IMPACT OF NITROGEN (N) FERTILIZATION ON THE REFLECTANCE OF COTTON PLANTS AT DIFFERENT SPATIAL SCALES

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ABSTRACT

In this study, the reflectance of cotton plants was measured at three different spatial scales and N treatment effects were examined and compared. The importance of this examination was to select the best spatial scale(s) for estimating chlorophyll or N content of cotton. Three spatial scales were identified: the individual leaf, the canopy, and the scene. Using a GER 1500 field spectroradiometer, spectral reflectance at those spatial scales was measured in extensive field campaigns in 2010 and 2011. The wavelengths at which N treatment effects were most pronounced are different at each spatial scale: 550 nm and 700 nm at the individual leaf level; 600 nm and 700 nm at the canopy level; and 685 nm and 690 nm at the scene level. Each spatial scale is subject to different inherent variations, where different components of vegetation and soil become major factors in determining the reflectance at that scale. Since the effects of N fertilization include both variations in chlorophyll content and ground cover, selecting only one scale may not be sufficient to capture the effects of N treatments. Therefore, a more practical approach may be to combine spectral data at spatial scales that respond to chlorophyll content (either the leaf or canopy level) with corresponding data at the spatial scale that best explains the variation in ground cover (the scene level).

Keywords: **cotton, reflectance, nitrogen, spatial scale, leaf, canopy, scene**

INTRODUCTION

In recent years, spectral reflectance methods have gained popularity among agronomy researchers for estimating chlorophyll or N nutrition of crops. For cotton, the scope of research includes the selection of the spectral wavelengths that are most sensitive to the parameters of interest, and also the exploration of sensors with either narrow contiguous hyperspectral bands, or those with broad multispectral bands. Most of these studies were performed either at the individual

leaf, canopy or scene levels (Tarpley et al., 2000; Buscaglia and Varco, 2002; Read et al., 2002; and Zhao et al., 2005).

Studies involving the sources of variability in plant canopies have long been a topic of interest in remote sensing studies. While many of these studies have focused on the selection of the best wavelengths at a certain spatial scale through correlation analysis, a limited number of research efforts have dealt with the effect of spatial scales in making scientific measurements related to N-nutrition (Baret, 1994; Asner, 1998; Daughtry et al., 2000; Blackburn, 2007; and Haboudane et al., 2008).

Early work has established the factors that contribute to the variations in reflectance measured at leaf and scene scales. In the spectral region of 500 – 750 nm (visible), reflectance is mainly a function of pigment absorption (Gausman et al, 1969; and Woolley, 1971). Studies by Billings and Morris (1951), Knipling (1970), and Woolley (1971) showed that reflectance in the 750 – 1300 nm (NIR) region is not attributed to chlorophyll absorption because this pigment is not affected by NIR radiation. The spectral spectra of leaves in the NIR region are characterized by a region of high reflectance caused by the leaf's internal structure and water content.

According to Kipling (1970), the reflectance of a plant canopy will be considerably lower than that of the individual leaves that comprise the canopy. Many additional are important in determining canopy reflectance in addition to single leaf reflectance, such as illumination and viewing angle, leaf orientation, amount of leaves, characteristics of the non-foliage (soil) background, and shadows (Knipling, 1970; Colwell, 1974; Baret, 1994; and Blackburn, 2007).

The factors affecting vegetation canopy reflectance, such as leaf display and leaf amount, are often described through the quantity leaf area index (LAI). Asner (1998) found that LAI has a pronounced effect on vegetation canopy reflectance in the NIR, but a minimal effect in the visible region. Daughtry et al., (2000) showed through simulation that LAI, background reflectance, and leaf optical properties are important parameters influencing scene reflectance. However, according to Maas (1997), scene reflectance is more a function of vegetation ground cover than vegetation canopy density (LAI).

