

2013

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OPTIMIZING YIELD AND CROP NITROGEN RESPONSE
CHARACTERIZATION BY INTEGRATING SPECTRAL
REFLECTANCE AND AGRONOMIC PROPERTIES
IN SUGARCANE AND RICE

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The School of Plant, Environmental, & Soil Sciences

by
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May 2013

Acknowledgements

I would like to thank my adviser Dr. Brenda Tubana, who has supported and guided me through my academic career. She has always been with me when I need help and advice. Additionally, I would like to thank my committee members Dr. Dustin Harrell, Dr. Collins Kimbeng, Dr. Bin Li, and Dr. Richard Johnson; Dr. Harrell for helping me established field experiments and providing me all the support I need for my rice study, Dr. Kimbeng and Dr. Johnson for sharing their knowledge for my sugarcane study and Dr. Li for your guidance with the statistical methods that I used for my research.

I would also like to thank Dr. Jasper Teboh, Dr. Josh Lofton, Marilyn Sebial and all soil fertility members for assisting with field activities under the hot and humid weather in Louisiana. Without their support, I would not have accomplished all my field research works. Additionally, I would like to give special thanks to my family and friends from all over the world who encourage me to achieve this process. A special thanks to my husband Yucheol Kim for all the support, understanding and love during my academic years.

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Abstract

Nitrogen (N) is one of the most important and limiting nutrients in crop production. The best management practices for N fertilization is always challenging due to its dynamic system in the nature. Remote sensing has emerged as one of the most useful technologies in modern agriculture for non-invasive monitoring of plant N status. The objectives of this research were to 1) determine the effect of water background turbidity and depth on red and red-edge reflectance based prediction models for biomass and grain yield in rice, 2) evaluate agronomic parameters of different sugar cane varieties in response to variable levels of nitrogen supply, and 3) determine the effect of sugarcane varieties on the relationships between spectral reflectance and agronomic parameters. Rice experiments were variety (CL152 and CL261) x N trial established in Crowley, LA in 2011 and 2012. Sugarcane experiments were variety (L 99-226, L 01-283, and HoCP 96-540) x N trial established in St. Gabriel and Jeanerette, LA from 2010 through 2012. Spectral reflectance and agronomic parameters were collected each week for three consecutive weeks beginning two weeks before panicle differentiation in rice and for four consecutive weeks beginning three weeks after N fertilization in sugarcane.

There was no significant effect of water background (turbid or clear) on the spectral reflectance at panicle differentiation, one week after panicle differentiation, and at 50 % heading ($p < 0.05$). Water depth slightly influenced the reflectance at red waveband but this effect was not carried over when vegetation indices were computed. Use of red-edge based vegetation indices improved the estimation of biomass and grain yield in rice. The effect of variety on the accuracy of the yield prediction model varied

depending on the transformation of reflectance within the red-edge and near infrared bands i.e., into normalized (NDVI) and simple ratio (SR) forms of vegetation indices. This result was associated with the behavior of near infrared wavebands on the geometrical structure of the plant canopy. There were no significant effects of variety on grain yield prediction models using derivative based red-edge indices. Our findings showed that red-edge based NDVI and SR are better predictors of rice grain yield than red-based NDVI and SR. Red-edge based NDVI or SR indices both have potential to predict rice grain yield and rice responsiveness to N fertilization. In sugarcane, the measured agronomic variables at early growth stage, i.e. biomass, tiller number, N content, height and FAI of three sugarcane varieties and their responses to N fertilizer were highly variable across year. The sugar yield response to N determined at harvest had stronger linear relationships with N response of biomass and N content at 4 to 5 weeks after N fertilization compared with N response of height and FAI. There were no differences in leaf spectral reflectance among varieties. In canopy level-spectral reflectance, wavebands at 450-500, 650-700, and 780-830 nm showed high correlation coefficient with agronomic parameters. The vegetation indices which have the potential for predicting biomass N uptake were red and red-edge based simple ratio and normalized difference vegetation index. Varietal effect on the models for estimating biomass and N uptake was significant only when red-based vegetation indices were used ($p < 0.05$). Addition of plant height in the model substantially improved biomass and N uptake estimation while diminishing the effect of variety. Remote sensing technology can be a potential tool to estimate biomass and N uptake in rice and sugarcane. The delivered information from this technology is useful to improve mid-season N management.

Chapter 1. Introduction

1.1 Nitrogen in Crop Production

Seventy-eight percent of the air we breathe consists of nitrogen (N) gas. Due to its inert structure, N is not directly available for most of plants. Plant dry matter contains about 5% N, the largest among essential mineral nutrients. Nitrogen is essential for production of amino acid which is the basic source of protein for plant metabolism to produce hormones, enzymes and membranes. Nitrogen is the fundamental element in chlorophyll pigments which is responsible for photosynthesis that accounts for 90 % of plant dry weight production (Poorter et al., 1990). Apart from yield, N also influences grain quality and disease resistance in crop production.

In the United States, the majority of N supply for crop production relies on chemical fertilizer; 40 to 60 % of grain yield productions are dependent on inorganic N fertilizer (Stewart, et al. 2005). In 2008, the price of N fertilizer reached the highest since 1960; the average cost of anhydrous ammonia fertilizer was \$ 755 per ton which was 1.5 times more than the previous year and 3 times more compared to ten years ago (Huang, 2009). This rise in N fertilizer price was linked with the increase of fuel (natural gas) cost. The Haber Bosch process, which is the industrial method of fixing atmospheric N gas into ammonia, requires a large amount of fuel as energy source. Funderburg (2001) reported that 1 ton of anhydrous ammonia fertilizer requires about 9500 cubic meter of natural gas. An increase in the price of gas from \$90 to \$250 per cubic meter increased the cost for fertilizer by \$136 per ton of fertilizer (Funderburg, 2001). Due to this rise in the cost associated with N fertilizer production, more than 55 % of N supplies in U.S.

was imported (USDA, 2011). With today's high yielding varieties and new production technologies, consumption of inorganic N fertilizer would likely continue to rise.

Producers need to improve utilization efficiency of applied N fertilizer to maximize profits.

With the increase in industrial N fertilizer use, environmental quality is also put at risk. Global warming is an environmental issue that has been discussed on a daily basis. Nitrous oxide derived from microbial transformation of nitrate (denitrification) in agriculture, is one of the 'greenhouse gases'; while its concentration in the atmosphere is relatively lower than CO₂, its global warming potential is 296 times that of a unit of CO₂ (IPCC, 2001). In the United States, about 70% of the total nitrous oxide emission originates from agricultural soil management associated with synthetic N fertilizer (USEPA, 2011). Also another form of gas such as ammonia from synthetic N fertilizer has been reported as the leading cause of acid rain (Vitousek et al., 1997). The loss of N fertilizer from agricultural fields has caused a negative impact on water quality. Nitrogen flux in rivers was reported to increase by 10 % and 27 % in northern America and developing countries, respectively, in recent decades due to N runoff from crop fields (Bouwman et al., 2005). This increase in N flux has resulted in eutrophication, a condition where oxygen level in large bodies of water is reduced ultimately affecting aquatic ecosystem. Several studies have also shown that N input in agricultural activities and nitrate contamination in the ground water were related (Burlart and Kolpin, 1993; Spalding and Exner, 1993; Zhang et al., 1996). The critical nitrate (NO₃-N) level for drinking water is set at 10 mg L⁻¹. The increase in ground water contamination by nitrate does reduce the accessibility for safe drinking water; consumption of nitrate

contaminated water has been associated with blue baby syndrome (Knobeloch et al., 2000).

By 2050, global population is estimated to be more than 9.3 billion (U.S. Census Bureau, 2001). With this expanding world population, it is essential to maximize food production per unit of land as well as per unit of applied fertilizer. However, the estimated worldwide N use efficiency (NUE) is only 33 % in cereal production (Raun and Johnson, 1999). This low N fertilizer use efficiency is attributed to dynamic N cycle in the soil system. There are numerous pathways by which applied N fertilizer can be lost from the soil system. On the other hand, there are many sources of N including N from mineralization, decomposition of organic matter and rainfall. Accounting what is currently available for plant uptake while minimizing N lost constitutes an efficient N management system.

In modern agriculture, efficient N management is a key component to sustain crop productivity and profitability (economics) while maintaining environmental quality.

1.2 Nitrogen Management

Nitrogen is dynamic in nature. Nitrogen transformation processes include amination, ammonification, immobilization, nitrification, and mineralization which are dependent on soil temperature, texture, moisture, organic matter and biological activity including plant uptake. There are N loss pathways which sit between these transformation processes. For example, $\text{NH}_4^+\text{-N}$, a product of ammonification process, if not taken up by the plant can be lost via ammonia volatilization depending on the soil pH and moisture or can be fixed on clay exchange sites. Most agricultural soils are aerobic resulting in the

conversion of NH_4^+ -N to nitrate through the nitrification process. Nitrate-N is subject to nitrate leaching and denitrification, the relative dominance of these two processes is dependent on moisture, temperature and soil physical properties. Vast amount of research have been conducted to establish the best N management practices. The cornerstone of the best N fertilizer management is the application of the right amount of N using the right source at the right timing in the right place (4 Rs).

1.2.1 Nitrogen Rate

Application of N fertilizer at the right rate is an integral part of crop production to maximize economical return as well as to minimize environmental risks. Historically, N rate recommendation has been determined based on crop response to N fertilizer in well-replicated field trials which further adjusted by soil types (Voss 1969; Univ. of Kentucky Coop.Ext. Serv., 2006). As environmental risk associated N fertilization increased, this system shifted into more field specific N management, i.e. N rate recommendation was determined based on yield goal and soil testing. Yield goal is the average of recent five years yield plus 30 % and then N rate is estimated based on the total amount of N that would be removed with a given yield goal level (Johnson, 1991). Setting a representative yield goal is one of the challenging aspects in determining the right amount of N fertilizer. Meisinger et al. (2008) listed different interpretations of ‘yield goal’ and gave examples associated with this problem, for example, study conducted by Schepers et al. (1986) showed that farmers generally overestimated yield by 2 Mg ha⁻¹ in corn production. Since yield goal generally does not consider the current

crop field conditions, they also noted the importance of adjusting yield goal based on plant growth and management zones.

Soil testing was also incorporated to yield goal-based N rate recommendation by subtracting the amount of available mineral N (nitrate and ammonia) from the estimated total amount of N removed (Stanford and Hanway, 1955; Bundy and Andraski, 1995). Oregon State University provided variable N rates depending on soil-mineralizable N (plant available ammonium, and nitrate) in winter wheat (Hart et al., 2006). However, some studies showed the challenge of soil test N to be unreliable in regions with high precipitation potential such as southern Louisiana. Breitenbeck (1990) reported the possibility of the low consistency in nitrate test in southern states since high humidity and precipitation enhanced the loss of mineralized N. Also it is difficult to determine the plant available N under the dynamic system of N in soil profile. Research conducted by Schmitt and Randall (1994) showed low reliability of total amount of nitrate in pre-plant sampling because it does not account for mineralized N for the entire plant growth duration.

The fundamental N management strategy has not been changed however, the improvement of technology and methods has provided tools to determine crop N-needs more precisely through monitoring plants and soils within-seasons. Pre-sidedress nitrate test (PSNT) measures soil surface nitrates concentration to help assess supplemental N that will be applied at mid-season. It is measured before rapid N uptake and when PSNT value is below the calibrated critical level certain amount of N is applied (Blackmer et al., 1991; Bundy and Sturgul, 1994). Similar to this approach, monitoring plant N content has been widely studied since N deficiency can be easily detected when older leaves turn

yellow or light green due to descending chlorophyll formation. The visual symptom associated with N deficiency was the basis of developing leaf color chart (LCC) for monitoring real-time N status in rice and wheat. In the early 1990's, the first LCC was established in Japan (Furuya, 1987) and further improved by the International Rice Research Institute (IRRI) and University of California (Witt et al., 2005). The youngest fully expanded leaf are monitored at specific interval (7-10 days in rice) and once the intensity of green color of leaves fell below the critical LCC score, additional N application is made (Singh et al., 2010). A similar concept was applied to corn, wheat and cotton by using chlorophyll meter (Bullock and Anderson, 1998; Singh, 2002; Wu et al., 1998). Leaf chlorophyll meter which emits lights at red and infrared bands, was developed to monitor N status by measuring relative chlorophyll content (SPAD 502 chlorophyll meter, Minolta Camera Col. Osaka, Japan). With the aid of this instrument, N rate recommendation based on N sufficiency index was developed for corn, cotton, rice and wheat (Follett et al., 1992; Schepers et al. 1992; Wood et al., 1992; Cabangon et al., 2011). This approach uses chlorophyll meter readings from non-N limiting reference strip and farmers practice to compute sufficiency index (SI): $SI = (\text{Average farmers practice reading} / \text{Average reference reading}) * 100$ (Shapiro et al., 2006). When SI values are below 95 %, additional N, at least 22 kg N ha⁻¹ in corn, is recommended and the N rate and critical level has been adjusted based on variety, growth stage, and growing conditions (Shapiro et al., 2006).

1.2.2 Nitrogen Timing

Plant N uptake rates vary depending on crops, growth stage, variety, and growing conditions. The optimum time of N application should coincide with the time where

intensive plant N uptake is taking place. Also, the source of N affects the duration on which ammonium and nitrate remain in the soil.

Generally, N is applied twice as pre-plant to supply vegetative growth and mid-season to supply N for grain formation in crop production (Raun et al., 2002; Bond and Bollich, 2007). Dry regions such as those areas receiving annual precipitations of less than 480 mm recommends one time N application as pre-plant (Leggett, 1959b). When annual precipitation exceeds 650 mm, the split application of N at fall and spring was reported to increase NUE and grain yield in wheat (Mahler et al., 1994). It is simply because split application reduces the risk of loss by leaching and denitrification before plant uptake. Under warm climates where temperature is optimum for nitrification, the pre-plant application would enhance the potential of leaching hence in-season application as sidedress or topdress after seeding is recommended.

