because the fertilizer costs in Sub-Saharan Africa (SSA hereafter) were inherently high.

Mozambique has lagged behind regarding any published information on AE_N with the exception of Howard et al. (1998), who collected a historic data-set of on-station trials across the countries that indicated that the mean AE_N for maize was 22 ± 14 kg kg^{-1}. The study also emphasized the large field-to-field variation depending among the agro-ecological zones and soil types. Empirical data particularly in rain-fed fields should be further accumulated so that the recommendations of fertilizer application are fine-tuned to a given environment for increasing the farmers' benefit from maize production in the region.

Secondly, it is important to understand the crop growth process as affected by fertilizer inputs. The N supply is generally considered to affect crop growth and productivity by altering the amounts of intercepted radiation through increases in canopy coverage and the radiation use efficiency (RUE) through increases in photosynthetic capacity with high leaf N content. Light-based crop growth analysis using these parameters is a fundamental approach to elucidate whether yield determinants are derived from the amount of light interception (LI) or the efficiency of the conversion of intercepted light energy. The process-based analysis should facilitate an understanding of the field-to-field variations in response of maize growth to fertilizer inputs.

However, the studies on the light-based analysis have been mostly concentrated on relatively favorable and high-yield conditions in the temperate regions (Sinclair & Muchow, 1999), while little experimental data are available for any crop production in rain-fed and inherently infertile field conditions in SSA. The lack of data is partly because it requires expensive light-quantum sensors and specific skills to select appropriate weather conditions and time of day for the measurements. Recently, a simple method using digital imagery analysis was applied particularly for soybeans to estimate the canopy LI (Purcell, 2000). The method, which does not require light-quantum sensors but solely a digital camera and the appropriate image-processing software (e.g. ImageJ), can permit the opportunity to conduct light-based crop growth analysis at multiple locations simultaneously and with less costs that are affordable even in poorly equipped SSA countries.

The objectives of the current study are firstly to identify the correspondence between the conventional method of using quantum sensors and the digital imagery analysis for estimating LI of the maize canopy at various growing stages, and secondly to understand the interactive effect of N application with field environment on the growth and yield of maize by applying this digital imagery technique at multiple locations in Northern Mozambique.

Materials and methods

Site description and experimental design

Field experiments were conducted at four locations in a single season in Mozambique. The locations included Nampula (15° 17′ S, 39° 19′ E, 372 m alt.), Gurue (15° 19′ S, 36° 42′ E, 691 m alt.), Mutuali (14° 57′ S, 37° 01′ E, 573 m alt.), and Lichinga (13° 20′ S, 35° 15′ E, 1397 m alt.) that covered a wide range of the pedo-climatic conditions of Northern Mozambique. The Nampula site represented a hot and semi-arid climate with sandy soils, whereas the Lichinga site had clayey and relatively rich soils in a cool and humid climate of the tropical highland near the Great Rift Valley. The pedo-climatic conditions of the other two sites were intermediate between Nampula and Lichinga, whereas Mutuali had more clayey and favorable soils in terms of the chemical properties than Gurue. The physical and chemical properties of the soils of each site are summarized in Table 1.

The experimental design below was commonly adopted at all of the sites. A locally recommended and open pollinated cultivar of maize (cv. Matuba) was grown under rain-fed conditions during the rainy season in 2012–2013. Two seeds per hill were planted at a density of 6.25 hills m^{-2} (80 cm × 20 cm) and thinned three weeks after planting to leave one plant per hill (See Table 2 for the planting dates at each site). A locally available type of NPK compound

Table 2. Averages of daily mean temperature and daily solar radiation from planting to tasseling (VT) and from VT to physiological maturity (R6) at the four experimental locations.

Location	Elevation (m)	Land use in previous season*	Planting date	Days		Mean temperature (°C)		Mean solar radiation (MJ m^{-2} day^{-1})	
				to VT	VT-R6	to VT	VT-R6	to VT	VT-R6
Nampula	372	fallow	19-Dec	45	46	26.9	26.2	23.6	23.8
Gurue	691	maize	11-Dec	52	36	25.0	24.8	19.5	21.7
Mutuali	573	fallow	9-Dec	51	37	25.1	24.5	17.9	19.3
Lichinga	1397	maize	6-Dec	71	46	20.2	19.7	15.6	17.0

Note. VT (Tasseling) and R6 (physiological maturity) were determined by visual observations.
*Experimental fields were left in fallow for several years and one year in Nampula and Mutuali, respectively.

fertilizer was uniformly incorporated at a rate of 3:6:3 g m^{-2} of N:P_2O_5:K_2O at one to two days prior to sowing. At the time of thinning, urea was side-dressed at three different N rates, i.e. 0 N (no application), 30 N (30 kgN ha^{-1}), and 80 N (80 kgN ha^{-1}). The side dressing treatments were replicated four times in a randomized complete block design with a plot size of 6.4 m × 5.6 m. Manual weeding and pest management were frequently conducted throughout the growing periods to avoid any biotic stress on crop growth.

Measurements of field environment, grain yield, biomass, and plant N uptake

The daily mean temperature, rainfall, and solar radiation were recorded by Watchdog 1525 micro stations (Spectrum Technologies Inc., Plainfield, IL, USA) at each site. Changes in the soil's water potential were recorded daily throughout the growing period using watermark soil moisture sensors that were connected to Watchdog 1400 data loggers (Spectrum Technologies Inc., Plainfield, IL, USA). Moisture sensors were installed at a depth of 20 cm in the middle of the maize rows, one sensor for each replicate within the 0 N treatment plots. The mean values of four replicates (four soil moisture sensors) were calculated to represent the soil's hydrological dynamics beneath the maize canopy at each site.

The grain yields were determined by harvesting from an area of 17.92 m^2 (4 rows × 5.6 m) in the middle of the plots approximately two weeks after the plants reached physiological maturity (R6). The grain yields were expressed in t ha^{-1} at a 0% moisture basis. The AE_N in the fertilized plots was calculated in the following equation as the increase in grain yield of maize per kg of fertilizer N input:

$$AE_N\left(\text{kg kg}^{-1}\right) = \frac{Y_{xN} - Y_{0N}}{x}$$

where Y_{0N} and Y_{XN} refer to the grain yields (kg ha^{-1}) in the 0 N treatment and in the given fertilization level (i.e. 30 and 80 N treatments), respectively.