This study was designed to compare the reflectance of cotton plants as influenced by nitrogen (N) fertilization at different spatial scales. The specific objective of this study was to determine the spatial scale(s) that best show N treatment effects for the purpose of estimating chlorophyll or N concentration of crops using remote sensing.

MATERIAL AND METHODS

Intensive field projects were conducted in 2010 and 2011 at the Texas Tech University Quaker Avenue Research Farm (Station 1), the Texas Agrilife Research and Extension in Lubbock (Station 2), and the Texas AgriLife and Extension Center in Halfway (Station 3). In 2010, the experimental study was conducted at Stations 1 and 2. However, in 2011, the experiment at Station 2 was terminated, and research efforts moved to Station 3. The experiment at Station 1 contained three levels of N application: 0, 67, and 134 kg ha^{-1}. At Station 2, there were 3 levels of N application: 0, 50 kg, and 101 kg ha^{-1}. N applications at Station 3 also consisted of three treatment levels: 0, 112 and 224 kg ha^{-1}. The soil types

at Stations 1, 2 and 3 are an Acuff sandy clay loam, an Acuff-Urban land complex, and a Pullman clay loam, respectively. All the experimental plots were well irrigated.

A GER 1500 spectroradiometer (SpectraVista Corp., Poughkeepsie, NY) was used to measure reflectance at three different spatial scales: the individual leaf, canopy, and scene. This spectroradiometer has a spectral range from 290 nm to 1030 nm, and a sampling interval of 1.5nm, with a 4° field of view. Canopy reflectance throughout this study is defined as the reflectance dominated by the leaf canopy alone and does not contain measurements of the background soil adjacent to the canopy. Scene reflectance includes soil background effects and is analogous to the reflectance measured by sensors aboard aircraft or satellites. In this study, scene reflectance was computed by averaging the reflectance measured along a transect spanning one row of cotton plants from the center of one furrow to the center of the next furrow, a distance equal to the row spacing.

For the leaf reflectance measurements, leaves were detached from the plant and immediately measured using an integrating sphere (LI-COR Inc., Lincoln, NE) attached to the GER 1500 via a fiber optic cable. The integrating sphere was used to achieve uniform, hemispherical illumination of the leaf samples. The leaf blade was mounted to the integrating sphere with the adaxial surface facing the inside of the sphere. Reflectance measurements from 15 leaves were taken per replication. Canopy reflectance measurements were made using the GER 1500 held at a constant distance (50 cm) above the top of the plant canopy centered on the plant row. Canopy measurements were made on clear days, with the time of data collection ranging from two hours before local solar noon to two hours after local solar noon. Prior to the collection of canopy reflectance at each replication plot, the spectroradiometer was calibrated using a reference panel of known reflectance. Six to ten canopy reflectance measurements were made for each plot. Scene reflectance measurements were made using the transect method as previously described (for additional details on this method, see Maas, 1997). In 2010, all measurements were made on two low-N and two high-N treatment plots. However, in 2011, scene reflectance was measured on each data acquisition date on at least 3 replications of the treatments. The exact number of measurements depended on the weather conditions during the acquisition day.

Chlorophyll meter readings and percent ground cover were also measured for the interpretation of reflectance data. A non-destructive method was used to measure the relative chlorophyll content, i.e., the Minolta SPAD 502 Chlorophyll Meter (Minolta Corp., Osaka, Japan).Overhead photographs for the calculation of percent ground cover were obtained using a Kodak digital camera (Eastman Kodak Company, Rochester, NY) attached to an adjustable pole that allowed the camera to be positioned approximately 3 m above the canopy. Ground cover was calculated using Adobe Photoshop (Adobe System Inc., San Jose, CA) software by dividing the image pixels containing canopy by the total number of image pixels. Canopy pixels were selected using the Select Range toolbox, and hand segmentations were performed following the procedure to eliminate the effects of volunteer plants, weeds, surface litter and shadows. Three to six images were taken at each replication plot.