In winter wheat, 80 % of total N uptake occurred by mid-season and 70% of its uptake is translocated to grain (Waldren and Flowerday, 1978). In corn, rapid N uptake occurred in the middle of vegetative growth stage, V8 to V12. The decrease of available N at this moment significantly decreased grain yield (Grima et al., 2010). Unlike corn and wheat, rice has unique growing condition wherein soil is flooded almost in its entire growth duration. A single N application is more effective than split application in rice; however, split application is still a predominant method to achieve even distribution of fertilizer and reduce the risk of N fertilizer loss associated with levee breakage during the growing season (Harrell et al., 2011). Other studies also mentioned the importance of timing in N application to prevent N loss and maximize yield as well as NUE (Bock and Hergret, 1991; Johnston and Fowler 1991.)

Another important aspect in terms of N timing is to improve grain quality. With mid-season fertilization, rice grain improved its protein content as well as yield. It is also reported that the increase of protein content in grain improved the resistance to damages from milling machine (Cagampang et al., 1996; Wopereis et al., 2002). Similar results have been reported in corn and wheat (Daniel and Triboi, 2000; Jaynes and Clovin, 2006).

1.2.3 Nitrogen Source

Urea, urea-ammonium nitrate (UAN), ammonium nitrate, and anhydrous ammonia are the major N fertilizers and these sources account for more than 75 % of total N fertilizer in the USA (USDA, 2011).

Urea [$\text{CO}(\text{NH}_2)_2$] is a dry N fertilizer consisting of 46 % N . Its advantages include 1) high N concentration compared to other N fertilizer, 2) easy to handle being in the solid form, and 3) not expensive; therefore the use of urea is cost effective in terms of storage, transportation and applications. However, N fertilizer containing ammonia or ammonium such as urea and UAN, is subject to losses through ammonia volatilization. Applied urea undergoes hydrolysis in the presence urease enzyme and produces NH_4^+ and HCO_3^- . Ammonium is further transformed to NH_3 gas and can escape to the atmosphere through ammonia volatilization depending on soil temperature, moisture, and pH. High temperature and alkaline soil pH enhance ammonia volatilization since it increases the concentration of dissolved NH_3 in the soil solution as well as enhances urease activity. Ammonia volatilization normally occurs when soil pH exceeds 7 but it can also occur at pH 6.5 when soil buffering capacity is low (Jones et al., 2007). The effect of placement, application timing and slow-release technology (e.g. the use sulfur-

coated urea and urease inhibitors) on amount of N losses through volatilization has been intensively studied (Meyer et al., 1960; Walters and Mazer, 1990; Dawar et al., 2010).

Urea-ammonium nitrate [$\text{CO}(\text{NH}_2)_2 + \text{NH}_4\text{NO}_3$] is a liquid N fertilizer (UAN) which contains about 28-32 % of N and accounts for about 41 % of total N consumption in the USA (USDA, 2011). Uniform distribution of N compared to solid sources is one of the advantages of using UAN as an N source. It allows simultaneous applications with other mix nutrients as well as pesticides. Row crop production generally prefers UAN for sidedressing. It still has potential to volatilize; however, the potential to volatilize is less compared to a single urea application since UAN contains lower % of ammonium N per unit of fertilizer (Cornell University Coop. Extension, 2009).

Ammonium nitrate (NH_4NO_3) formed by anhydrous ammonia and nitric acid, contains 33 % of N. The advantage of using ammonium nitrate is less potential of losing N by ammonia volatilization since half of the N is in NO_3^- form. Nitrate is mobile in the soil due to its negative charge and therefore it is immediately available for plant uptake. Two to 3 weeks after N fertilization, ammonia nitrate fertilizer recorded 4 to 5 times less amount of N volatilized compared with urea (Whitehead and Raistrick, 1990; Jones et al., 2007). Due to explosion hazard with ammonium nitrate when combined with oxidizable C, there are government regulations that restricts its sale and transportation in many regions of the world (IPNI).

Anhydrous ammonia (NH_3) is generally managed as liquid under high pressure. It has the highest N content (82 %) among N fertilizers and therefore considered the cheapest N fertilizer source. Due to its hazardous form, extra care must be taken during handling and special equipment is required (Raun and Zhang, 2006). Since anhydrous

NH_3 is a gas at normal pressure it needs to be injected into certain depth of soil to react with water and organic materials. Therefore, the benefits of applying anhydrous NH_3 would be minimized under light texture soils which contain less clay and organic matter (McDowell and Smith, 1958). Because loss is minimized once it is incorporated into soil, it can be applied long before planting. Fall application of anhydrous ammonia for corn for the following spring allows producers to reduce the amount of field operation during spring therefore allows them to finish all field operations such as planting on a timely manner (Kyveryga et al., 2004).

1.2.4 Nitrogen Placement

The placement of N fertilizer depends on the N source and application timing. At pre-planting, N fertilizer is usually applied by broadcast method in a uniform manner on the soil surface either as UAN and urea forms (Mahler, 2001). To minimize losses through volatilization, broadcasted N fertilizer should be incorporated into soil by plowing at 15 cm deep (Jones, 2007). Broadcasting N fertilizer is relatively easy and cost effective with no requirement for specialized equipment. On the other hand, broadcast N application requires extra amount of N fertilizer. In addition, this type of application may support growth of weed and without incorporation with soil, there is high potential of losing N by ammonia volatilization (Randall, 1997).

Banding application which places the solid fertilizer close to seed at planting is not a common method of N application (Mahler, 2001). However, under conservation tillage practice (no-tillage or minimum tillage practice) system banding N fertilizer is a preferred N placement method. Banded-N fertilizer showed remarkable benefits when

there are plant residues on the ground and in cold growing environment where root growth is slow (Hoeft and Ritchie, 1997). By placing fertilizer in band and close to roots, seedling roots can take nutrients efficiently. Total N application rate can be less than broadcast but the risk of leaching is higher and may require additional investment to modify equipment.

Unlike placing fertilizer on the ground, foliar N application using dilute N-containing solutions to the leaves is mainly practiced to improve grain quality in crop production. Foliar application can alleviate moisture or diseases stress by enhancing quick N absorption from leaf pores (Mattson, 1980). Also, it reduces the potential loss by nitrate leaching or denitrification (Powlson et al., 1989; Poulton et al., 1990). Uptake of N from leaf surface can be independent from soil conditions or root growth; therefore, foliar N applications are beneficial in areas under saline or dry conditions (Seth and Mosluh, 1981; Seth and Prasad, 1965). The effectiveness of foliar N application in improving protein content has been reported in wheat (Finney et al., 1957; Endres and Schatz, 1993; Bly and Woodard, 2003). Higher grain protein in barley was obtained using foliar-applied urea compared with broadcast NH_4NO_3 (Bulman and Smith, 1993). Since N demands are high during the entire plant growth, it is difficult to supply adequate amount of N without burning the leaves. Therefore, foliar N is normally taken only as a supplement.

1.2.5 Nitrogen Management in Rice and Sugarcane in Louisiana

In Louisiana, current N rate recommendation in rice is based on yield goal established from multi-year N response trials across sites in the southern United States,

with which further adjusted by soil type, cultural practice and variety (Norman et al., 2000; Harrell et al., 2011). Generally, N application is made twice at pre-flood and mid-season (Bond and Bollich, 2007). Nitrogen fertilization rate ranges from 77 to 176 kg N ha⁻¹ and two third of total N is applied at pre-flood and the rest of them is applied at mid-season (Saichuk et al., 2012).

Since nitrate-N can be lost under flooded condition due to denitrification, ammonium based N fertilizer such as urea, is generally applied. Under drained or aerated condition, ammonium-N is easily converted into nitrate or lost by ammonia volatilization, hence immediate flooding after N application is recommended. However, it is challenging to flood the entire field within a few days following N fertilizer application. Therefore use of urease inhibitor, ammonium sulfate, and nitrification inhibitor are recommended to prevent ammonia volatilization and denitrification. Agrotain is one of several urease inhibitors which contains 25% of NBPT (n-butyl thiophosphoric triamine). Incorporating NBPT with urea resulted in reduced ammonia volatilization by 15 to 20 % compared with conventional urea application (Dillon et al., 2012; Norman et al., 2004). Application of ammonium sulfate is an alternative way to reduce the loss but the relatively low concentration of N (21 %) compared to urea (46%) requires large amount of fertilizer application. Nitrification inhibitor (dicyandiamide, DCD), can slow down ammonia oxidation. Kumar et al. (2000) compared the application of urea and ammonium sulfate with and without DCD. Their research obtained 11 to 26 % reduction of nitrous oxide emission when those N fertilizers were amended with DCD. The benefits of using these materials in N fertilization in rice have been reported (Carreres et al., 2002; Linnquist et al., 2012).

Sugarcane is considered a semi-perennial crop, in which the initial crop planted around August to September is harvested approximately 14 months later (plant cane). Then sugarcane is harvested on an 11 month cycle for an additional 3 to 4 years (ratoon sugarcane). Nitrogen fertilizer is applied only once between early April to the beginning of May prior to intensive growth of sugarcane. The LSU AgCenter N rate recommendation is based on soil type and crop age (Legendre et al., 2000). Recommended N rate is between 67 to 110 kg N ha⁻¹ for plant cane and it is between 88 to 132 kg N ha⁻¹ for ratoon cane (Legendre et al., 2000). Higher N rate is applied in ratoon cane due to a higher response to applied N. Normally after the last ratoon cane is harvested; field is left fallow for a year or planted to soybean as cash crop or for N credit. During this period, plant available N may increase either due to mineralization turnover or N credit from growing soybeans.

Sugarcane quality and yield are easily affected by N management. Wiedenfeld (1995) reported that the excess amount of N application decreased sugar yield, juice purity as well as recoverable sucrose. Similar result was reported by Chapman et al. (1994) and Borden (1942). This reduction of sugarcane quality at high N rate is associated with the higher mortality of primary stalks. However, based on 5-years N response trial in Louisiana, Tubana (unpublished data) reported that 75% of site-year showed over-fertilization. The timing of N application also influences sugarcane quality and yield. The decrease of cane and sugar yield were reported when the application was made at early or late of optimum timing (Wiedenfeld, 1997) therefore proper N management is essential for sugarcane production.

As listed above, numerous studies have been conducted to identify the best N management practices to satisfy different crops, tillage systems, and growing conditions. However, temporal and spatial variability in fields and crop response to N makes it difficult to archive maximum benefit from applied N.

1.3 Temporal and Spatial Variability

Temporal variability in soil N is mainly attributed to climatic factors such as precipitation and temperature which affect soil moisture and biological activity. On the other hand, spatial variability in soil N is generally explained by the distribution of soil types within a field; the inherent properties of different soil types are developed based on climate, topography, parent material and biological activity. Human activities (field operation, fertilization, management practices) are also considerable factors which influenced soil properties in a long-term. From an agronomic stand point, soil N is grouped into total N, inorganic N and organic N. Inorganic N, which constitutes nitrate-N and ammonium-N, is the plant-available N. Total N is relatively stable over time while inorganic N is highly variable over time and space.

The basic sources of inorganic N are atmospheric deposition, mineralization associated with ammonification and nitrification, and inorganic fertilization. The total N deposition was estimated to be $10 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ in the Unites States and Europe (Holland et al., 1999). The atmospheric deposition causes spatial and temporal variation at local scales depending on format of deposition (wet or dry), topography and canopy structure (Ollinger et al., 1993). Soil organic matter (SOM) and plant residue from previous year affect inorganic N availability through N mineralization. Soil organic matter is converted

to ammonium-N by bacteria and fungi through the process called ammonification. In this process, pH, oxygen availability, and temperature are driving factors. When pH is more than 6, bacteria are the dominant microorganism driving ammonification process while it is fungi when soil pH is lower than 6. Myers (1974) showed the linear increase of ammonification rate from 20 °C to 50 °C. Salvetti et al. (2006) also reported the impact of oxygen availability and temperature on ammonification process. Ammonium-N produced by ammonification is then transformed to nitrite-N and nitrate-N by nitrification. The study conducted by Cahn et al. (1994) showed spatial variability of mineral N was substantial and the pattern of variability was different across time. Due to its seasonal change, they address that timing of soil sampling may be critical to determine N fertilizer requirements. Due to high variability in fields, the Illinois Soil N Test determines corn N recommendation based on amino sugar-N fraction of SOM instead of using mineral N (Ruffo et al., 2005). Shahandeh et al. (2005) reported similar result that N mineralization and nitrate level were highly variable within field and across seasons in corn field. Excessive N application in previous year was reported to stimulate net N mineralization and enhance leaching of mineral N (Stevens et al., 2005). Limited or excessive moisture, disease stress, or insect damage at intensive plant growth may substantially decrease yield potential. Under such condition, the amount of required N would likely reduce. Therefore, it is also important to know that plant response to N fertilization changes depending on plant growing environment. Mamo et al. (2003) examined the spatial variability in economically optimum N rate for corn. Their research reported that reduction in N application rate and potential economic benefits were different between each crop terms suggesting that temporal variability can be attributed to

different climatic factors. A long-term study conducted by Raun et al. (2011) from 1970 to 2010 also showed that yield and N response of grain yield substantially varied. In addition there was no relationship observed between yield, N response, and year for both corn and wheat crops.

Nitrogen cycle is dynamic in nature and addition and loss of N from soil profile as well as plant N response are highly variable within field and across seasons. Most of N management approach established many years ago do not account for these variability. An integrated N management approach using instruments that allow us to non-destructively monitor in-season crop N health status combined with simple field procedure to perceive the amount of soil N available for the cropping season is essential to improve N use efficiency.

1.4 Nitrogen Assessment Using Remote Sensing Technology

In the early 1980's, precision agriculture, detecting and treating a spatial and temporal variability, emerged as component of modern agriculture in the United States. The technologies such as geographic positioning system (GPS), remote sensing using satellite or ground-based sensor and yield monitoring device, enable us to assess large area of crop and field conditions in short period of time without taking samples. Also, continuous numerical data collected from remote sensing technology amplify the potential analysis with different parameters. This section is focused on the use of remote sensing technologies to monitor crop N status and its application for better N management.