Within the yield sampling areas, we selected six average-sized adjacent plants as a subsample and cut them at ground level to determine the harvest index (HI) of each plot as the ratio of grain weight to the total aboveground biomass. The total aboveground biomass was calculated as the quotient of the grain yield determined by the yield samplings from the area of 17.92 m^2 and HI determined by the subsamples. The total N uptake was calculated by summing the products of dry weights and N concentrations of the plant tissues (grain, cob residue, and culm) of the subsamples. The dry weight and N concentration of the plant tissues were determined after oven drying at 80 °C to a constant weight and using an automatic and highly sensitive NC analyzer, Sumigraph NC-220F (SCAS, Japan), respectively.

Measurement of canopy LI with digital imagery analysis

Fractional LI of the maize canopy was estimated by digital imagery analysis following the methodology of Purcell (2000). Briefly, digital photographs were taken twice per plot above the maize canopy to include at least two consecutive rows with approximately 1-week intervals from emergence to R6. The camera was mounted approximately 3.0 m above the ground using a ladder and inclined more than 70° from the horizon (Supplementary Figure 1). Then, fractional green areas of the individual images were determined using ImageJ (http://imagej.nih.gov/ij/) and averaged as the fractional canopy coverage (CC, %) of each plot at that time. The green area of each image was extracted with the fixed hue setting at 50–120 in the HSB (Hue, Saturation and Brightness) color model. The saturation and brightness values were adjusted to include green leaves by visually confirming with the original photographs. In the current study, we have established an extensive research team that enabled frequent photo shootings of maize canopy simultaneously at these remote and multiple locations.

To interpolate these observed CC values with the digital imagery analysis, daily changes in the CC of each plot were estimated using the CC model in AquaCrop (http://www.fao.org/nr/water/aquacrop.html). The model simulates the CC values at time '*t*' by the following equations during the period of canopy development:

$$\mathrm{CC_t} = \begin{cases} \mathrm{CC_0}\, e^{\mathrm{t\,CGC}} & for\ CC \le \frac{\mathrm{CC_{max}}}{2} \cdots (1) \\ \mathrm{CC_{max}} - 0.25 \frac{(\mathrm{CC_{max}})^2}{\mathrm{CC_0}} e^{-\mathrm{t\,CGC}} & for\ CC > \frac{\mathrm{CC_{max}}}{2} \cdots (2) \end{cases}$$

and by the following equation during the periods of canopy decline to maturity:

$$\mathrm{CC_t} = \mathrm{CC_{max}}\left[1 - 0.05\left(e^{\frac{\mathrm{CDC}}{\mathrm{CC_{max}}}} - 1\right)\right] \qquad \cdots (3)$$

where $\mathrm{CC_0}$, $\mathrm{CC_{max}}$, CGC, and CDC indicate the initial CC at emergence, the maximum CC, the canopy growth coefficient, and the canopy decline coefficient, respectively. In the current study, we put the first measurements as the $\mathrm{CC_0}$ of each replicate. The other model parameters, i.e. $\mathrm{CC_{max}}$, CGC, and CDC, were determined by the least square approach to minimize the sum of square errors between the observed and simulated CC values ($\mathrm{CC_{obs}}$ and $\mathrm{CC_{sim}}$, respectively, hereafter) for each replicate.

The cumulative intercepted radiation (CIR) was determined by summing the product of the daily fractional LI ($\mathrm{CC_{sim}}$) and daily incident radiation from planting to physiological maturity (R6) of maize. Then, the radiation use efficiency (g $\mathrm{MJ^{-1}}$) was calculated by dividing the cumulative aboveground biomass (g $\mathrm{m^{-2}}$) by CIR (MJ $\mathrm{m^{-2}}$) at R6 for each replicate. Here, we assumed that the cumulative aboveground biomass at R6 was equal to the total aboveground biomass determined at harvest. The proportion of intercepted radiation was also calculated as the ratio of CIR to the total amount of incident radiation at R6.

Validation of digital imagery analysis for the maize canopy

The methodology to estimate LI by extracting the green coverage of the canopy in the digital photographs was originally developed for soybeans, in which the results showed a close agreement with LI measurements made with quantum sensors (Purcell, 2000). To confirm the validity of this methodology for the maize canopy, we tested the correlation between $\mathrm{CC_{obs}}$ measured by digital imagery analysis and LI measured by a conventional method using quantum sensors. The LI was calculated as LI = 1 − (I/I_0), where I and I_0 indicate the photosynthetically active radiation (PAR) at ground surface and at the top of the canopy, respectively. I and I_0 were simultaneously measured by placing a line quantum sensor (Li-191SA, Li-COR, Lincoln, NE) diagonally across two maize rows at the soil surface and a point quantum sensor (Li-190SA, Li-COR, Lincoln, NE) above the maize canopy. At maturing stage, the line quantum sensor was placed slightly upwards to avoid the shade from dead leaves close to the ground surface. The LI measurements were conducted between 1000 and 1400 h and averaged six replicates in each plot. The CC–LI matching measurements were repeated for a total of 56 times at different growing stages of maize in the four experimental sites and additionally at two other sites.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed to determine the individual and interaction effects of the location (Nampula, Gurue, Mutuali, and Lichinga; df = 3) and N treatment (0, 30, and 80 N; df = 2) on the measured variables. Tukey's honestly significant difference (HSD) test was conducted to compare the mean values at the 5% level of probability. JMP 8 software (SAS Institute Inc.) was used to perform the statistical analysis.