The raw leaf spectrum data were averaged over 10-nm intervals. The purpose of the averaging procedure was to reduce random noise in the raw spectral data resulting from a lower signal-to-noise ratio associated with the use of the integrating sphere. This produced a smoother, more meaningful reflectance spectrum. Canopy and scene measurements did not require this smoothing, since the target was illuminated by ambient sunlight. Scene reflectance was computed by averaging the individual spectra obtained along a transect and included measurements over the plant canopy and adjacent soil.

RESULTS AND DISCUSSION

To facilitate the interpretation of reflectance spectra, results reported here are focused on two main spectral regions: the visible and red-edge region (450-740 nm) and the NIR region (750-900 nm).

Leaf reflectance

Reflectance in the visible region varied from 8 to 19% across the wavelength range. The spectral curves peaked near 550 nm, with two distinctive chlorophyll absorption bands around 480 nm and 680 nm. Overall reflectance in the visible range decreased with increasing amounts of applied N (Figs. 1a to 1c) and, therefore, chlorophyll content. For leaves measured from the zero- N plots throughout the growing season, reflectance in all wavelengths was consistently the highest, followed by reflectance from the medium- and high-N plots. Visual differences among the reflectance spectra were most apparent at 550 nm (green region). Ratio analysis (Figs. 1d to 1f) also successfully displayed sensitivity to chlorophyll content at 700 nm (red-edge region). Regardless of the N treatment, strong absorption could be observed at 480 nm and 680 nm, where the ratios between N rates were close to one (Figs. 1d to 1f). This finding agreed with the results of other studies that, at the leaf level, reflectances at the blue and red wavelengths are easily saturated, and did not exhibit sufficient sensitivity for estimating chlorophyll and N concentrations (Gitelson and Merzlyak, 1996; and Sims and Gamon, 2002). It appeared that, at the leaf scale, green and red-edge reflectance showed higher sensitivity that is preferred for N estimation in cotton (Thomas and Gausman, 1977; Tarpley et al., 2000; Zhao et al., 2005; and Buscaglia and Varco. 2002).