1.4.1 Plant and Spectral Reflectance

Remote sensing in crop production commonly utilizes visible and near infrared reflectance from plant canopies to diagnose plant health. Near infrared wavelength relates with cell and plant geometrical structure while visible wavelength shows unique signatures of plant pigments.

Near-infrared represents the wavelength between 750 to 1350 nm in electromagnetic spectrum which is related to characteristics of plant cell structure as well as canopy structure (Sims and Gamon, 2002). In terms of leaf-base analysis, the arrangement of mesophyll cell, intercellular air spaces, and the thickness of leaves have been known to alter the near-infrared reflectance (Gates, 1970; Delucia and Nelson, 1993; Slaton et al., 2001). On the other hand, at canopy level, ratio of soil and vegetation reflectance is importance for interpretation of near-infrared reflectance. Plant reflects 60 to 80 % of near-infrared while soil reflects 20 to 30 % of incoming light depending on soil texture, organic matter and moisture contents (Arnold et al., 2002). Decreasing in near-infrared reflectance indicates increase of soil exposure which is also interpreted as reduction of plant biomass on the ground. Since estimation of plant biomass is important for monitoring plant growth as well as for predicting yield, combining reflectance at near-infrared with other spectral wavelengths has been studied (Raun et al., 2001; Harrell et al., 2011).

Visible wavelength is between 400 to 750 nm and it has a strong relationship with photosynthetic pigments. Plant pigments are generally classified into chlorophylls, carotenoids, and anthocyanins. Chlorophyll is the most abundant pigments which absorbs solar energy (sunlight) and converts it into chemical energy. Strong spectral absorbance

by chlorophyll pigments was reported in the red and blue wavebands (Blackburn, 1998). The maximum absorbance occurs between 660 and 680 nm, however, these wavebands are not recommended for estimating chlorophyll content due to the saturation of absorption at low chlorophyll contents. Since the light from the blue region is also absorbed by other pigments (e.g. anthocyanin), red-edge waveband (680 to 740 nm) has gained research attention (Blackburn, 1998). Red-edge point (REP) which is the maximum of the first derivative reflectance between red and near infrared region, has been reported as a good indicator of plant chlorophyll content (Fiella and Peñuelas, 1994; Blackburn, 2007). Carotenoids are yellow, orange, and reddish pigments which are also called as ‘accessory’ pigment because they absorb light at wavebands where chlorophyll does not. There are more than 700 carotenoids and major pigments are lutein, β -carotene, neoxanthin and violaxanthin (Johnson et al., 2008). Under low light intensity, violaxanthin functions as an antenna pigment and transfers solar energy into chlorophyll while under excess light, violaxanthin is converted into another form of carotenoids protecting plant metabolism by enhancing energy dissipation (Demmig-Adams and Adams III, 1996; Frank et al., 1997). Lutein and violaxanthin are yellow color pigments which absorb strong light at 410 to 430 nm (Cuttriss and Pogson, 2004). The overlap of light absorption between chlorophyll and carotenoids makes it difficult to estimate carotenoid content in leaf. Anthocyanin is not a pigment which takes a role during photosynthesis but takes a role in protecting plant from excess light or UV light (Gould et al., 1999; Mendez et al., 1999). It absorbs blue, some from green, and the longest wavelength among pigment (Schwinn and Davies, 2004; Merzlyak et al., 2003). Since the absorption is similar to chlorophyll, the estimation of anthocyanin is not easy; however,

low absorbance of blue light which is equivalent to high reflectance of blue light may indicate plant stress. The detection of plant stress is important to estimate plant growth which eventually influences yield and therefore N requirement.

Vegetation index using multiple spectral reflectance has been developed to monitor plant stress and growth. Major vegetation indices are listed in Table 1.1. Normalized difference vegetative index (NDVI) and simple ratio (SR) are the most commonly used vegetation index to estimate plant biomass, leaf area index (LAI), N concentration and yield in several crops while the decrease of NDVI sensitivity was reported as plant coverage or biomass increased on the ground (Carlson and Ripley, 1997; Blackburn, 1998; Gitelson et al., 2002; Hansen and Schjoerring, 2003). Soil adjusted vegetation index (SAVI) was developed as a modification of NDVI to reduce the impact of soil reflectance since texture; moisture and SOM can alter spectral reflectance. The implementation of soil reflectance in SAVI reduced interference at low plant coverage compared to NDVI (Qi et al., 1994). High accuracy of predicting N contents and biomass at dense plant canopy has been reported using REP (Meer and Jong, 2006). As mentioned earlier, REP is the point of red-edge bands and its position tends to be at longer wavelength when reflectance are coming from high chlorophyll contents canopy or healthy plant (Biewer et al., 2009). Elvidge and Chen (1995) also reported the reduction background effect using REP.

1.4.2 Nitrogen Assessment Using Remote Sensing Technology

Numerous studies have been conducted to relate interactions among plant pigments, canopy structure and light reflectance and use these relations to non-destructively assess plant N status. Others have used this technique to treat yearly and

Table 1.1. Spectral vegetation indices and its properties.

Vegetation Index	Acronym	Formula	Properties	Reference
Simple ratio	SR	R_{780}/R_{650}	biomass	Serrano et al., 2000
Normalized difference vegetation index	NDVI	$(R_{780}-R_{650})/(R_{780}+R_{650})$	biomass	Blackburn, 1998
Red edge	REP	wavelength at the maximum point of first derivative reflectance between 700 to 750 nm	Chlorophyll content, N concentration, biomass	Meer and Jong, 2006
Structural independent pigment index	SIPI	$(R_{800}-R_{445})/(R_{800}-R_{680})$	Carotenoids	Peñuelas et al., 1996
Soil adjusted vegetation index	SAVI	$(R_{780}-R_{650})/(R_{780}+R_{650}+L)*(1+L)$ where the parameter L depends on the area of soil coverage	Adjustment of NDVI to reduce the impact of soil reflectance	Qi et al., 1994
Water index	WI	R_{900}/R_{970}	water content	Peñuelas et al., 1997

destructively assess plant N status. Others have used this technique to treat yearly and within field variability with respect to N requirement. Success of monitoring leaf N status using canopy reflectance has been reported in many crops such as rice, wheat, and corn (Scharf and Lory, 2009; Takebe et al., 1990; Zhao et al., 2003; Xue et al., 2004). Scharf et al. (2009) reported that reflectance at blue and red bands is good to predict economically optimal N rate and measurements of visible and near-infrared bands may be useful to determine variable-rate N recommendations for side dressing in corn. Nitrogen response index (a measure of crop response to N fertilization), and yield potential were estimated using NDVI and this information were put into a working algorithm for determination of mid-season N rate in corn and wheat (Raun et al., 2002). This approach has been reported to improve NUE (Raun et al., 2002; Tubana et al., 2011). Miao et al. (2009) evaluated conventional vs. optimized N fertilization based on sensor using N loss and net economical return in wheat as performance measures. The outcome of this study showed that sensor based N rate recommendation reduced N loss by about 100 kg ha⁻¹ and increased net economical return by about \$100 ha⁻¹ compared to conventional N fertilization

Determination of SOM and water content using remote sensing was also conducted to determine N fertilizer need. Generally, soil containing high organic matter or water, absorbs more light; therefore, decrease spectral reflectance (Zheng and Schreier, 1988). Using this concept, Chen et al. (2000) estimated SOM using remotely sensed data on soil surface. Determining SOM can provide estimation of soil total N and therefore plant available N however; Scharf et al. (2002) indicated that this approach would require to account for N mineralization.

Precision N management using remote sensing technology has been shown to improve NUE, economical return, and environmental quality in crops like wheat and corn. Accounting for both spatial and temporal variability has the potential to improve N management in rice and sugarcane. Remote sensing technology is a useful tool that could be used to understand and establish the quantitative relationships of N-related agronomic parameters and spectral reflectance characteristics of rice and sugarcane with varying amount of N supply.

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Chapter 2. Effect of Water Background Turbidity and Depth on Red and Red-Edge Reflectance Based Prediction Models for Biomass and Grain Yield in Rice

2.1 Introduction

Water and nitrogen (N) are the most limiting inputs in crop production. Since rice is grown in a flooded environment, N is often considered the most important input limiting grain yield. Currently in the mid-southern United States, rice N rate recommendations are variety dependent and are determined from multi-site N response trials across sites in the southern United States, which further adjusted depending on soil type, cultural practice, and variety (Norman et al., 2000; Harrell et al., 2011). Generally, two N fertilizer applications are done seasonally in drill-seeded delayed flood rice. The first is made just before permanent flood establishment and the second is applied mid-season. Fertilizer N recommendations made in this manner can potentially over- or under-estimate N rate due to the lack of consideration for spatial and temporal variability. Remote sensor technology is one method which has recently been investigated as a tool to help predict optimum mid-season N application rates and address the spatial and temporal variability that exists (Harrell et al., 2011; Tubana et al., 2011).

Remote sensor-based N management has been studied in many crops including corn, wheat, and cotton (Raun et al., 1999; Tubana et al., 2008). Wells et al. (1989) found that biomass production is closely related with rice grain yield. Harrell et al. (2011) successfully predicted rice grain yield by estimating above ground biomass using normalized difference vegetation index (NDVI). They showed that 42% of the total variability in grain yield can be explained by NDVI collected at panicle differentiation (PD). Calculated NDVI from second derivative analysis also showed a high potential for

predicting rice grain yield (Shibayama and Akiyama, 1991). Along with predicting grain yield at mid-season, many researchers have also monitored plant N status using spectral reflectance (Sims and Gamon, 2002; Xue et al., 2004; Zhang et al., 2006; Stroppiana et al., 2009).

The N rate recommendation derived from spectral indices has been tested and shown promise to increase nitrogen use efficiency (NUE). Sensor-based N fertilization algorithm reduced the traditional N rate by 33% while maintaining a similar rice grain yield (Xue and Yang, 2008). Dobermann et al. (2002) conducted site-specific N management in 179 fields based on SPAD chlorophyll meter reading. Their approach was to modify N rate depending on the critical value of SPAD meter at specific growing stage and variety. It resulted in an increase in NUE by 30-40 %. A similar approach was tested based on index using NDVI readings from non-limited N fertilizer field and NDVI reading from farmers practice (Xue and Yang, 2008). According to these studies, determining optimum mid-season N rates using remote sensing technology is highly feasible.

Unlike corn, wheat or other crops, the water background in rice is a unique feature which may require additional consideration when using sensor technology. A water-compared to a soil-background may affect the spectral reflectance and vegetation indices values. Normally, water transmits most of incident radiation in the visible wavelength, which results in small reflection of light. Contrary to pure water, water in paddy rice may be turbid due to suspended soil sediments resulting from walking in the paddy and using a hand held sensor. In turn, the turbidity may alter the absorption and reflection of light. Water absorbs near-infrared wavebands, thus the reflectance in that region decreases as

the area of exposed surface water increases in rice fields. There is also a potential interference on spectral reflectance readings especially with low rice biomass observed during early growth stages. Under low plant biomass, red wavelength was not a reliable estimate of Leaf Area Index (LAI) due to the presence of algae (Vaesen et al., 2000). Hoshi et al. (1984) showed the increase in water depth reduced the spectral reflectance due to increased radiant absorption in the water. The findings of these studies warrant further research of similar scope to explore the possibilities of increasing the accuracy and precision of rice grain yield predictive models established from canopy reflectance readings. Understanding and addressing the effect of the water background and depth on spectral reflectance readings in rice production can improve the use and application of this technology for midseason N applications.

The most commonly used and tested vegetation index, NDVI, has raised concern when biomass, and LAI are high or when crop reached complete canopy closure. The sensitivity of NDVI decreased as plant canopy ground coverage increased (Gitelson et al., 2002). In fact, the use of current rice yield prediction model using NDVI in Louisiana is limited when grain yield exceeds 8000 kg ha^{-1} (Harrell et al., 2011). This phenomenon was also detected in a variety of crops including corn and cotton (Jackson and Pinter, 1986; Galvao et al., 2005). To overcome the problem, an alternative spectra regions called red-edge has gained attention. Red-edge is the wave bands between red and NIR and approximately refers to 680 nm to 740 nm. In these regions, scientists are focusing on the red-edge point (REP), defined as the maximum of the first derivative reflectance between red and NIR regions. Meer and Jong (2006) showed that REP has strong correlation in N concentration at dense plant canopy ground coverage. The vegetation

index associated with red-edge could be one of the potential bands to determine physiological status.

Varietal differences in yield and physiological N response can be involved in adjusting N rate recommendation. Abundant varieties have been available in each southern state and N rate recommendations are slightly different depending on variety (Walker and Street, 2003; Roberts and Wilson, 2012; Saichuk et al., 2012). Regarding to the sensor based application, it is important to know the effect of variety on the spectral reflectance. Differences in geometrical canopy structure and foliar chemical compositions give a unique signature on spectral reflectance. Darvishesefat et al. (2011) showed the differences in spectral signatures among varieties in rice. Jackson and Pinter (1986) obtained 20 % higher reflectance values in wheat with planophile canopies (non-erect) as compared with erectophile canopies (erect). Similar results were also observed in sugarcane Galvão (2005).

It is essential to investigate the effect of the water background, depth, and variety on predicting rice yield at differing physiological status of development using red edge spectral reflectance. Comparing prediction models by different variety and vegetation index is also required to diagnose the feasibility and applicability of this technology. The findings from this study are essential to refine the rice yield potential model as well as developing a working algorithm for N rate recommendations.

2.2 Materials and Methods

2.2.1 Location and Experimental Design

A study was established at the LSU AgCenter's Rice Research Station located in Crowley, Louisiana. Experimental plots were established under conventional tillage on a

Crowley silt loam (fine, smectic, thermic Typic Albaqualfs). The experiment consisted of 7 pre-flood N rates (0, 34, 68, 101, 135, 168, and 202 kg ha⁻¹) with four replications arranged in a randomized complete block design. For each replication, one unplanted plot was added as a reference. Two varieties, CL152 (an early maturing, semidwarf long-grain) and CL261 (an early maturing, short stature medium grain), were tested. The CL152 is generally taller than the CL261. Rice was drill-seeded on March 16th in 2011 and on March 19th in 2012 at a depth of 4 cm a seeding rate of 300 seeds m⁻² using a small-plot grain drill. Each plot was 1.38 by 4.8 m². Once rice seedling reached 4 to 5-leaf stage, N fertilizer in the form of urea (46 % N) was broadcasted and permanent flood was established one day later.