Results

Weather conditions and phenology development

Table 2 shows the daily mean temperature and daily solar radiation during the periods of the vegetative development stage (planting to tasseling, VT) and reproductive development stage (VT to physiological maturity, R6) of maize at each experimental site. The durations of the vegetative stage tended to be shorter with higher mean temperatures. There was a close linear relationship between the rate of phenology development (1/day) and daily mean temperature from planting to VT with the intercept (base temperature) at 8.3 °C. The durations of reproductive stage had the same tendency except that the Nampula site had a long reproductive period, despite its highest mean temperature. The Lichinga site had a low temperature and long growth duration which reflected its high elevation relative to the other sites. As a result, the growth duration from planting to R6 was 91, 88, 88, and 117 days in Nampula, Gurue, Mutuali, and Lichinga, respectively.

All four sites received consistent rainfall during the vegetative stage while Nampula was exposed to a long dry spell (no rain for 13 consecutive days) in the late growth stage (Figure 1). The changes in soil's water potential also indicated the severe drought stress in the reproductive stage in Nampula and relatively moist field conditions throughout the growth periods in the other three sites. The extended reproductive period in Nampula was probably due to drought stress. The soil's water potential slightly declined below −50 kPa for five days just before R6 in Gurue. The daily solar radiation was greater in the order of Nampula, Gurue, Mutuali, and Lichinga, and the values in Nampula were greater than those in Lichinga by 51% and 40% at the vegetative stage and reproductive stage, respectively.

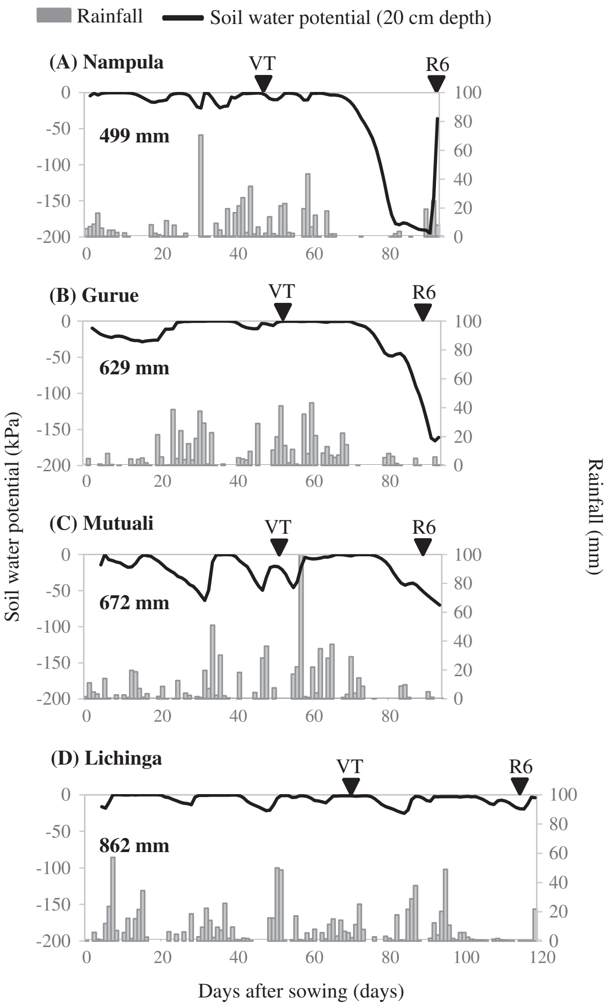


Figure 1. Daily rainfall and soil's water potential at a 20-cm depth beneath the maize canopy in (A) Nampula, (B) Gurue, (C) Mutuali, and (D) Lichinga.

▾ indicate the growth stages of maize (VT: tasseling; R6: physiological maturity). The numeric numbers in bold within the graphs indicate total amounts of rainfall from planting to R6 at each location. *The figure is partly extracted and modified from Tsujimoto et al. (2015).

Grain yield and biomass production

The grain yields and aboveground biomass differed greatly among the treatments by locations in the ranges of 1.6–4.9 and 5.4–13.6 t ha^{-1}, respectively (Table 3). In the 0 N treatments, the maize productivity tended to be greater in the order of Lichinga, Mutuali, Gurue, and Nampula. The ANOVA results indicated that the yield responses to the N application differed among the locations. The effect of N application was relatively small at the drought-stressed Nampula (the yield difference between the 0 and 80 N treatments were 0.5 t ha^{-1} in the means and not statistically significant), whereas in the other three locations, the application of 80 kgN ha^{-1} significantly increased the grain yields by 1.7–2.4 t ha^{-1} compared with those in the 0 N treatment. The AE$_N$ was 5.9 and 6.6 with the 30 and 80 N treatments in Nampula, respectively, while those values ranged between 25.2 and 35.3 and between 20.6 and 29.9 with the 30 and 80 N treatments, respectively, in the other three sites (Table 3). The increased rates in grain yield and aboveground biomass with the N application were particularly large in Mutuali and Gurue. The HI tended to increase with increases in the N application rates (Table 3). In the location means, the Nampula and Gurue had relatively low HI. However, the range of HI among the treatments and locations was relatively small compared with those of the grain yield and aboveground biomass.

CC–LI matching

Figure 2 plotted the relationship between the LI measured by the quantum sensors and CC$_{obs}$ estimated by digital imagery analysis concurrently taken at various stages of the maize canopy. Except for the measurements within 10 days of physiological maturity (closed circles), these two parameters were highly correlated and fell very close to the one-to-one line in a range from 0.25 to 0.85. When the maize canopy approached physiological maturity, the CC$_{obs}$ values were underestimated against the LI, particularly in the drought-stressed Nampula. The correlation between CC$_{obs}$ and LI in the maturity period was slightly improved by adjusting the leaf extraction range toward the red color in the imagery analysis, i.e. by changing the hue settings of extraction from 50 to 120 to 30 to 120 in ImageJ (the cross marks in Figure 2).

Canopy development

The dynamics of CC development were depicted from planting to R6 for each location in Figure 3. Here, the final observations of each replicate were replaced by the LI values measured with quantum sensors because of the underestimation in leaf areas when extracted by digital imagery analysis at the leaf senescence period.