Reflectance in the NIR region substantially differed from that in the visible region and was characterized by a flat plateau. Reflectance values in this region were less variable than their counterparts in the visible region, and ranged from 55% to 57% throughout the growing season (Figs.1a to 1c). In contrast to the pattern shown in the visible region, the relationship between NIR reflectance and the amount of N applied was not as consistent. For all sampling dates except DAP 64, the ratio of reflectance between the low and high N treatments, and between the medium and high N treatments, was close to one (Figs. 1d and 1f). Leaves with low N content tend to have more compact cell structure (Ollinger, 2010) and, therefore, the N-deficient treatment should be expected to produce thinner leaves
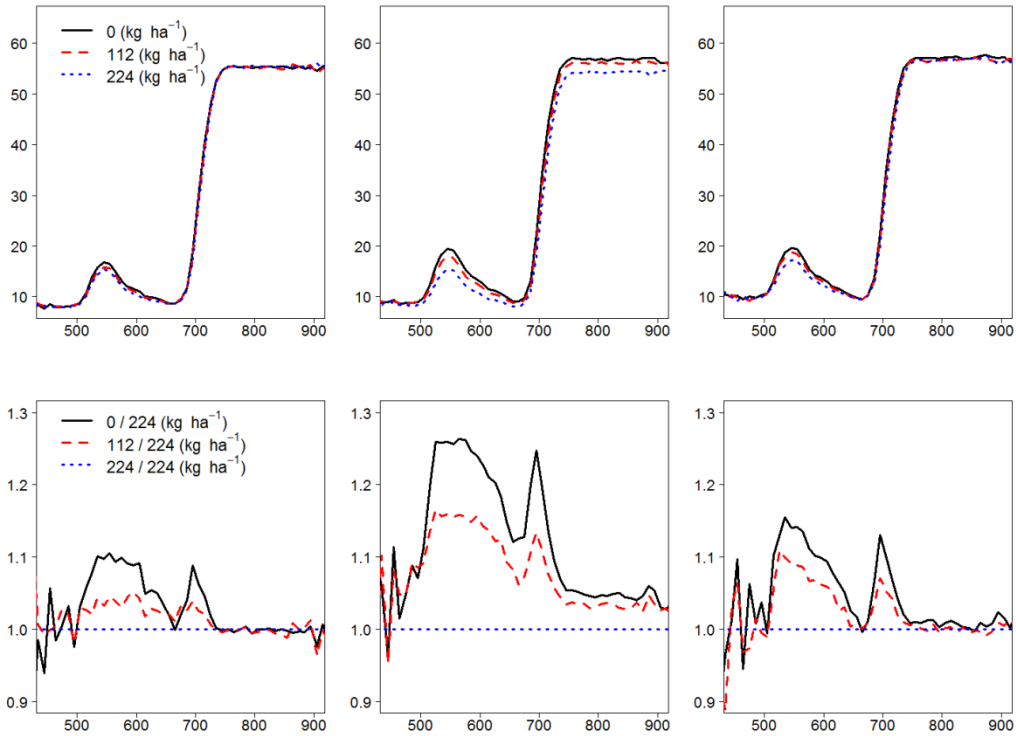
with lower NIR reflectance. Since the NIR reflectance did not appreciably change across N treatments, it is possible that the N treatments in this study did not significantly affect the internal structure of the leaves. The possibility that leaf internal structure was not affected by the N treatments, as suggested by the measurements at the leaf scale, the high relative differences observed at the red-edge wavelengths was likely controlled more by chlorophyll absorption on the visible end than by scattering by leaf internal structure on the NIR end (Horler et al., 1983).

On DAP 64, NIR reflectance for the leaves receiving no N application was slightly higher than reflectance of high-N treatment leaves (Fig. 1b). Myers and Allen (1968) attributed this increase in reflectance to smaller and fewer cells within the stressed leaves that consequently had a reciprocal effect on the number of air spaces.

(a)   (b)   (c)

(d)   (e)   (f)

Canopy reflectance

In the visible region, the general shape of the curves for cotton canopy reflectance was similar to that for leaf reflectance (Figs. 2a to 2c). However, the range of reflectance values was slightly less (approximately 4 to 12% across the visible wavelengths). Decreased overall reflectance in the visible region was associated with the more complex nature of the plant canopy, in which reflectance was affected by leaf display characteristics and shadows within the canopy.

Two distinctive chlorophyll absorption bands are observed at around 480 nm and 685 nm, with a peak near 550 nm. Early in the planting season, relative differences in the reflectance of the cotton canopy in the visible region were somewhat inconsistent with the amount of applied N (Fig. 2a), although the actual differences were small. The N-deficient canopy reflected the highest visible radiation, followed by the medium- and high-N treatments. Consistent effects of N treatments on the canopy reflectance spectra were observed starting with the DAP 64 measurements (Figs. 2b and 2c), where the reflectance was inversely related to the amount of applied N.

While the maximum relative differences at the leaf level occurred at a broad range of green and a narrow range of red-edge wavelengths, the wavelengths at which they were most different were observed to shift to a broad range of wavelengths for the canopy, from 510 nm to 700 nm (green to red-edge regions). Two distinctive peaks are observed near 600 nm (yellow-orange) and 700 nm (red-edge), as shown in Figs. 2d to 2f. The strong blue and red absorption features observed at the leaf scale also became weaker at the canopy scale. Minimum absorption can be seen in the red wavelengths due to the presence of the two peaks at 600 nm and 700nm. The weakened absorption features at these two wavelengths could be associated with the combined effects of specular and increased bidirectional reflectance from leaves facing the sun and shadows cast by one leaf onto another at the top of the canopy. This shift is associated with the change from the leaf to the canopy scale, which allows more factors to affect the measured reflectance. Shifts in the wavelengths sensitive to N treatment effects were previously reported by Read et al., (2002).