2.2.2 Sampling Area and Data Collection

After the rice seedlings reached the 3 leaf-stage 1 m x 1 m x 0.3 m (1 x w x h) galvanized borders were carefully pressed onto the ground around each plot to a depth of 2.5 cm creating a 1 m² sampling area. The borders protected the sampling area from disturbance while taking measurements (reflectance readings, digital picture and depth of water) under a clear, non-turbid water background. Reflectance readings were then re-measured with a turbid water background. To make the water turbid, water inside the 1 m² sampling area was carefully mixed with a meter stick. Whole plant samples were taken for biomass yield, and total C and N determination at each sampling period. Reflectance and biomass measurements were taken each week for three consecutive weeks beginning two weeks before PD (about 1500 cumulative growing degree days, GDD). At maturity, whole plots were harvested using a small plot combine to determine grain yield. Detailed field activities were listed on the Table 2.1.

Table 2.1. Agronomic practices and growing conditions established at Crowley, LA in 2011 and 2012.

	2011			2012		
	Date	DFP*	CGDD**	Date	DFP*	CGDD**
Planting	16-Mar	0	0	19-Mar	0	0
N fertilization	20-Apr	36	385	23-Apr	40	441
Panicle Differentiation	23-May	76	927	25-May	64	851
Panicle Differentiation+1wk	6-Jun	82	1044	30-May	73	936
50% Heading	13-Jun	89	1169	6-Jun	79	1035
Harvest	5-Aug	143	2166	1-Aug	136	2028

Canopy reflectance measurements were taken using the Ocean Optics Jaz spectrometer (Ocean Optics, Dunedin, FL), which detects continuous wavebands from 300- to 1100-nm with an optical resolution of 1.5 nm. Incident light (downwelling irradiance) and the outgoing light (upwelling) from a 1m² white steel plate coated with barium sulfate was determined and used to correct environmental noise interference before plant canopy measurement was done. Dark readings were measured by covering the sensor with a cap and fabric material. The distance between the fiber optic sensor and target (white plate or rice canopy) was determined to make sure that the field of view covered a 1 m² area (sampling area size). The distance between the rice canopy and fiber optic sensor was calculated based on the lens' field of view using trigonometry functions. The cosine corrector and Gershun tube with 28 degree field of view was attached to the fiber optic sensor. Since the field of view was 28 degree, the height required to cover 1 m² was computed by multiplying Tangent 14° with the length of the adjacent side.

Digital pictures taken from sampling area were analyzed using Integrated Land and Water Information System (ILWIS) software (The Faculty of Geo-Information Science and Earth Observation of the University of Twente, Netherlands) to compute the percentage of ground coverage by plant. A digital camera was attached to the hand held self-telescopic stand stick and height was maintained at 1.5 m to take a top-view shot of the plots.

Biomass samples (three 1-m long rows) were cut at the soil level at each sampling date. Biomass samples were then oven-dried at 60°C for 48 hours, weighed, ground, and analyzed for total C and N analysis using the dry combustion method (Elementar Americans Inc., Mount Laurel, NJ). Grains sub-samples were also processed and analyzed for total C and N.

2.2.3 Spectra Reflectance and Indices

Simple ratio (SR) based on red [(1.1) SR_{red}] and red-edge [(1.2) $SR_{red-edge}$], and NDVI based on red [(1.3) $NDVI_{red}$] and red edge [(1.4) $NDVI_{red-edge}$] were computed using the following formulas:

$$SR_{red} = \frac{\rho_{780}}{\rho_{670}} \quad (1.1)$$

$$SR_{red-edge} = \frac{\rho_{780}}{\rho_{670}} \quad (1.2)$$

$$NDVI_{red} = \frac{\rho_{780} - \rho_{670}}{\rho_{780} + \rho_{670}} \quad (1.3)$$

$$NDVI_{red-edge} = \frac{\rho_{780} - \rho_{730}}{\rho_{780} + \rho_{730}} \quad (1.4)$$

With the concept of derivative analysis, red-edge points (REP) were also determined by using: maximum first derivative analysis by polynomial fitting technique [(1.5) REP_{DF}], Linear interpolation technique [(1.6) REP_{LI}], Linear extrapolation technique [(1.8) REP_{LE}] and the Lagrangian technique [(1.7) REP_{LAG}].

5th order polynomial fitting technique

Polynomial function was fit to spectra reflectance between red to near infrared (670 to 780 nm) using TableCurve 2D v5.01 software. The maximum first derivative reflectance was computed as REP_{DF}.

$$\rho(\lambda) = a_0 + \sum_{i=1}^5 a_i \lambda^i \quad (\text{Skidmore, 2006}) \quad (1.5)$$

where λ (wavelength) is from 670 to 780 nm.

Linear interpolation technique

The linear interpolation method was used to estimate REP by employing reflectance at four different wavebands (Cho and Skidmore, 2006; Guyot, 1988). The benefit of this method is that it does not require continuous wavebands for derivative analysis. The reflectance between red and near infrared is assumed to be simple straight line and REP is determined by the linear method (Meer and Jong, 2006).

$$\text{REP}_{\text{LI}} = 700 + 40 * \frac{(\rho_{\text{REP}} - \rho_{700})}{(\rho_{740} - \rho_{700})} \quad (1.6)$$

$$\text{where } \rho_{\text{REP}} = \frac{\rho_{670} + \rho_{780}}{2} \quad (1.6a)$$

Linear extrapolation technique

The linear extrapolation technique was developed by Cho and Skidmore (2006). This method eliminates the problem from the double-peak which can be observed in high N

treated plant or when chlorophyll concentration is high using the first derivative analysis. The two straight lines, one from near infrared and other from red points, were computed based on the first derivative reflectance and the intersection of those straight lines was considered as REP.

$$REP_{LE} = \frac{-(b_1 - b_2)}{(a_1 - a_2)} \quad (1.7)$$

where near infrared line: 1st derivative reflectance $(\lambda) = a_1\lambda + b_1$

red line: 1st derivative reflectance $(\lambda) = a_2\lambda + b_2$

To determine the near infrared lines, 725 and 750 nm bands were selected while 680 and 700 nm bands were selected for red line.

The Lagrangian technique

$$REP_{LAG} = \frac{A(\lambda_i + \lambda_{i+1}) + B(\lambda_{i-1} + \lambda_{i+1}) + C(\lambda_{i-1} + \lambda_i)}{2(A + B + C)} \quad (1.8)$$

$$\text{where } A = \frac{\text{Derivative reflectance}_{(i-1)}}{(\rho_{i-1} - \rho_i)(\rho_{i-1} - \rho_{i+1})} \quad (1.8a)$$

$$B = \frac{\text{Derivative reflectance}_{(i)}}{(\rho_i - \rho_{i-1})(\rho_i - \rho_{i+1})} \quad (1.8b)$$

$$C = \frac{\text{Derivative reflectance}_{(i+1)}}{(\rho_{i+1} - \rho_{i-1})(\rho_{i+1} - \rho_i)} \quad (1.8c)$$

For this study, $\rho_{i-1} = 710 \text{ nm}$, $\rho_i = 730 \text{ nm}$, $\rho_{i+1} = 750 \text{ nm}$ were selected.

2.2.4 Data Analysis

Statistical analysis was performed using SAS 9.3. (SAS Institute, 2009) and R (Comprehensive R Archive Network, 2008). The regression analysis model was built to

identify the impact of water background (1.9) and depth (1.10) on the reflectance using R at each wavelength with the following equations:

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 \quad (1.9)$$

where b_1 = coefficient of water background

b_2 = coefficient of plant biomass

X_1 = 0 if water is clear, =1 if water is turbid

X_2 = dry plant biomass kg ha^{-1}

Y_i = spectral reflectance at each wavelength

$$Y_i = b_0 + d_1 W_1 + d_2 W_2 \quad (1.10)$$

where d_1 = coefficient of water depth

d_2 = coefficient of plant biomass

W_1 = depth of water

W_2 = dry plant biomass kg ha^{-1}

Y_i = spectral reflectance at each wavelength

The analysis of variance (ANOVA) and analysis of covariance (ANCOVA) was performed with PROC MIXED procedure. First the effect of variety on the yield, biomass and plant coverage was determined using ANOVA. The effects of variety on the relationship between spectral indices and agronomic parameters at different growth stage were also investigated by ANCOVA with the following equation:

$$Y_i = b_0 + b_1 I + b_2 V + b_3 I * V \quad (1.11)$$

where b_1 = coefficient of vegetation indices based on red-edge reflectance

b_2 = coefficient of variety

b_3 = coefficient of variety*vegetation indices

I = vegetation indices based on red-edge reflectance

$V = 0$ if variety is CL261, $V=1$ if variety is CL152

Y_i = grain yield kg ha^{-1}

If Y-intercepts (b_2) are significantly different, this indicates that at the same vegetation index value, grain yield of variety CL152 is significantly different from CL261. If slopes (b_3) of the regression lines are significantly different, this indicates that the increase of yield per unit increase of vegetation is different between CL152 and CL261.

2.3 Results and Discussion

2.3.1 The Effect of Water Background and Depth on the Spectral Reflectance

During the sampling period, from PD to 50 % HD, canopy coverage from planted plots ranged from 35 to 100 % (Figure 2.1). During this period there was no significant effect of the water background (turbid or clear) on the spectral reflectance ($p > 0.05$; Figure 2.2). The graph on Figure 2.3 shows the coefficient of water background on the linear regression. Since the upper and lower 95 % confidence interval of the coefficient includes zero across all wavelength, it can be concluded that water background had no effect on spectral reflectance measured from 400 to 900 nm. The study conducted by Vaesen et al. (2000) showed a similar result. Their study examined the effect of water turbidity only on vegetation indices, not each wavelength; the relationship between LAI and NDVI_{red} or SR_{red} was not influenced by the water background.

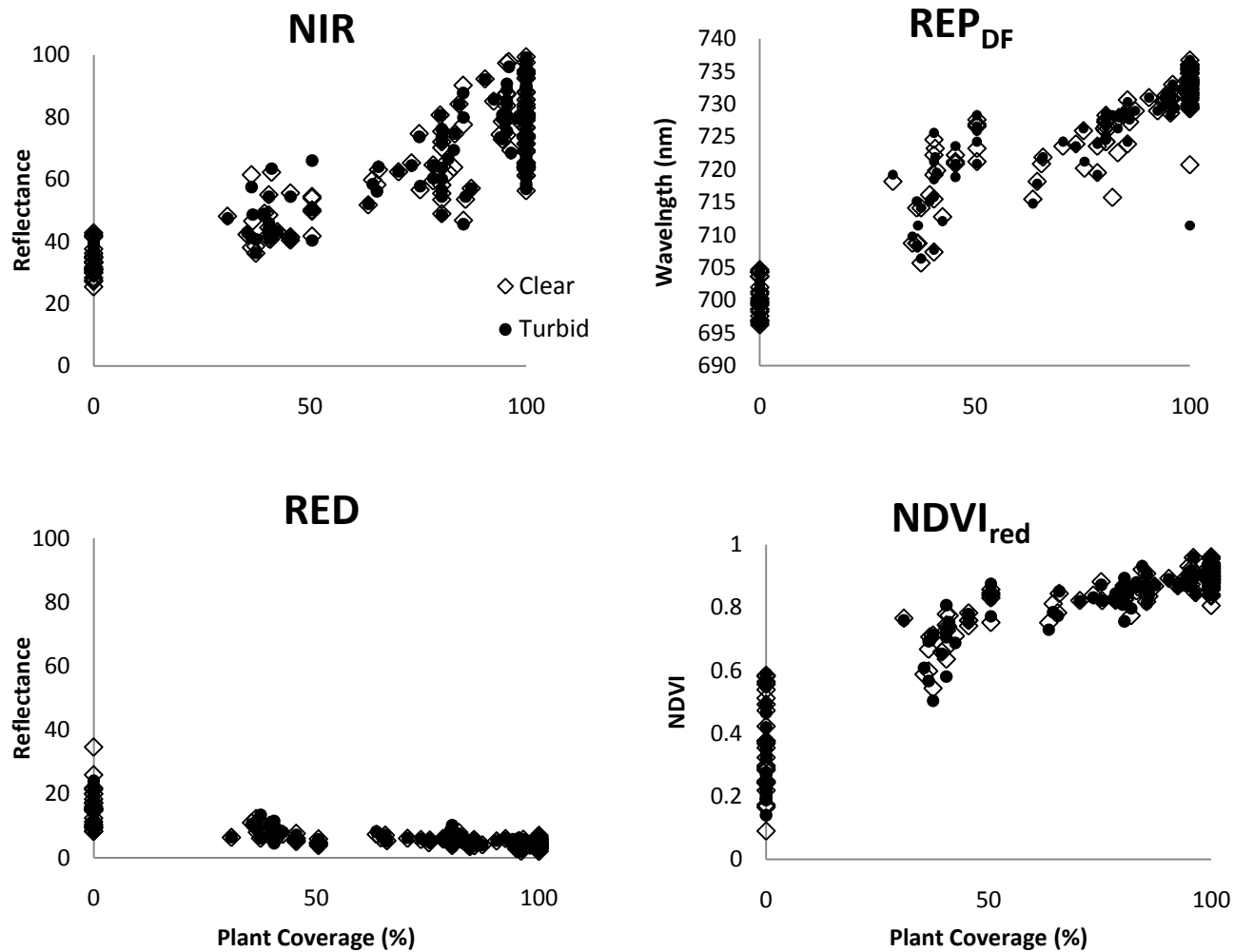


Figure 2.1. The near-infrared (NIR), red (RED) spectral reflectance , normalized difference vegetation index (NDVI_{red}), and red-edge position (REP_{DF}) based on polynomial technique under clear and turbid water at different plant coverage.