The simulation curves were adequately fitted to the observations with the RMSE range of 0.8–2.4. At the warm climatic conditions, i.e. Nampula, Gurue, and Mutuali, the figures showed a sharp increase in CC$_{sim}$ soon after planting and irrespective of the N application rates. A comparison of the crop growth coefficient (CGC) also indicated that the canopy development rates were greater than at the cool climatic condition of Lichinga but not affected by N application rates at these three sites (Table 4). On the other hand, the CGC values were small and significantly improved by the N application in Lichinga. The ANOVA results also suggested the interactive effect between the N treatment and location on the CGC. The days from planting to reach at half of the CC$_{max}$ also indicated the slow canopy development at the Lichinga site compared to the other sites (Table 4).

Table 3. Comparison of grain yield, aboveground biomass at maturity, HI, and agronomic nitrogen use efficiency (AE_N) as affected by different N application rates at the four experimental locations.

Location	Treat-ment	Grain yield t ha^{-1}	Aboveground biomass t ha^{-1}	HI	AE_N kg kg^{-1}
Nampula	0 N	1.6^{ef}	5.4^{eg}	0.30^{ab}	-
	30 N	1.8^{ef}	6.2^{eg}	0.29^{ab}	5.9
	80 N	2.1^{def}	7.1^{cefg}	0.30^{ab}	6.6
Gurue	0 N	1.7^{f}	6.6^{fg}	0.25^{b}	-
	30 N	2.8^{de}	9.4^{abcde}	0.30^{ab}	35.3
	80 N	3.9^{abc}	11.6^{abd}	0.34^{ab}	27.3
Mutuali	0 N	2.5^{cdef}	7.2^{defg}	0.35^{a}	-
	30 N	3.4^{bcd}	11.0^{abc}	0.31^{ab}	29.7
	80 N	4.9^{a}	13.6^{ab}	0.36^{a}	29.9
Lichinga	0 N	2.8^{cdef}	9.2^{bcdef}	0.31^{ab}	-
	30 N	3.6^{abcd}	11.0^{abcd}	0.33^{ab}	25.2
	80 N	4.5^{ab}	13.1^{a}	0.34^{a}	20.6
Treatment mean					
	0 N	2.2^{c}	7.1^{c}	0.30^{b}	
	30 N	3.0^{b}	9.5^{b}	0.31^{ab}	
	80 N	3.9^{a}	11.3^{a}	0.34^{a}	
Location mean					
	Nampula	1.8^{b}	6.2^{c}	0.30^{b}	
	Gurue	2.8^{ab}	9.2^{b}	0.30^{b}	
	Mutuali	3.6^{a}	10.6^{ab}	0.34^{a}	
	Lichinga	3.6^{a}	11.1^{a}	0.33^{ab}	
ANOVA summary					
Location (L)		***	**	**	
N application (N)		***	***	*	
L x N		**	*	ns.	

Note. Within each column, values with the same letter do not significantly differ at 5%.
*$p < 5\%$; **$p < 1\%$; ***$p < 0.1\%$. ns. not significant.

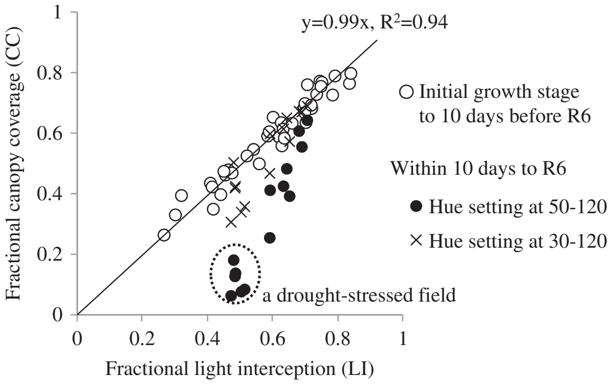


Figure 2. Fractional canopy coverage (CC) versus fractional LI in the measurements during various growth stages of maize at six sites by year experiments.

*Open circles and closed circles represent the measurements from initial growth stages to 10 days before physiological maturity (R6) and within 10 days of R6, respectively. For the measurements within 10 days of R6, adjusted values of fractional canopy coverage were shown in the cross marks by changing the lower limit of the hue setting from 50 to 30 to extract leaf areas of the images. The linear regression line was fitted to the open circle values.
**The hue setting in the ImageJ has the full-scale range from 0 to 255 that corresponds to the attribute of a visual sensation according to which an area appears to be one of the perceived colors, starting from red at 0, through green, blue, and then by way of the purples back to red again at 255. The Hue of 50–120 corresponds to the green area. The Hue of 30–50 is a transitional range from green to yellow and orange.

The crop declining coefficient (CDC) was not affected by the N application but by the location. Among the location means, the Nampula site showed a sharp reduction in CC_{sim} with the highest mean CDC. The sharp decline in CC_{sim} was probably associated with the occurrence of a long dry spell in its late growth stage at the site.

Cumulative intercepted radiation

CIR from planting to R6 ranged between 691 and 970 MJ m^{-2} with a coefficient of variation (CV) at 9.3% among the treatment by location (Figure 4(A)). The CIR increased with greater amounts of N application, particularly in Lichinga (by 19% with the 80 N treatment relative to the 0 N treatment). In the other three sites with warm climatic conditions, the effect of N application on CIR was relatively small with the increased rates of 12–13% by the 80 N treatment relative to the 0 N treatment.

In a comparison of the location means, the CIR values were the lowest in Lichinga, despite the longer growing period than the other sites. This result occurred because the Lichinga site was the lowest both in the daily incident radiation (Table 2) and in the proportion of intercepted radiation (Table 4). The low proportion of the intercepted radiation was also evidence of the slow initial canopy development at the site (Figure 3(D)). The Nampula site also showed a low proportion of intercepted radiation (42.5% in the location mean) due to the sharp decline in CC_{sim} during the long dry spell, whereas the CIR values were the greatest with large amounts of the incident radiation.