Overall NIR canopy reflectance ranged from 50% to 59%. Except for DAP 78, NIR reflectance consistently increased with the increase in the amount of N fertilization. The increased NIR reflectance was the result of increasing leaf biomass and subsequently, increased scattering by the leaves. On DAP 78 (Fig. 2f), the ratios between different N rates were substantially less than 1. As cotton canopies progress toward the boll filling stage, the production of new leaves is reduced, which would reduce the overall NIR canopy reflectance.
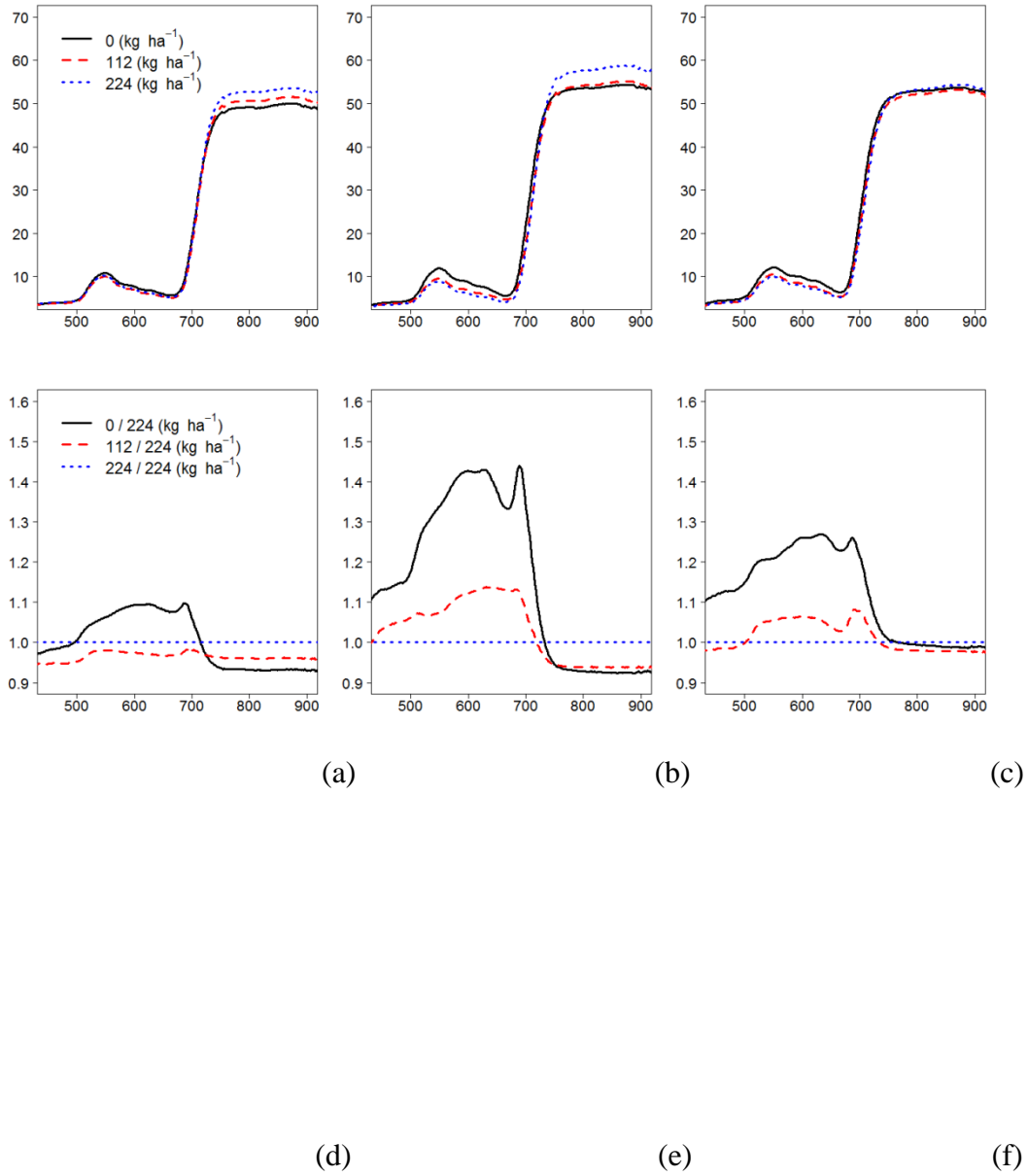
(a) (b) (c)