Since LAI and biomass accumulation is positively related, their results support the finding of our study, i.e. there was no effect of water turbidity on the relationship between spectral reflectance and rice biomass.

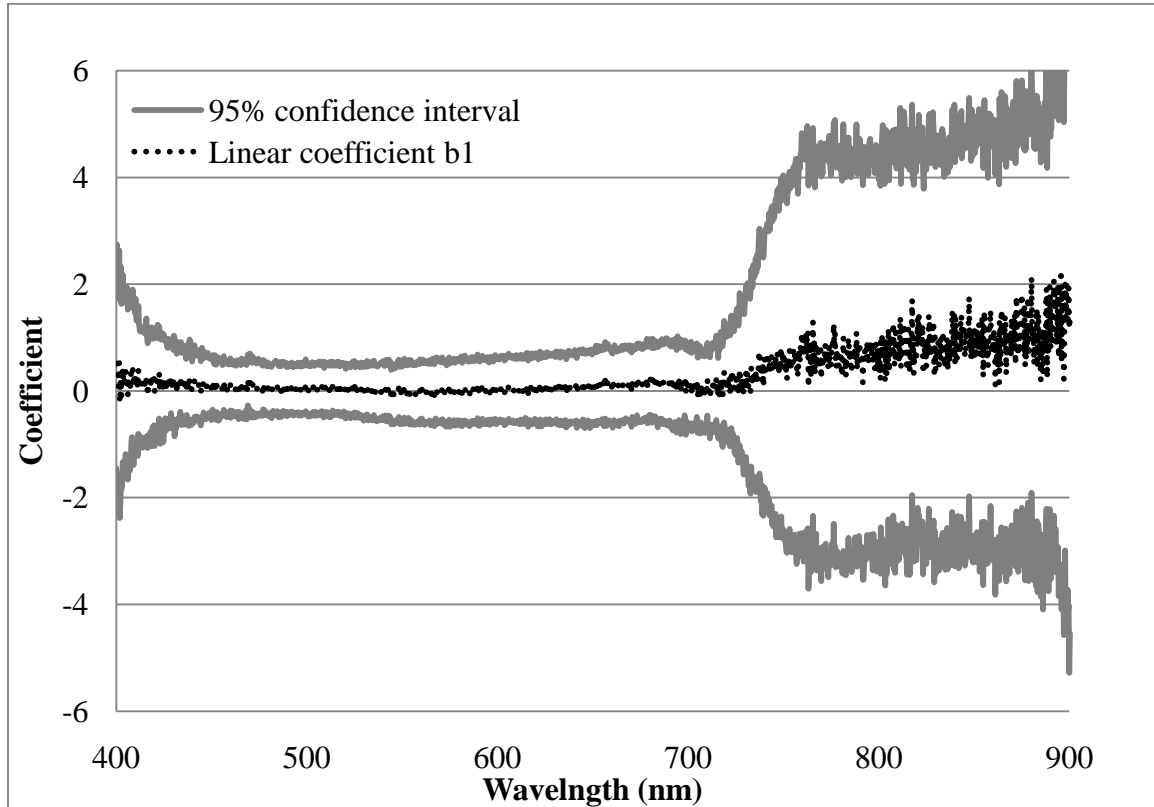


Figure 2.2. The effect of water background on the spectral reflectance from 400 to 900 nm.

No significant effect of water depth on the spectral reflectance was observed except at red wavelength ($p > 0.05$; Figure 2.3). Theoretically, an increase in water depth should decrease reflectance due to the increased radiant absorption in the water (Hoshi et al., 1984). However, our results indicate otherwise, the water depth increase enhanced red reflectance. One of the potential reasons relies on the relationship between water depth and growth of algae and weeds. For both cropping years, growth of ducksalad

(*Heteranthera limosa*) on water surface was evident at the research sites. According to Sen et al. (2002), the water depth can affect the population or growth of certain weed species; the increase in water depth subsidizes weed growth. This implies an increase in red reflectance since weed or algae interference increases absorbance of red. Further, even the coefficients of near infrared and green regions were not significant; this assumption can explain the slight negative coefficient values of the present study. Since the population of algae or weed was not carefully studied nor counted as the above-ground biomass accumulates, further research is required to understand this behavior of red reflectance associated with water depth. The slight impact of water depth on the spectral reflectance between 650 to 700 nm can be affected to NDVI_{red} or SR_{red} vegetation indices. To clarify this, the statistical regression model with the following equation was performed at each sampling time.

$$Y_i = b_0 + d_{11}W_1 + d_{22}W_2 \quad (1.12)$$

where d_{11} = coefficient of water depth

d_{22} = coefficient of plant biomass

W_1 = depth of water

W_2 = dry plant biomass kg ha⁻¹

Y_i = NDVI red or SR red

According to the model, water depth had no significant effect on NDVI_{red} (p = 0.11, 0.97 and 0.09 at PD, PD+1wk and 50% HD, respectively) and SR_{red} (p = 0.73, 0.33 and 0.06 at PD, PD+1wk and 50% HD, respectively). This may have resulted from the relative small shifts of red reflectance as the plant biomass or coverage increased. As

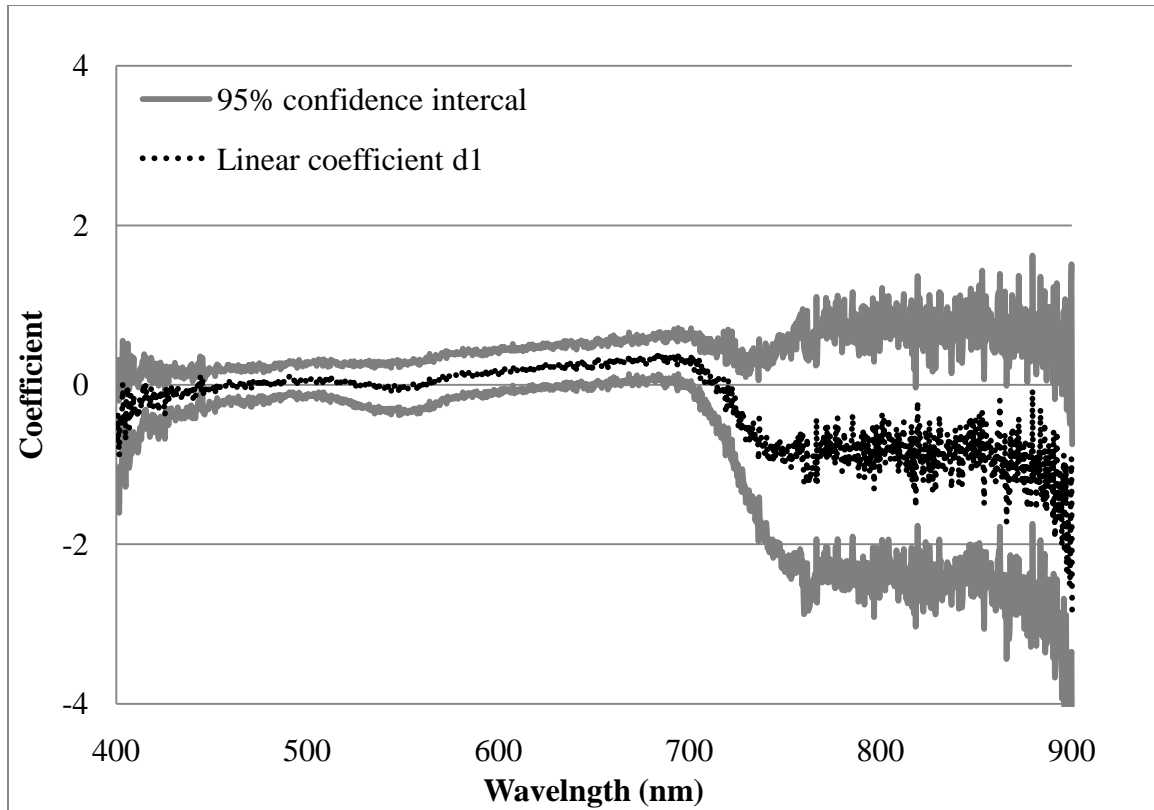


Figure 2.3. The effect of water depth on the spectral reflectance from 400 to 900 nm.

shown in Figure 2.1., the change of red reflectance associated with plant coverage which ranged from 35 to 100 was extremely small compared with NIR reflectance. This behavior of red reflectance is related to its saturation point at relatively low chlorophyll contents (Smis and Gamon, 2002). Therefore, if the red band is used as a single red wavelength, the effect of water depth would be significant on the spectral reflectance readings but when expressed as a vegetation index in combination with other bands, the effect of water on the spectral reflectance can be negligible, especially at mature plant growth stages. From a practical stand point, substantial plant coverage is achieved at PD, the growth stage where N fertilization is commonly done in the mid-southern United States rice production systems and the optimal time for sensing to estimate in-season

yield and N response index according to Harrell et al. (2011) and Tubana et al. (2011), respectively. Therefore, the problem associated with water depth is expected to be minimal.

2.3.2 The Relationship Between Vegetation Indices and Agronomic Parameters

There were significant differences on dry biomass, N uptake, plant coverage, and grain yield between the two varieties in 2011 but not in 2012 (Table 2.2, 2.3, 2.4, and 2.5). The variety CL261 had higher biomass, N uptake and plant coverage at PD in 2011. The effect of variety on biomass as well as % of plant coverage was significant at PD and PD+1wk ($p > 0.05$; Table 2.3 and 2.5). In terms of N uptake, the significance difference was only observed at PD (Table 2.4). Therefore, the varietal effect on those agronomic parameters were more evident until PD+1wk but once plant growth stage reached 50% HD, the effect was not observed. In 2012, blast disease caused by the fungal pathogen (*Pyricularia oryzae*) substantially decreased plant vigor which ultimately reduced biomass production and N uptake. This was especially true for CL261 which is classified as very susceptible to blast (Table 2.3 and 2.4). This reduction in mid-season biomass production directly affected the grain yield. Grain yields were decreased by about 3000 kg ha⁻¹ for each N rate in 2012 (Table 2.2). The effect of Variety x N rate was only observed in 2012, which can be attributed to the different level of resistance of the two varieties to blast disease. The high yield associated with high biomass at mid-season supports the concept of estimating above ground biomass at mid-season to predict yield. However, in addition to this fact, Harrell et al. (2011) discussed about the implementation of additional elements

Table 2.2. Analysis of variance for the effect of variety and N rate on rice grain yield at Crowley, LA in 2011 and 2012.

Treatment	2011	2012	
	Grain Yield kg ha ⁻¹		
Variety			
CL152	† 8911b		§
CL261	9373a		§
Nitrogen Rate kg ha ⁻¹		CL152	CL261
0	6404d	3611d	3851e
44	8111c	5832c	5114d
88	9747b	7354b	6727c
132	10380ab	8454a	7383b
176	11067a	8794a	8002a
Variety x N Level	NS	***	

NS Not significant at $\alpha=0.05$ level.

*** Significant at $\alpha<0.001$ level.

† Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis.

§ Significant at Variety x N level; therefore means are listed by variety at each N rate.

in predicting yield since biomass is not only one parameter which always carried over at harvest. For example, they mentioned the risk of decreasing in yield due to lodging and disease infection with increasing biomass production.

The red-edge based vegetation indices had better relationship with biomass, N uptake, and grain yield compared with red-based indices (Table 2.6). At PD, the r values of the linear relationships of biomass was 0.74 and 0.79 for SR_{red} and $NDVI_{red}$ while it improved to 0.84 when $SR_{red-edge}$ and $NDVI_{red-edge}$ were used. With regards to biomass, the degree of improvement using red-edge based indices declined as the rice grew. The

Table 2.3. Analysis of variance for the effect of variety and N rate on biomass at panicle differentiation (PD), panicle differentiation + 1 week (PD+1wk), and 50 % heading (50%HD) in rice at Crowley, LA in 2011 and 2012.

Treatment	2011			2012		
	Biomass kg ha ⁻¹					
	PD	PD+1wk	50%HD	PD	PD+1wk	50%HD
Variety						
CL152	3743b	5024b	10837a	3181a	3565a	3284a
CL261	4913a	6574a	12090a	3350a	3671a	3181a
Nitrogen Rate kg ha ⁻¹						
0	2345c	3233b	6605c	1315d	1727c	1312d
44	3850b	4892b	10486bc	2445c	3335b	2445c
88	4853ab	7137a	12863ab	3453b	4000ab	3453bc
132	5007a	6813a	12886ab	4148ab	4421a	4148ab
176	5586a	6921a	14476a	4968a	4609a	4804a
Variety x N Level	NS	NS	NS	NS	NS	NS

NS Not significant at $\alpha=0.05$ level.

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis.

Table 2.4. Analysis of variance for the effect of variety and N rate on N uptake (kg ha^{-1}) at panicle differentiation (PD), panicle differentiation + 1 week (PD+1wk), and 50 % heading (50%HD) in rice at Crowley, LA in 2011 and 2012.

Treatment	2011			2012			
	N uptake kg ha ⁻¹						
	PD	PD+1wk	50%HD	PD	PD+1wk	50%HD	
Variety							
CL152	§	91a [†]	176a	76a	72a	53a	
CL261	§	107a	189a	79a	73a	54a	
Nitrogen Rate kg ha ⁻¹	CL261	CL152					
0	29c	33c	40b	78c	18d	22c	13d
44	50c	63bc	65b	138bc	43cd	50bc	29d
88	92b	87b	116a	191ab	74bc	80ab	52c
132	96ab	132a	126a	238a	117ab	94a	73b
176	118a	160a	148a	269a	135a	116a	100a
Variety x N Level	*	NS	NS	NS	NS	NS	NS

NS Not significant at $\alpha=0.05$ level.

* Significant at $\alpha<0.05$ level.

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis.

§ Significant at Variety x N level; therefore means are listed by variety at each N rate.

Table 2.5. Analysis of variance for the effect of variety and N rate on plant coverage (kg ha⁻¹) at panicle differentiation (PD), panicle differentiation + 1 week, and 50 % heading (HD) in rice at Crowley, LA in 2011 and 2012.