Radiation use efficiency

RUE (g MJ^{-1}), calculated by dividing the cumulative aboveground biomass by CIR at R6, varied greatly between 0.62 and 1.59 g MJ^{-1} (Figure 4(B)) and showed a close correlation with the grain yield (figure not shown, $R^2 = 0.87$***). The CV in RUE was much greater at 35.3% than that in CIR among the treatment by location. The N application significantly improved the RUE at all of the sites and at Mutuali and Gurue in particular with the increased rates of 71 and 59%, respectively, in the 80 N treatment relative to the 0 N treatment. In Lichinga, the RUE was relatively high at 1.33 g MJ^{-1} even under the 0 N treatment and increased to 1.59 g MJ^{-1} (+20%) with the 80 N treatment. The RUE values and the effect of the N application on RUE were both limited in a drought-stressed Nampula. In a comparison of the location means, the RUE values were greater at the sites (in the order of Lichinga, Mutuali, Gurue, and Nampula) where the field environments were endowed with adequate rainfall throughout the growing period (Figure 1)

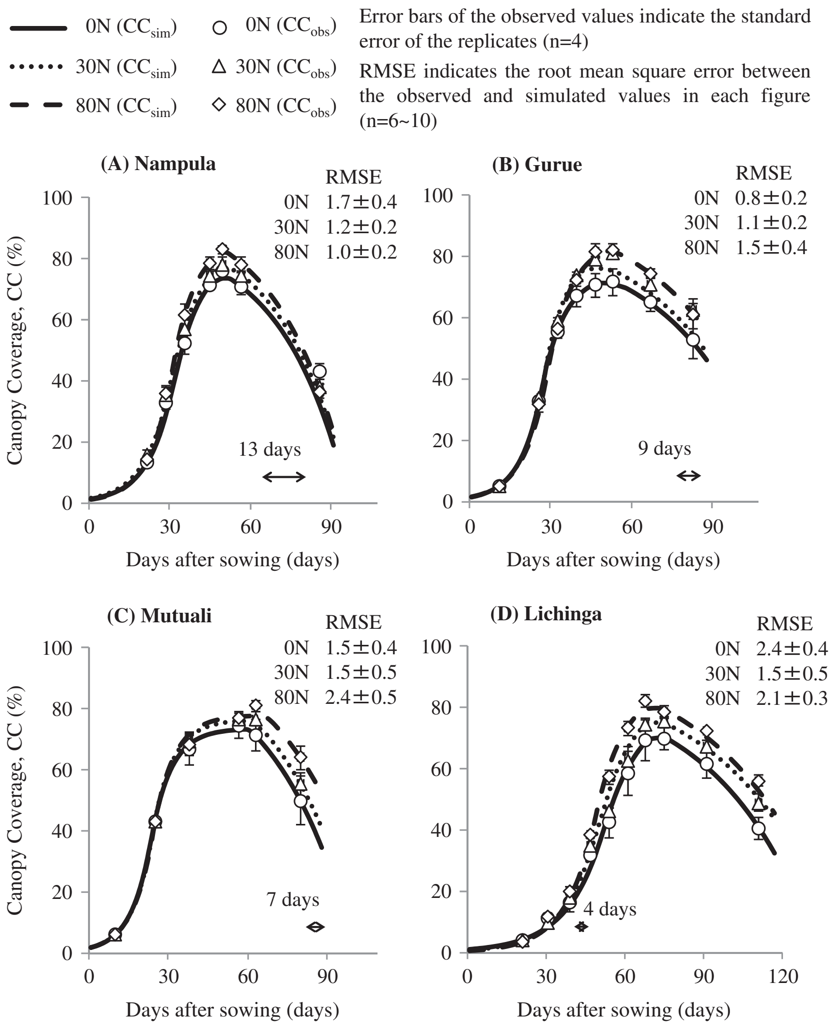


Figure 3. Changes in the observed and simulated values of the canopy coverage with different N application rates in the four experimental locations.

*Solid arrow within each figure indicates the periods of the maximum consecutive days without rainfall at each experimental site. It should be noted that the final observation values were all replaced by the LI values measured with quantum sensors.

and with relatively rich soils in terms of clay contents, total carbon contents, and CEC (Table 1).

Figure 5 plotted RUE against total N uptake at maturity. Greater N application rates linearly increased both N uptake and RUE at Gurue and Mutuali. The Lichinga site showed modest improvement in RUE by the N application while the increased amounts in total N uptake were equivalent to the above-said two sites. In Nampula, greater N application slightly but significantly increased total N uptake, whereas there was no significant treatment effect on RUE.

Discussion

Maize grain yield and response to nitrogen application

Our experiments provided the empirical data of the maize yield and AE_N as a response to different N application rates at various agro-environments in Northern Mozambique. The results suggested that, except for the Nampula site that was exposed to a long dry spell, the side dressing of urea at the initial growth stage had a positive effect on grain yields of maize (Table 3). The AE_N values at these sites (28 ± 5 kg kg^{-1}, n = 6) were comparable to those (23 ± 19 kg kg^{-1}, n = 324) shown in an extensive review of researcher-led fertilizer trials of maize across the SSA countries (Vanlauwe et al., 2011).

An economic survey at the study sites allowed us to estimate the ratio of fertilizer (urea N) to output (maize) prices at approximately 7.4 by utilizing the retail price of urea (65.2 MZN per kg of N) and an average of farm-gate prices of maize (8.8 MZN per kg of grain) in the main harvest season. This ratio is comparable with what has been reported in the same region in the 2010/2011 cropping season (World Bank, 2012), in which the report concluded that the ratio was too high for farmers to have

Table 4. Comparison of the canopy growth coefficient (CGC), canopy decline coefficient (CDC), observed maximum canopy coverage, proportion of radiation intercepted, and days to $1/2CC_{max}$ as affected by different N application rates at the four experimental locations.