(d) (e) (f)

Fig. 2. Canopy reflectance measurements for different N rates on (a) DAP 36, (b) DAP 64, and (c) DAP 78. Relative ratios between different N treatments on (d) DAP 36, (e) DAP 64, and (f) DAP 78.

Scene reflectance

In contrast to the characteristic spectral curves for leaf and canopy reflectance, which are dominated by chlorophyll absorption and scattering by leaf biomass, reflectance spectra at the scene scale are more variable over the growing season in response to the increasing amount of vegetation in the scene. Early in the growing season, the reflectance spectra increase linearly in a manner similar to the characteristic soil reflectance curve (Fig. 3a). This is an indicator of the absence of chlorophyll absorption, especially at the red wavelengths, due to the domination of the soil background signal over the canopy. On DAP 36 (Fig. 3a), reduced absorption at the red wavelengths for the medium- and high-N treatment curves was visible, indicating the presence of a higher amount of canopy cover resulting in more chlorophyll absorption per ground area. Field measurements confirmed that those two treatments had greater ground cover than the N-deficient plots. The relationship between the amounts of N fertilization and reflectance in the visible region was consistent throughout the growing season. The high-N treatment had the lowest visible reflectance, followed by the medium- and low-N rates. The difference among different N treatments was small early in the growing season when the ratio of soil to plant cover was large. Differences increased starting at DAP 64 (Fig. 3b) as the cotton canopy grew.

At the leaf and canopy levels, the highest ability to discriminate between N treatments occurred at the green, yellow-orange and red-edge wavelengths. The sensitivity at these wavelengths was not as great at the scene scale (Figs. 3a to 3c), especially when the signal from the canopy began to dominate the signal contributed by the soil component of the scene. The relative differences were most pronounced at the red-edge (685 – 690 nm), although considerable differences extended through the range from 580 to 700 nm (Figs. 3d to 3f).

The NIR reflectance at the scene scale was significantly lower than at the other two scales. The overall reflectance ranged from 32 – 43% throughout the growing season. Compensating factors such as viewing geometry, leaf orientation, shadows and the non-foliage background could be account for this lower reflectance (Knipling, 1970). In comparison to the measurements made at the leaf and canopy levels, the relationship between N levels and NIR reflectance was the most consistent at this scale. The highest N fertilization rate resulted in the highest NIR reflectance, and vice-versa. As more cotton canopy obscured the soil surface as a result of high N fertilization, less soil reflectance contributed to the scene reflectance. The high proportion of canopy to soil surface, or percent ground cover, produced this increase in the NIR reflectance.