Treatment		2011			2012		
		Plant Coverage %					
Variety		PD	PD+1wk	50%HD	PD	PD+1wk	50%HD
CL152		§	81b [†]	85a	83a	84a	86a
CL261		§	84a	83a	81a	82a	85a
Nitrogen Rate kg ha-1		CL261	CL152				
0		39d	39c	41c	44c	29c	32d
44		67c	76b	78b	78b	88b	76c
88		84b	96a	95a	100a	93ab	95b
132		93a	97a	100a	100a	99a	100a
176		99a	100a	100a	100a	100a	100a
Variety x N Level		*	NS	NS	NS	NS	NS

NS Not significant at $\alpha=0.05$ level.

* Significant at $\alpha<0.05$ level.

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis.

§ Significant at Variety x N level; therefore means are listed by variety at each N rate.

Table 2.6. The coefficient of correlation (r) between vegetation indices and each agronomic variable at panicle differentiation (PD), panicle differentiation +1 week, and 50 % heading (HD).

Vegetation Index	Biomass			N Uptake			Yield		
	PD	PD+1wk	50% HD	PD	PD+1wk	50% HD	PD	PD+1wk	50% HD
SR _{red}	0.72	0.77	0.66	0.56	0.76	0.75	0.72	0.83	0.83
SR _{rededge}	0.84	0.83	0.70	0.83	0.84	0.75	0.82	0.85	0.89
NDVI _{red}	0.79	0.76	0.61	0.67	0.75	0.64	0.82	0.86	0.84
NDVI _{rededge}	0.84	0.79	0.64	0.83	0.84	0.73	0.85	0.88	0.90
REP _{DF}	0.75	0.69	0.57	0.78	0.76	0.62	0.71	0.81	0.85
REP _{LI}	0.81	0.69	0.61	0.85	0.82	0.72	0.78	0.78	0.84
REP _{LAG}	0.44	0.29	0.39	0.45	0.32	0.41	0.43	0.38	0.62
REP _{LE}	0.75	0.76	0.58	0.75	0.84	0.62	0.72	0.88	0.85

SR – simple ratio

NDVI – normalized difference vegetation index

REP – red-edge position

DF-5th order polynomial fitting technique

LI-linear extrapolation technique

LAG-lagrangian technique

LE-linear extrapolation technique

improvement of predicting N uptake and grain yield using red-edge based indices was more evident. Across sampling periods, about 50 % of total variability in N uptake was explained by red based vegetation indices such as $NDVI_{red}$ and SR_{red} while red-edge based vegetation indices explained about 69% of total variability in N uptake (Table 2.6). Similar results were observed between vegetation indices and grain yield. Red-based spectral indices can explain 49 to 72 % of total variability in grain yield while the range improved to 64 to 90 % using red-edge based vegetation indices.

The relationships of $NDVI_{red-edge}$ or $SR_{red-edge}$ with measured parameters (biomass, N uptake and grain yield) had the highest r values across sampling periods in biomass, N uptake and grain yield. The red-edge position reflectance readings (REP_{DF} , REP_{LI} , REP_{LAG} and REP_{LE}) computed from derivative analysis were also closely related to those agronomic parameters. The advantage of red-edge reflectance has been reported in many studies (Curran et al., 1990; Mutanga and Skidmore, 2004; Cho et al., 2008). One of its advantages over red-based indices such as $NDVI_{red}$ is increased sensitivity of detecting plant physiological status at high plant biomass or coverage. The poor estimation using $NDVI$ is associated with the absorption of red light approaching saturation at full plant canopy coverage (Thenkabail et al., 2000; Smis and Gamon, 2002). As shown in Figure 2.4, the $NDVI_{red}$ reached plateau at lower biomass level, approximately 5000 kg ha^{-1} , compared with REP . This indicates that the amount of biomass beyond 5000 kg ha^{-1} is not a function of $NDVI_{red}$. On the other hand, red-edge based index remained a function (quadratic) of biomass even when biomass weighed more than $10,000 \text{ kg ha}^{-1}$. This result demonstrated the improvement of estimating plant physiological condition using red-edge position waveband at mid-season in rice.

In general, derivative-based red-edge indices are considered better predictors of biomass, however, in our study the degree of their relationships with biomass varied across sampling times. For example, the low r values in REP_{LAG} at PD demonstrate the several outliers which are potentially associated with the complex computation of red-edge position with the use of multiple wavebands. Several studies also noted the relatively higher complexity of computing REP_{LAG} compared with REP_{DF} , REP_{LI} or REP_{LEP} (Cho and Skidmore, 2006; Shafri et al., 2006). Also Curran et al. (1990) summarized the importance of holding light assumptions, such as illuminations should be independent from the leaf reflectance, when red-edge positions were measured. Under dynamic environment systems, those assumptions might be violated and eventually affect the readings at red-edge positions.

The red-edge vegetation indices expressed in normalized and ratio forms $SR_{red-edge}$ and $NDVI_{red-edge}$, had no derivative analysis involved, and yielded constant r values across sampling periods (Table 2.6). In addition to reducing background variation, the feasibility in data comparison due to the standardization is the advantage of using those normalized or ratio-based vegetation indices (Daughtry et al., 2000; Malingreau, 1989). Unlike REP computation which involves continues or multiple wavebands, $SR_{red-edge}$ and $NDVI_{red-edge}$ simply require only two wavebands. With respect to the fact that applying remote sensing technology in nutrient management is still considered cost-inhibitive, the use of fewer bands would facilitate in developing affordable remote sensor system in crop production.

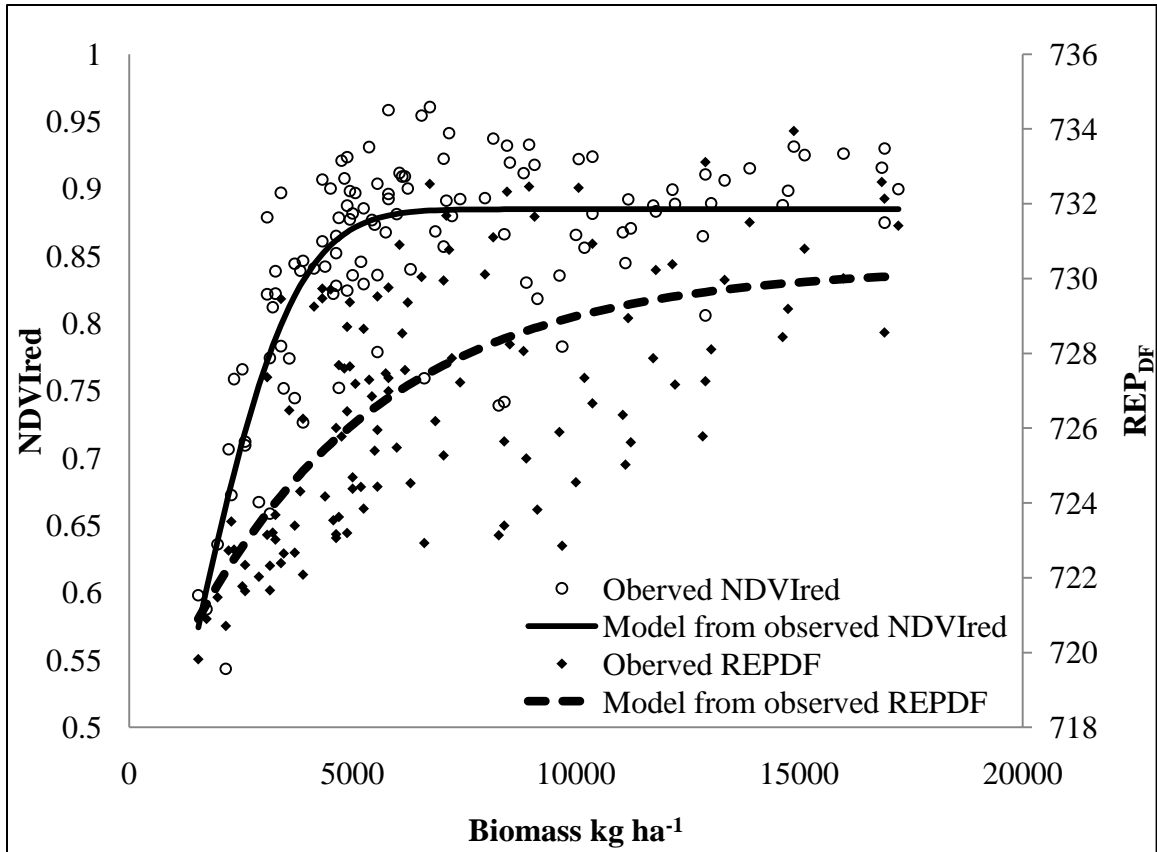


Figure 2.4. The comparison of normalized difference vegetation index (NDVI) and red-edge position (REP_{DF}) based on polynomial fitting technique for the relationship with biomass.

2.3.3 The Effect of Rice Variety on the Yield Prediction Model Using Red-Edge Reflectance

The coefficient table for linear regression to determine the effect of variety on the relationship between red-edge based spectral indices and grain yields were summarized in Table 2.7. Differences in variety were more evident when grain yield was predicted using red-edge normalized or ratio-based vegetation indices. For example, the suggested models for predicting yield using $SR_{red-edge}$ at PD were

$$\text{Predicted grain yield} = -5712 + 8055 * SR_{red-edge} \text{ for variety CL152}$$

$$\text{Predicted grain yield} = -3092 + 6005 * SR_{red-edge} \text{ for variety CL261.}$$

This formula indicates that the 0.1 unit increase of $SR_{red-edge}$ corresponds to a predicted grain yield increase of 806 kg ha⁻¹ in variety CL152 but it was 600 kg ha⁻¹ in variety CL261. Also when $SR_{red-edge}$ is assumed to be one, the base line of grain yield is 2324 kg ha⁻¹ for variety CL152 and 2913 kg ha⁻¹ for variety CL261. This implies that the corresponding increase in grain yield by one unit increase in $SR_{red-edge}$ would be different depending of variety. This interpretation can apply not only PD stage but across sampling periods when grain yield prediction model is established using $SR_{red-edge}$. This result raises the discussion of spectral resolution predicting grain yield. When the detection limit of $SR_{red-edge}$ is assumed to be 0.1 then the minimum unit that $SR_{red-edge}$ can differentiate grain yield is 806 kg ha⁻¹ for CL152 and 600 kg ha⁻¹ for CL261. One unit increase of $SR_{red-edge}$ corresponds to large range of yield increase in variety CL152, therefore a higher resolution of spectral reflectance would be required compared with variety CL261 in terms of estimating certain unit increase in grain yield. For example, to detect 1000 kg ha⁻¹ difference in grain yield, $SR_{red-edge}$ need to show at least 0.1 differences for variety CL152 as compared to 0.2 for variety CL261. That difference caused by variety would be challenging when remote sensing technology is carried over to practical application in nutrient management. Accounting the varietal effect when establishing the grain yield prediction model using $SR_{red-edge}$ improved R^2 value from 0.67 to 0.73, 0.72 to 0.74, and 0.79 to 0.83 compared with simple regression model at PD, PD+1wk, and 50% HD, respectively. This result showed that the effect of variety on the relationship between $SR_{red-edge}$ and grain yield changed with plant growth stage. The addition of individual variety parameters improved the model allowing to explain 5 % more in total variation in grain yield. Although there was a significant effect of variety

on the grain yield prediction model using $SR_{\text{red-edge}}$ the improvement in predicting yield was not evident. Therefore, one simple model can be sufficient to predict yield based on $SR_{\text{red-edge}}$.

When $NDVI_{\text{red-edge}}$ was used, there was no effect of $I*V$ indicating that there was no corresponding differences in predicted grain yield per unit increase of $NDVI_{\text{red-edge}}$ between varieties (Table 2.7). However, there are still effects of variety on the prediction model as shown by different intercept. As shown in Table 2.7, when grain yield was regressed by red-edge position (REP_{DF} , REP_{LI} , REP_{LG} , and REP_{LE}) coefficients, b_2 and b_3 , were not significant. It indicated there was no effect of variety or interaction of variety and vegetation index on the yield prediction model. Therefore, contrary to normalized or ratio-based indices, all of REP indices which defined as the point of red-edge waveband did not require the separated model based on varieties.

The influence of variety on the relationship between grain yield and spectral reflectance readings and their vegetation indices can be explained by the inherent differences in physical and physiological attributes among varieties. Generally, reflectance at the near infrared band is associated with the plant geometrical structures while reflectance within the visible wavelength, especially red and blue, is highly related to absorption of major two pigments, chlorophyll *a* and *b*. Reflectance at red-edge position, which is the waveband from pigment absorption to reflective region, is reported to be a good indicator of biomass, N content, and chlorophyll content (Elvidge and Chen , 1995; Meer and Jong, 2006; Cho et al., 2008). As Jackson and Pinter (1986) observed differences between planophile (non-erect) and erectophile (erect) canopies in wheat.

Table 2.7. The coefficient table for linear regression to determine the effect of variety on the relationship between red-edge based spectral indices and grain yields at panicle differentiation (PD), panicle differentiation +1 week, and 50 % heading (HD) at Crowley LA, in 2011 and 2012.