Location	Treatment	Canopy growth coefficient (CGC)	Canopy decline coefficient (CDC)	Observed max canopy coverage	Proportion of radiation intercepted[1]	Days to $1/2CC_{max}$[2]
				%	%	days
Nampula	0 N	0.11^{bcd}	3.36^{ab}	76.0^{a}	40.0^{de}	32.2^{bc}
	30 N	0.11^{cd}	3.56^{a}	78.0^{a}	42.8^{bcde}	33.7^{b}
	80 N	0.12^{abc}	3.72^{a}	83.1^{a}	44.8^{abcde}	33.7^{b}
Gurue	0 N	0.12^{abc}	2.37^{de}	72.6^{a}	46.4^{abcde}	27.1^{cde}
	30 N	0.12^{abc}	2.51^{cde}	81.2^{a}	49.1^{abcd}	28.0^{bcde}
	80 N	0.12^{abc}	2.61^{bcde}	82.8^{a}	52.0^{ab}	29.6^{bcd}
Mutuali	0 N	0.14^{a}	3.27^{abc}	73.5^{a}	47.3^{abcd}	23.5^{e}
	30 N	0.13^{a}	3.19^{abcd}	77.6^{a}	50.8^{abc}	23.9^{de}
	80 N	0.13^{ab}	3.02^{abcde}	81.3^{a}	53.7^{a}	24.3^{de}
Lichinga	0 N	0.07^{f}	2.27^{e}	72.1^{a}	36.5^{e}	52.1^{a}
	30 N	0.08^{ef}	2.30^{e}	75.5^{a}	40.7^{cde}	50.3^{a}
	80 N	0.09^{de}	2.33^{e}	82.4^{a}	43.5^{bcde}	49.2^{a}
Treatment mean						
	0 N	0.11^{a}	2.82^{a}	73.6^{c}	42.6^{b}	31.7^{a}
	30 N	0.11^{a}	2.90^{a}	78.3^{b}	45.8^{a}	32.0^{a}
	80 N	0.12^{a}	2.92^{a}	82.4^{a}	48.5^{a}	32.2^{a}
Location mean						
	Nampula	0.11^{b}	3.55^{a}	79.0^{a}	42.5^{b}	33.2^{b}
	Gurue	0.12^{ab}	2.50^{b}	78.9^{a}	49.2^{a}	28.2^{c}
	Mutuali	0.13^{a}	3.16^{a}	77.5^{a}	50.6^{a}	23.9^{d}
	Lichinga	0.08^{c}	2.30^{b}	76.7^{a}	40.2^{b}	50.5^{a}
ANOVA summary						
Location (L)		***	***	ns.	***	***
N application (N)		$p = 0.0503$	ns.	***	***	ns.
L × N		***	ns.	ns.	ns.	ns.

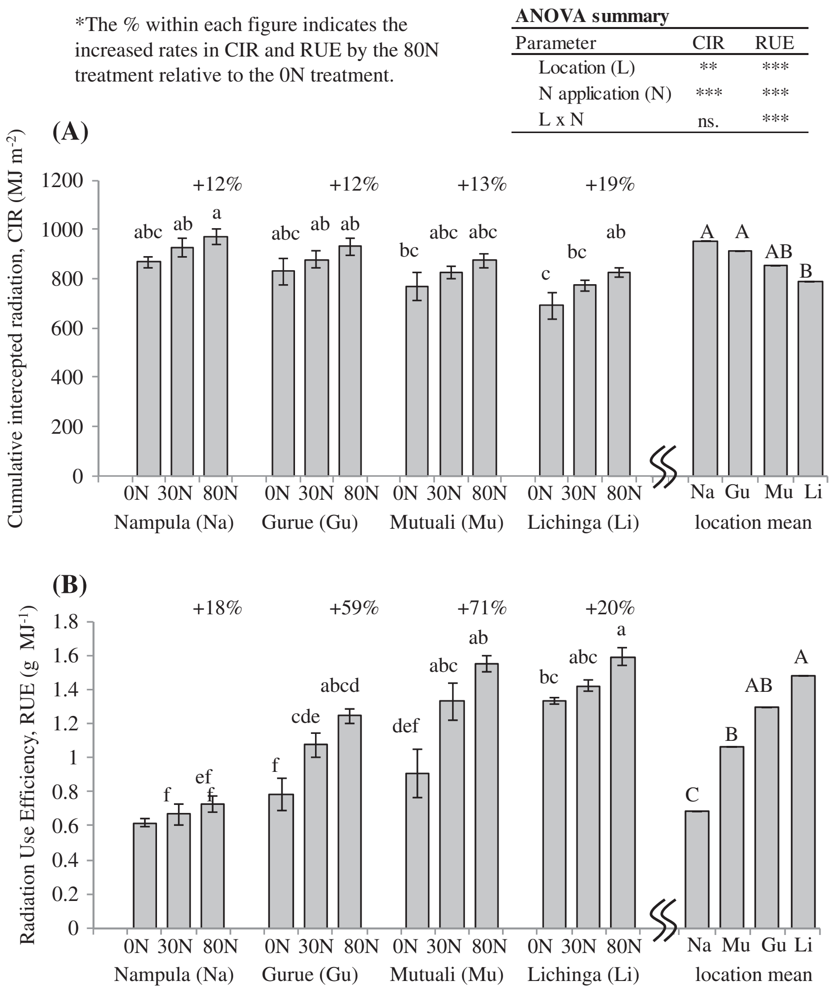
Note. Within each column, values with the same letter do not significantly differ at 5%.

*$p < 5\%$.; **$p < 1\%$.; ***$p < 0.1\%$. ns. not significant; [1]Proportion of radiation intercepted was calculated as the ratio of cumulative intercepted radiation (CIR) to cumulative incident solar radiation from planting to physiological maturity. [2]Days to $1/2CC_{max}$ indicate the duration from planting to when the simulated canopy coverage (CC_{sim}) reaches half of the maximum canopy coverage (CC_{max}).

any incentives to use fertilizer in Mozambique. However, when the same level of AE_N can be consistently achieved, as shown in our three experimental sites, the application of urea would be beneficial and attractive to farmers for maize production. Yanggen et al. (1998) indicated that the ratio of the extra output value over the cost of fertilizer input should be higher than 2 and preferably 3–4 for farmers to consider financial incentives using mineral fertilizer in the SSA. In our study, the value/cost ratio above 2, 3, and 4 can be achieved when the AE_N is 14.8, 22.2, and 29.6 kg kg^{-1}, respectively.