(a)   (b)   (c)
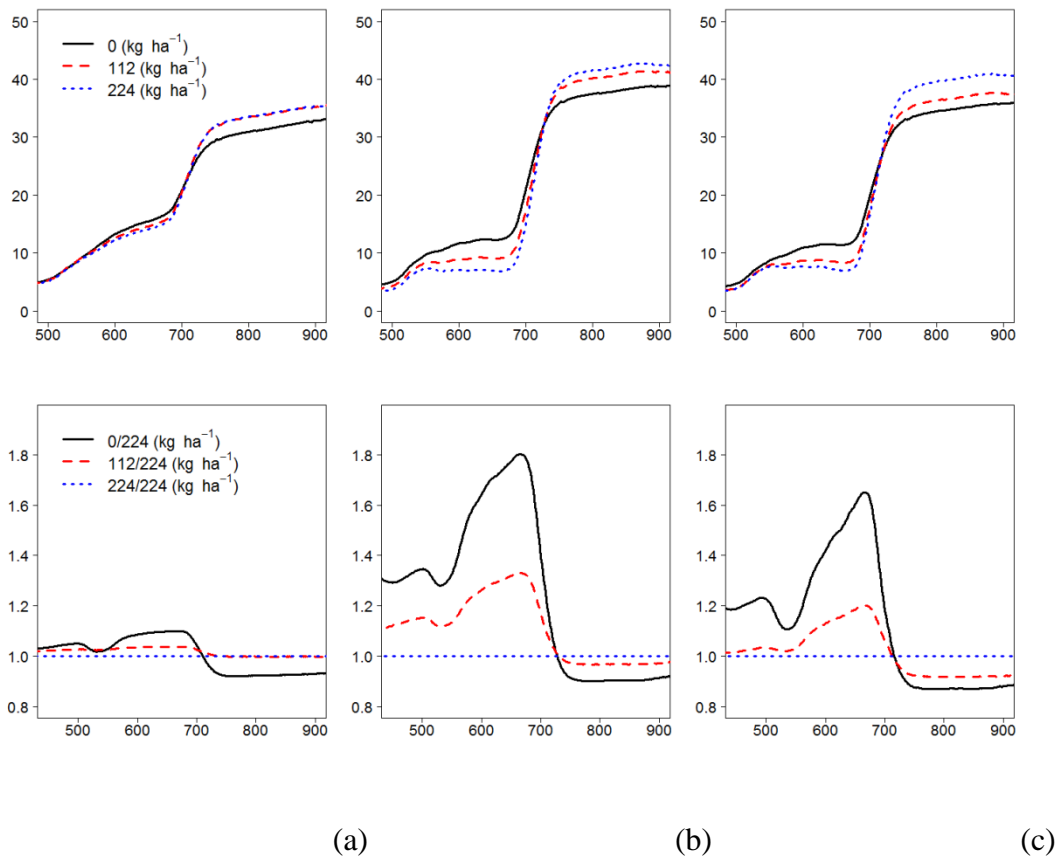
CONCLUSIONS

Effects of N fertilization changed significantly with spatial scale. The wavelengths at which N treatments were most pronounced were different at each spatial scale: 550 nm and 700 nm at the individual leaf scale; 600 nm and 700 nm at the canopy scale; and 685 nm and 690 nm at the scene scale. Each spatial scale is subject to different inherent variations, where different components of vegetation and soil become factors in determining reflectance at that scale.

Given the changing properties of the spectral signatures as a result of N fertilization and spatial scales, three essential conclusions are proposed. First, the selection of scale at which N treatment effects are most represented is a crucial step prior to the estimation of chlorophyll or N content. N fertilization affected not only crop chlorophyll concentrations but also the biomass production, expressed as ground cover. Chlorophyll content information is best represented at either the leaf or canopy scale, while effects related to the variation in ground cover is best represented at the scene scale. For estimating chlorophyll or N concentrations and a

Fig. 3. Scene reflectance measurements for different N rates on (a) DAP 36, (b) DAP 64, and (c) DAP 78. Relative ratios between different N treatments on (d) DAP 36, (e) DAP 64, and (f) DAP 78.

This study also demonstrates that the differences in reflectance measurements made at different spatial scales might explain previously unsuccessful attempts to use indices created at one scale but applied to another. Finally, these results also emphasizes the importance of understanding how a particular sensor system views the crop, since measurements made at different spatial scales are subject to different inherent variations.

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