Indices	Growth Stage	R^2	P value	Coefficients			
				b_0	b_1	b_2	b_3
$SR_{red-edge}$	PD	0.73	<0.001	-3092	6005	-2620	2050
	PD+1 wk	0.74	<0.001	-5310	7154	608	-
	50% HD	0.83	<0.001	-4238	6432	-3058	1999
$NDVI_{red-edge}$	PD	0.73	<0.001	596	25639	890	-
	PD+1 wk	0.76	<0.001	-117	27617	573	-
	50% HD	0.82	<0.001	-648	28587	508	-
REP_{DF}	PD	0.51	<0.001	-124104	182	-	-
	PD+1 wk	0.64	<0.001	-175646	252	-	-
	50% HD	0.72	<0.001	-185746	266	-	-
REP_{LI}	PD	0.63	<0.001	-401254	798	563	-
	PD+1 wk	0.6	<0.001	-394877	544	-	-
	50% HD	0.71	<0.001	-371885	522	-	-
REP_{LG}	PD	0.18	<0.001	-54026	84.9	-	-
	PD+1 wk	0.15	<0.001	-23469	43	-	-
	50% HD	0.38	<0.001	-83653	125	-	-
REP_{LE}	PD	0.52	<0.001	-100169	149	-	-
	PD+1 wk	0.78	<0.001	-149918	216	-	-
	50% HD	0.72	<0.001	-137077	199	-	-

$$*Y_i = b_0 + b_1 I + b_2 V + b_3 I * V$$

where

b_1 = coefficient of vegetation indices based on red-edge reflectance

b_2 =coefficient of variety

b_3 =coefficient of variety*vegetation indices

I =vegetation indices based on red-edge reflectance

$V=0$ if variety is CL261, =1 if variety is CL152

Y_i =grain yield $kg\ ha^{-1}$.

- effect (I , V or $I*V$) is not significant at $\alpha=0.05$ level

SR – simple ratio

NDVI – normalized difference vegetation index

REP – red-edge position

DF-5th order polynomial fitting technique

LI-linear extrapolation technique

LAG-lagrangian technique

LE-linear extrapolation technique

Galvão (2005) also reported the varietal effect on infrared spectral reflectance in sugarcane. Based on their study, distinct differences were observed in the green (550 nm) and near infrared (NIR) (800 nm~) bands. Therefore, in our study, the distinct differences of biomass accumulation in varieties affected the reflectance reading at the near infrared region and then carried-over to both $NDVI_{red-edge}$ and $SR_{red-edge}$ vegetation indices computation; this was not the case for REP. It is important to note as well that the regression lines describing the relationship of $SR_{red-edge}$ and grain yield for each variety had different slopes, but not when $NDVI_{red-edge}$ was used as a predictor. This difference can be explained by analyzing the mathematical expression of these two forms of vegetation indices. The weighted impact of near-infrared and red-edge reflectance readings when expressed in normalized form (as in NDVI) is eliminated. This explains why the distribution of NDVI readings was narrow even if there was a wide range in reflectance readings at near infrared than the red-edge position. Unlike NDVI, SR is simply a ratio which utilized reflectance readings within the near infrared and red-edge position without normalizing the values. This tends to result in a wide distribution of SR values. The distinct behavior of these two vegetation indices was reported by several researchers (Tubana et al. 2011; Chang et al., 2005).

2.4 Conclusions

Water background (turbid or clear) did not significantly alter spectral reflectance at PD, PD+1 weeks, and 50% HD. Water depth slightly influenced the behavior of red reflectance but this effect was not carried over when vegetation indices, SR_{red} or $NDVI_{red}$ were computed. The red-edge based vegetation indices had better relationships with

measured agronomic parameters as compared with red based indices. Vegetation indices expressed in normalized or ratio forms computed from derivative spectral analysis (REP_{DF} , REP_{LI} , REP_{LAG} and REP_{LE}), resulted in consistent r values across sampling periods.

The effect of variety on the accuracy of the yield prediction model varied depending on the transformation of reflectance within the red-edge and near infrared bands i.e., into normalized (NDVI) and ratio (SR) forms of vegetation indices. This result was associated with the behavior of near infrared wavebands on the geometrical structure of the plant canopy. There were no significant effects of variety on grain yield prediction models using derivative based red-edge indices. Our findings showed that red-edge based NDVI and SR are better predictors of rice grain yield than red-based NDVI and SR. Red-edge based NDVI or SR indices both have potential to predict rice grain yield and rice responsiveness to N fertilization.

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Chapter 3. Agronomic Parameters of Different Sugarcane (*Saccharum* spp.hybrids) Varieties in Response to Variable Levels of Nitrogen Supply

3.1 Introduction

In Louisiana, sugarcane is one of the most valuable row-crops. It is grown in 22 out of 64 parishes and contributes \$2.2 billion to Louisiana economy (Legendre, 2000; the American Sugar Cane League, 2012). The cost of sugarcane production has increased dramatically in the last decade due to gas and fertilizer costs. In 2004, the average cost of N fertilizer and total sugarcane production was only \$ 0.62 kg⁻¹ and \$ 488 ha⁻¹, respectively (Salassi and Deliberto, 2009). In 2010, the estimated sugarcane production costs increased to \$685 ha⁻¹ due to high fuel (\$ 0.72 L⁻¹) and N fertilizer (\$1.12 kg⁻¹) price (Salassi and Deliberto, 2010). Therefore in less than ten years, the production costs have increased by about 40 %, with the cost of N fertilizer contributed the most impact. With these large increases in production costs paired with minimal increase of sugar price, producers need to maximize the agronomic benefits for every unit of N fertilizer applied. With increasing public concern on environmental quality, high production costs, and low N fertilizer use efficiency in crop production, it is essential to improve N management in sugarcane production.

Nitrogen fertilizer is applied only once between early April until the beginning of May for Louisiana sugarcane production systems. Sugarcane is considered a semi-perennial crop, in which the initial crop planted around August to September is called plant cane and is harvested approximately 14 months later. Then sugarcane which is harvested on 9 to 11 month cycle for an additional 3 to 4 years, is called ratoon sugarcane. Older ratoons are generally harvested 1st close to a 9 month growing season. Currently, LSU Ag-Center N rate recommendation is based on soil type and crop age. Plant sugarcane N recommendations are between 67 to 110 kg N ha⁻¹ and are between 88 to 132 kg N ha⁻¹ for ratoon sugarcane crop. (Legendre et al., 2000). However,

temporal and spatial variability can make N rate requirement highly variable for maximum sugarcane production. Based on a 5-year N response trial, Tubana (unpublished data) reported that 75% of site-year showed over-fertilization, 38% of the 75 % fields showed no response to applied N. Several researchers have shown plant sugarcane does not typically respond to N fertilization due to the adequate mineralized N during the fallow period (Wiedenfeld, 1995; Muchovej, 2004).

Inappropriate N rate and timing has the potential to adversely affect sugarcane yield and quality. Wiedenfeld (1995) reported that the increase of N application decreased sugar yield, juice purity as well as recoverable sucrose. The reduction of sugarcane quality at high N rate may be associated with the higher mortality of primary stalks. Borden (1945) reported that high N rate application resulted in production of dense stalk population, which eventually led to higher mortality of primary stalks. Excess plant-available N was one of the potential reasons for the increased number of immature stalks (suckers) at later growth stages of sugarcane (Salter and Bonnett, 2000). This increased number of immature stalks at harvest can dilute cane sugar which ultimately can result in decreased sucrose content and economic value. On the other hand, inadequate N at tillering (rapid stalk production) eventually reduced the overall sugarcane biomass production. This is simply due to decreased plant growth early in the season can directly impact biomass accumulation, canopy photosynthesis, as well as sustained growth later in the season. Additionally, since sugar yield is directly related to biomass production, decreased biomass production can result in decrease sugar yield. Due to inappropriate timing of N application, reduction of sugar yield has been also reported (Wiendenfeld, 1997; Lofton et al., 2012).

Mismanagement of N fertilizer does not only affect sugarcane production but is also considered as one of the major factors contributing to degraded environmental conditions. The

majority of sugarcane production is located within central and southwestern Louisiana where 12 major drainage basins in Louisiana are located (Southwick et al., 2002). The analysis of water samples collected from canal, bayou and river around sugarcane fields showed an increase of nitrate level one month after the N application (Southwick et al., 2002). The increased N added to waterways could be a source of eutrophication (Rabalais, 2002) as well as increased level of nitrate in drinking waters which is associated with human blood disorder called methyhemoglobinemia (blue baby syndrome) (Knobeloch et al., 2000). Therefore it is essential to have proper N management to increase sugarcane yield and quality while sustaining the quality of our environment.

Several researchers have demonstrated the use of plant N response to refine their method of projecting crop N requirement (Peterson et al., 1993; Varvel et al., 1997; Raun et al., 2010). One method that has shown promising results is called a response index (RI) defined by Johnson and Raun (2003) as the yield ratio of the highest yielding N fertilized plot and a check plots which received no N. Mullen et al. (2003) elaborated stating that yield RI of corn can be predicted using normalized difference vegetative index (NDVI) when readings collected at V8 leaf growth stage. Furthermore, Harrell et al. (2011) and Tubana et al. (2011) showed that NDVI reading within three weeks after panicle initiation in rice can estimate yield and RI. Similar to corn and rice, Lofton et al. (2012) found that NDVI taken during late tillering could be used to estimate sugarcane N response for both cane and sugar yields. Given the varietal diversity and the recognized distinction of these varieties, it is imperative to determine the impact of these varietal characteristics on the estimation of RI (Rostron, 1989; Urquiaga et al., 1992; Gavão et al., 2005; Laborde et al., 2008; Johnson et al., 2008). The recent study by Lofton et al. (2012) did not investigate the effects of varieties on mid-season plant agronomic N response and its relationship with cane and sugar yield. While many researchers have recognized these distinct

differences among sugarcane varieties, there is no documentation on the pattern of cane varieties response to N nor how these variables measured early in the season relates to sugar yield response to N. Therefore, the objective of this study was to evaluate and relate the N response of select agronomic parameters of three cane varieties to N response of measured sugarcane yield.

3.2 Materials and Methods

3.2.1. Experimental Design

Data were collected from a study established at the LSU AgCenter Sugar Research Station located in St. Gabriel (30°15'13"N 91°06'05"W), Louisiana. The soil associated with the experiment was Canciene silty clay loam (Fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquept). The experiment consisted of a complete factorial treatment structure between three varieties and four N rates in a randomized complete block design and each treatment was replicated four times.

The three varieties consisted of a variety that has an erectophile canopy structure, HoCP96-540, a planophile canopy (poor erectness), L 99-226, and one intermediate variety, L 01-283. The four N application rates were 0, 45, 90, and 135 kg ha⁻¹. The plot size was 12 m by 5.4 m containing three bedded rows. Bedded rows were opened wherein three whole cane stalks were placed side by side for each run; each run (three cane stalk) were overlapped with the next run of three stalks by 8 cm or including at least two mature internodes. In the middle of April, liquid urea-ammonium nitrate (UAN; 32-0-0) was knifed-in near the shoulder of each bed at 15 cm depth. For weed management, metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) and atrazine (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) were applied in early spring based on LSU Ag-Center recommendations.

3.2.2. Sampling Methods and Data Management

Data was collected every week for four consecutive weeks from three weeks after N fertilization (WKN). For each sampling time, the following data were collected from a 1 m section of the two outside rows: plant biomass, number of stalks, N concentration in plant biomass, plant height, total length of leaf, and opening length of leaf (length from bottom of leaf to where it bent). For plant biomass sampling, a 1-m long sampling area within a row was selected for each plot. All sugarcane plants in the sampling area were cut at the base. Biomass samples were oven-dried at 60°C for 48 hours, weighed, ground and analyzed for total C and N using dry combustion method (LECO Corp., St. Joseph, MI). Length of leaf and opening length were collected to discriminate the geometry of plant canopy within variety. Using these information, foliar angle index (FAI) was established based on the equation (3.1);

$$\text{FAI} = \text{Leaf opening length (cm)} / \text{Total leaf length (cm)} \quad (3.1)$$

High FAI value indicates erectophile canopy structure while low value indicates planophile canopy structure.

At harvest, sugarcane stalks were cut from each plot using a Case IH 8800 Series sugarcane harvester (Case IH Agriculture, Racine, WI). Prior to harvest, ten whole stalk subsampled were randomly selected from the middle row of each plot then shredded and analyzed for sugar quality parameters using Spectracane Near Infrared System (Bruker Corporation, Billerica, Massachusetts) to determine theoretical recoverable sugars (TRS). Following this analysis shredded stalks were dried at 60°C for 48 hours, ground to pass a 2-mm sieve, and analyzed for total N using dry combustion method.

The dates of planting, fertilization, sampling and harvest are summarized in Table 3.1. From those collected agronomic parameters, response index (RI) was computed using the following equations (3.2).

RI_{45} = Agronomic parameter at 45 kg N ha⁻¹ plot/check plot (3.2a)

RI_{90} = Agronomic parameter at 90 kg N ha⁻¹ plot/check plot (3.2b)

RI_{135} = Agronomic parameter at 135 kg N ha⁻¹ plot/check plot (3.2c)

where agronomic parameters are sugar yield, dry biomass, number of tillers, %N, height and FAI

check plot = 0 kg N ha⁻¹

3.2.3. Data Analysis

Statistical analysis was performed using SAS 9.3. (SAS Institute, 2009). Analysis of variance (ANOVA) was performed to determine the effect of variety, on agronomic variables for each sampling periods. Simple regression analysis was performed with PROC REG procedure to see the relationship between response index computed from agronomic parameters.

Table 3.1. Dates of field activities at St. Gabriel, LA from 2010 to 2012.

	2010	2011	2012
	Plant Cane (PC)	1 st Ratoon (1R)	2 nd Ratoon (2R)
N fertilization	22-Apr	13-Apr	16-Apr
1st sampling	11-May	9-May	1-May
2nd sampling	29-May	16-May	7-May
3rd sampling	3-Jun	23-May	14-May
4th sampling	10-Jun	31-May	21-May
Harvest	22-Nov	3-Nov	11-Nov

3.3 Results and Discussions

3.3.1. Climatic Conditions

Climatic conditions were highly variables across three years (Figure 3.1). The 2011 was generally dry year; cumulative precipitation at the time of N fertilization was about 200 mm which was only half of what was received in 2012. Throughout at the sampling period in 2010

and 2012, small amounts of precipitation were recorded and by the end of 6 WKN, accumulated rainfall reached about 600 mm. This was not the case in 2011 where no precipitations were recorded during the entire sampling period. In 2010, low temperatures from January to May resulted in low cumulative growing degree days (CGDD) at the scheduled time of N fertilization. However, a drastic increase in temperature was recorded at 3 WKN. At 4 WKN, CGDD in 2010 surpassed what was accumulated in 2011 and 2012.