The effect of N application was compromised and not economically profitable in Nampula, which is probably because the water stress overwhelmed the N deficiency in determining the grain yield. This result implies that adequate rainfall during the growing season is fundamental to achieve any profits from fertilizer application. In addition, sandy soils with low carbon content might have also confounded the fertilizer use efficiency at the Nampula site. Previous studies indicated that both carbon content and soil texture would play an important role in enhancing the fertilizer use efficiency of maize in the degraded soils of SSA (Kurwakumire et al., 2014; Vanlauwe et al., 2011; Wopereis et al., 2006). In the on-farm trials of Eastern Zimbabwe, Kurwakumire et al. (2014) reported poor responses of maize to applied N when the organic carbon and clay contents of the soils were less than 4.0 g kg^{-1} and 10%, respectively, even under adequate rainfall conditions. The carbon (organic) contents of soils are considered to be attributable to the capture of fertilized N, which may improve the synchrony between the supply and demand of N by the crop and thereby improve the fertilizer use efficiency. In addition, N applied as a top-dressing is prone to leaching in light-textured soils.

Our previous survey identified that both rainfall and the clay and carbon contents of soils tended to be high westward in the mid–high-elevation inlands of Northern Mozambique where Lichinga, Gurue, and Mutuali are located (Tsujimoto et al., 2011, see also Tables 1 and 2). Therefore, selective urea application in these areas should be one approach to enhance the fertilizer use efficiency and benefits from the fertilizer inputs, and to increase the productivity of maize in the region. However, it should be noted that weed management and planting densities were under optimal conditions for all of the experimental sites in the current study. Dimes et al. (2015) indicated that appropriate weed management and plant stands would need to be the first step for the improvement of maize productivity in Eastern and Southern Africa, or the benefits of small fertilizer applications would be compromised.



*The % within each figure indicates the increased rates in CIR and RUE by the 80N treatment relative to the 0N treatment.

ANOVA summary

Parameter	CIR	RUE
Location (L)	**	***
N application (N)	***	***
L x N	ns.	***

Figure 4. Comparison of (A) CIR and (B) RUE for the different N application rates at the four experimental sites.
*Values with the same letter do not differ significantly at 5% by the Tuley's HSD test. The error bars indicate the standard error of the replicates ($n = 4$).

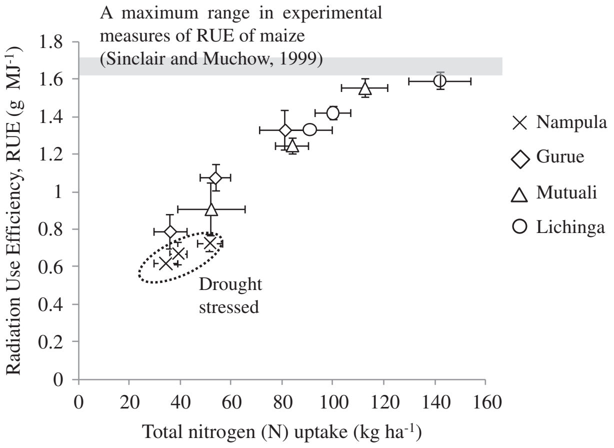


Figure 5. Relationship between total nitrogen uptake and RUE from planting to the physiological maturity of maize.
*The error bars indicate the standard errors of replicates ($n = 4$).

Validity of the imagery analysis for estimating LI of maize canopy

Our results showed a close 1:1 relationship between the CC_{obs} values from the digital imagery analysis and LI values measured by quantum sensors. Theoretically, this result is not surprising because the method itself has been successfully used in soybeans and wheat in previous studies (Caviglia et al., 2004; Purcell, 2000). However, to our knowledge, this is the first empirical data to show the validity of the digital imagery analysis for the tall canopy of maize (up to 2.3 m in plant height in the current study) across various N management practices and field environments in the tropics.

It should be noted that the digital imagery extraction caused an underestimation of LI at the maturity stage and particularly at the drought-stressed Nampula site (Figure 2). This was probably because the discoloring leaves in the process of senescence were excluded from the fractional green areas in the digital imagery analysis while they intercepted the incident solar radiation in the measurements with quantum sensors. We avoided the shade of senesced leaves near to the ground surface by placing the line quantum sensors upwards during the reproductive stage, but partially discoloring and upper leaves still caused the discrepancy between CC_{obs} and LI when close to maturity or after the severe drought stress in Nampula. The adjustment of the leaf extraction range toward the red color slightly improved the correlation between CC_{obs} and LI in the maturity period. However, this manipulation also caused difficulties in differentiating the leaves from the soil areas, and besides it has a risk of underestimating RUE by including non-active leaves.

It is generally regarded that the estimation of RUE during the reproductive stage often causes measurement error due to the biomass loss through leaf abscission or to the light interception by non-active leaves (Muchow et al., 1993). In case of the imagery analysis as well, the boundaries between active and non-active leaf areas of maize were not clear during the period of rapid color change. The results indicate that the digital imagery technique can be applicable as a simple and inexpensive method to determine the fractional LI of maize except when the leaf discoloration is accelerated in the physiological process of senescence and also by drought stress.

Effect of N treatment by the field environment on RUE

The measurements of CC with digital imagery analysis and its interpolation utilizing the AquaCrop model simplified the light-based analysis to assess the physiological basis of variation in maize yields in different N application rates at multiple locations. The results showed that the yield variations of maize among the N treatments and locations were highly correlated with RUE from planting to R6. Generally, RUE is limited by the leaf photosynthesis rate, which is highly relevant to factors such as leaf N content and leaf stomatal conductance (Sinclair & Horie, 1989; Sinclair & Muchow, 1999). In the current study, we did not conduct the periodic measurements of the leaf N content, whereas the RUE among treatments and locations showed close and curvilinear responses against the total N uptake at maturity (Figure 5). This responsive curve in Figure 5 coincides with a previous finding by Muchow and Davis (1988) in which the RUE of maize grown in a wide range of N application rates linearly increased with higher N uptake at maturity up to approximately 100 kgN ha^{-1}, and then leveled off at a maximum value of RUE around 1.4–1.5 g MJ^{-1}. The plateaued range of RUE in these two studies fitted to the physiological ceiling of RUE for maize. Sinclair and Muchow (1999) reviewed a number of studies and concluded that the maximum RUE for maize in the field ranged from 1.6 to 1.7 g MJ^{-1} during the vegetative growth stage and from 1.3 to 1.7 g MJ^{-1} for the whole growing period.

The lower range of RUE in the whole growing period than in the vegetative growth period is generally explained by the physiological reduction of the leaf photosynthetic capacity in the process of leaf senescence and N allocation to the grain in the reproductive stage. Previous studies indicated that the differences in RUE among various N application rates have become particularly significant in the reproductive stage when the RUE gradually declined along with the leaf N content depending on the N deficiency status of the treatments (Muchow & Davis, 1988; Vos et al., 2005). Although we did not measure the RUE in the vegetative stage and in the reproductive stage separately, a large variation of RUE found in the current study could be also attributable to the degrees of N deficiency in the reproductive stage at a given N application rate and field environment. For instance, consistent N supply from relatively fertile soil (the highest in clay content and total C and N contents among the sites, see Table 1) and the moist soil condition (Figure 1) in Lichinga might have contributed to the large N uptake at maturity and maintenance of high RUE in the entire growth period (Figure 5). On the other hand, the insufficient N supply might have caused gradual N deficiency and reduction in RUE in later growth stage in the 0 N treatment in Gurue having sandy soils with limited contents of carbon. It should be noted that a humid climate with low and diffusive radiation could have also favored high RUE at the Lichinga site relative to the other sites as indicated in previous studies (Hammer & Wright, 1994; Sinclair & Shiraiwa, 1993).

At the Nampula site, the drought stress probably constrained the RUE irrespective of the N application rates. The soil's water deficit is commonly acknowledged as a major factor to affect leaf photosynthesis and RUE (Bolaños & Edmeades, 1993; Muchow, 1989).

Effect of N treatment and field environment on CIR

The effect of N application on the CIR was modest relative to that on RUE. The result is consistent with previous findings that maize generally strives for maintenance of the leaf area at the expense of decreased leaf N concentration and decreased photosynthetic capacity when exposed to N limitation (Gastal et al., 2015). Vos et al. (2005) referred to this type of response as 'maize strategy', whereas some broadleaf species, such as potatoes, maintain the leaf N concentration and RUE at the expense of decreased leaf area at the N deficiency. Lemaire et al. (2008) proposed that C3 species were more likely to maintain leaf N content and RUE at the expense of leaf area than C4 species.

Relatively small variation in CIR could also be relevant to the fact that the canopy development was vigorous irrespective of the N application rates at the low–mid-elevation sites where the climate was endowed with a high temperature and adequate rainfall during the vegetative growing stage (Figure 3(A–C)). The result of Muchow and Davis (1988) in the semi-arid tropics of Australia demonstrated that the amounts of intercepted radiation were relatively stable in the broad range of N application rates between 60–420 kgN ha^{-1}, and that the yield variation was mostly derived from the RUE differences, particularly during the reproductive stage. These results together with a discussion in the previous section imply that an additional N supply is particularly important for maintaining high leaf N content and RUE to late growth stage to achieve the high grain yield of maize while LI can be ensured with vigorous canopy development in the tropics.

Contrary to the warm sites, the effect of N application on CIR was relatively large at Lichinga (Figure 4(A)). A slight improvement of the initial canopy development with the N application caused cumulative differences in CIR during the long growth period at Lichinga (Figure 3(D)). The stagnant canopy development in Lichinga, which has the highest RUE among the sites, agrees with the conventional understanding that leaf appearance and expansion are more sensitive to low temperatures than leaf photosynthesis or RUE of maize (Duncan & Hesketh, 1968). White and Reynolds (2003) reviewed some studies (Duncan & Hesketh, 1968; Kiniry et al., 1989) and reported little variation of maize RUE over a broad temperature range between 19 and 27 °C, whereas the leaf growth rate is linearly decreased by lowering temperature in the same range.

Our experimental result in Lichinga implies that the amelioration of initial canopy development should be important to further increase maize yield while the RUE is relatively high at the cool climate highlands of Northern Mozambique. The proportion of radiation intercepted (36.5–43.5% in Table 4) in Lichinga was still low relative to the other sites or previous high-yield experiments (Lindquist et al., 2005; Muchow, 1989).

Therefore, a manipulation of planting density can be one aspect of further studies to understand the relationship between the initial canopy development and maize yield in the highland areas. Higher planting density generally shortens the time from planting to maximum canopy coverage. Westgate et al. (1997) demonstrated the advantage of the higher planting density of maize in a range of 5.0–12.5 plants m^{-2} to accelerate the canopy closure and to increase the intercepted radiation and grain yield in a cool climate condition of Northern USA. Edwards et al. (2005) also indicated the positive effect of increasing the planting density on maize yield in a range of 5–20 plants m^{-2} when growing short-season hybrids with limited amounts of cumulative thermal units and LI in mid-south USA.

Conclusion

Our study demonstrated the validity of digital imagery analysis to readily estimate the LI of the maize canopy except under leaf discoloring periods. The method enabled us to conduct a light-based analysis of maize growth at multiple sites and under poorly equipped conditions in the rain-fed fields of Northern Mozambique. As a result, we identified that the top-dressing of urea at small rates is beneficial to increase maize yields largely due to the RUE enhancement under warm and humid climates. In cool and high-elevation sites in the western highlands, the N application improved not only RUE but also LI by accelerating the stagnant initial canopy development. Conversely, in the semi-arid and sandy areas in the east, the effect of N application is most likely unreliable due to the risk of drought stress. Although the physiological responses of maize to N supply have been analyzed in many studies, the quantitative and empirical data across various field environments in a local scale should help develop effective fertilizer management plans, enhance farmers' incentives for fertilizer inputs, and increase productivity of maize in the target region.

Disclosure statement

No potential conflict of interest was reported by the authors.

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