3.3.2. Sugar Yield and Its Response Index to N

Within year variation in temperatures and precipitations affected sugar yield. The average sugar yield in 2011 was 6.7 Mg ha^{-1} which was more than two times lower than (15.6 Mg ha^{-1}) in 2010 (Table 3.2). Generally, sugar yields declines with crop age. However, the substantial decrease in sugar yield in 2011 is probably associated with the lack of moisture during vegetative growth; the sugar yield dropped by more than 50 % in 2011 and had the lowest yield across the harvest years. Additionally, the cooler weather at early growth stage may inhibited plant growth and decreased sugar yield. There was a significant interactive effect (N rates x Variety) on sugar yield ($p < 0.05$). This indicates that the effect of N level on sugar yield was not consistent across varieties. Response index values for the 45, 90 and 135 kg N ha^{-1} application rates showed the varying magnitudes of sugar yield increases of different varieties due to N (Figure 3.2). For example in 2010, variety L 99-226 increased RI with N rate while variety HoCP 96-540 tended to decrease RI with increase N rate. Variations in pattern of RI with N rate were also evident across varieties in 2011. The RI values were similar across different N rates in variety HoCP 96-540 but increased in variety L01- 283.

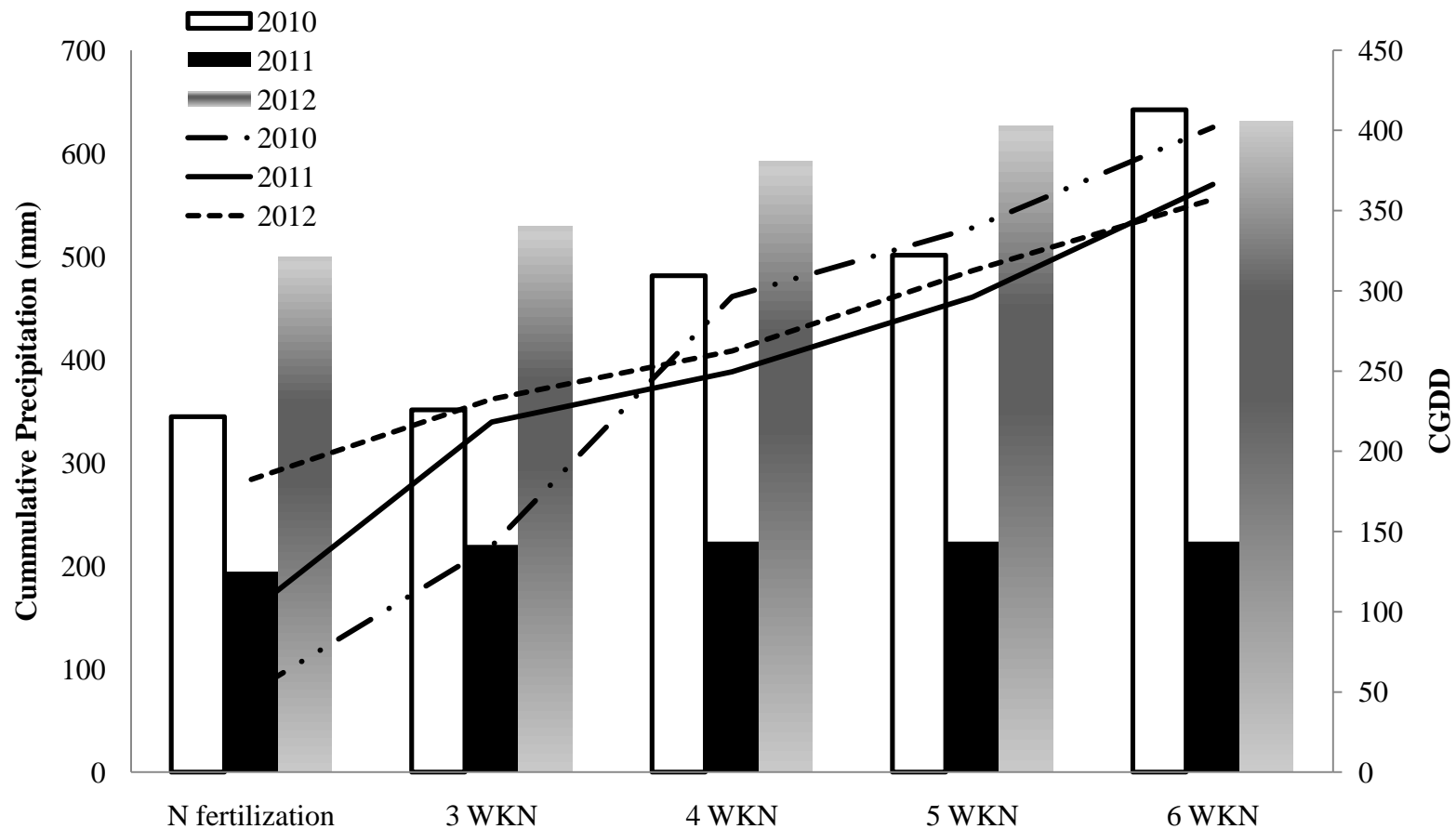


Figure 3.1. Cumulative precipitation and cumulated growing degree days from beginning of year at N fertilization and during sampling periods at St. Gabriel, LA 2010-2012.

CGDD- cumulative growing degree days from beginning of year computed by $(\text{maximum daily temperature} + \text{minimum daily temperature})/2 - 18^{\circ}\text{C}$

WKN- weeks after N fertilization

Table 3.2. Analysis of variance for the effect of variety and N rate on sugar yield of cane at St. Gabriel, LA 2010-2012.

		Sugar Mg ha ⁻¹		
		L 99-226	L 01-283	HoCP 96-540
	N kg ha ⁻¹			
2010 (PC)	0	13.13c [†]	13.29b	13.12b
	45	15.16b	16.09a	17.04a
	90	15.24b	17.18a	16.83a
	135	17.82a	17.16a	15.55a
2011 (1R)	0	5.03d	4.60c	4.86b
	45	6.26c	6.23b	7.25a
	90	8.19a	8.08a	7.31a
	135	7.35b	8.30a	7.50a
2012 (2R)	0	5.37d	5.17d	5.11d
	45	6.22b	7.52c	6.88c
	90	8.38a	8.46b	8.01b
	135	7.44b	9.54a	8.81a

[†]Same letter within column and year indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis

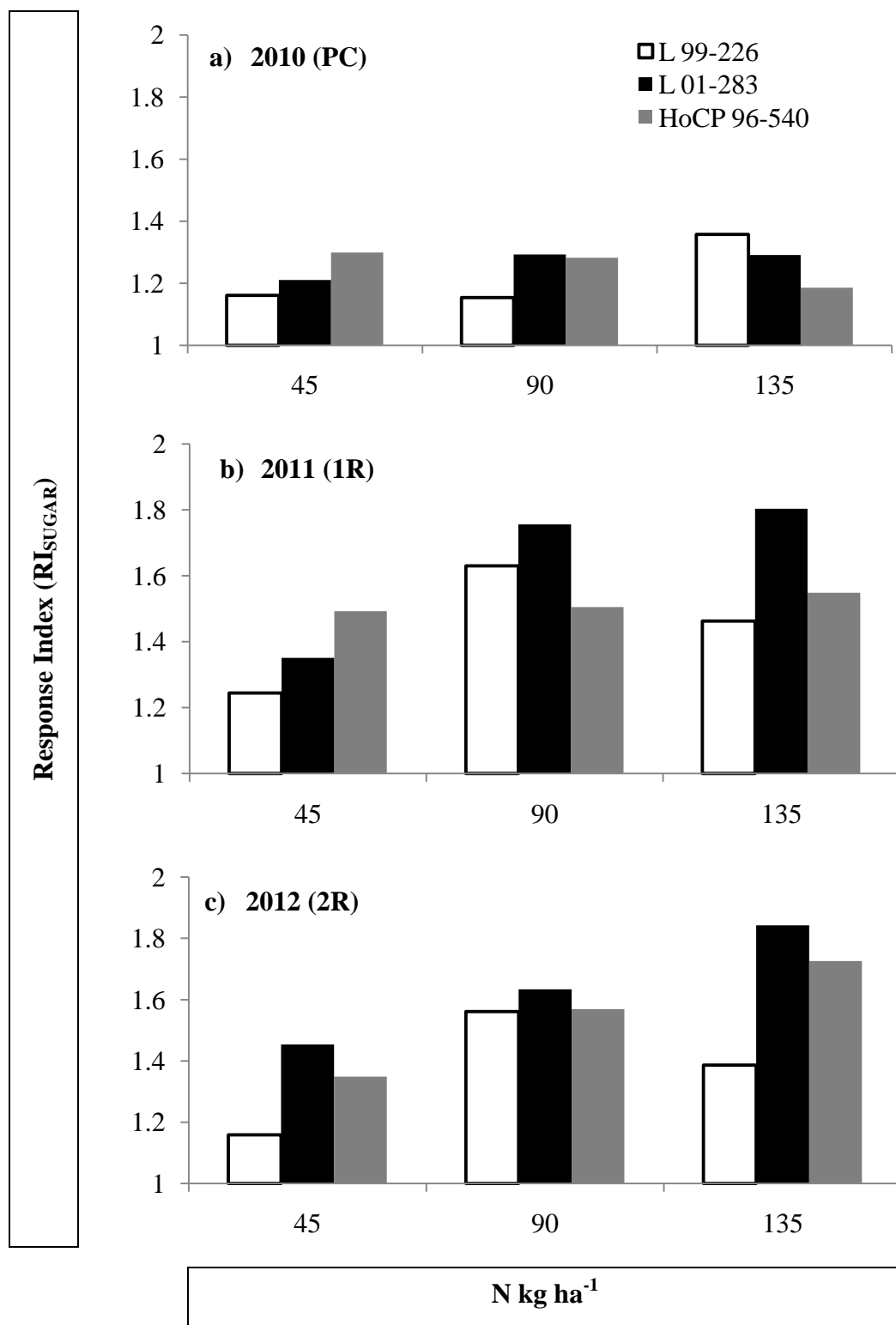


Figure 3.2. Ratio of sugar yield where sugar yield at $0\ kg\ N\ ha^{-1}$ as denominator and 45, 90 , and 135 $kg\ N\ ha^{-1}$ as numerator (Response Index- RI_{SUGAR}) for 3 different varieties in St. Gabriel, LA from 2010 (a) to 2012 (c).

All sugarcane varieties in all years showed a response to applied N (Figure 3.2). In 2010, plant cane-sugar yield response to applied N ranged from 10 to 50%, while sugar yield response to applied N for ratoon cane (2011 and 2012) ranged from 25 to 80 % (Figure 3.2). This low to non-responsiveness to applied N of plant cane is typically due to large amount of plant-available N from mineralization during the fallow period (Wood, 1964; Wiedenfeld 1995; Muchovej and Newman, 2004). In 2010, sugar yield obtained was higher than in 2011 or 2012 while sugar yield in 2011 and 2012 was more responsive to applied N. Our findings suggest that high sugar yield is not always associated with higher N supply or N responsiveness and vice versa. The higher yield with lower response to N in 2010 while the reverse outcome was obtained in 2011 and 2012 supports the concept that yield and RI being two independent components required for prediction of crop N requirement (Raun et al., 2010).

3.3.3. Mid-Season Agronomic Variables and Its Response Index to N

The agronomic variables measured and their responses to N levels were highly variable across year. Tables 3.3 to 3.7 showed the analysis of variance for each measured agronomic parameters and Figure 3.3 to 3.7 showed the agronomic response to N for each variety at each sampling time. The significant effect of varietal differences was found for biomass, height and FAI ($p < 0.05$). The effect of N rates was not evident in most of agronomic parameters in 2010. As mentioned previously, the plant cane crop does not typically respond to applied N due to available N from organic matter mineralization during the fallow period. On the other hand no response of biomass and height to N rates in 2011 could be explained by lack of moisture due to the extremely low precipitation during the sampling periods.

Biomass

In 2010 (plant cane), significant varietal differences in biomass were only observed at 5 and 6 WKN ($p < 0.05$; Table 3.3). The following growing season (ratoon crops), variety L01-283 consistently had the highest biomass produced across sampling time. In 2011 (1st ratoon), variety L 99-226 produced similar biomass as L 01-283, which was significantly higher than HoCP 96-540 ($p < 0.05$).

Even though there was no significant interactive effect (Variety x N rate) on the dry biomass across sampling times and years ($p > 0.05$; Table 3.3), response of dry biomass to each N rate where 0 kg N ha⁻¹ as base level (Response Index-RI_{BIOM}) was dependent on varieties (Figure 3.3). For example, variety L 99-226 resulted in the highest RI_{BIOM} value of 1.71 with 45 kg N ha⁻¹ at 5 WKN in 2010. However, the highest RI_{BIOM} value was 1.70 for L 01-283 with 135 kg N ha⁻¹ at 6 WKN and for HoCP 96-540, it was 1.40 with 135 kg N ha⁻¹ at 5 WKN. This indicated that only 45 kg N ha⁻¹ was required to increase 70 % of biomass for L 99-226 but it required 135 kg N ha⁻¹ to achieve similar increases for L 01-283. Furthermore, while HoCP 96-540 required similar N as L 01-283, the yield increase associated with applied N was only 40%. The majority of maximum RI_{BIOM} were detected between 5 to 6 WKN for each variety across years. Similar results about varietal effects on N response and biomass accumulation were reported by Gascho et al. (1986).

Height and Foliar Angle Index

Varieties had a significant ($p < 0.05$) effect on both height and FAI in both 2011 and 2012 (Table 3.4 and 3.5). The sugarcane varieties used in the current study were described as erect (HoCP 96-540), intermediate (L01-283), and planophile (L99-226) canopy structure. It can be inferred from Table 3.4 that among these varieties, L01-283 has the tallest structure while L 99-226 being the shortest. The highest FAI was observed in L 01-283 followed by HoCP 96-540

and L 99-226 (Table 3.5). Since greater value of FAI indicates erectophile canopy, the order toward erectness of the canopy structure was L 01-283 > HoCP 96-540 > L 99-226. In addition, the results presented in Table 3.5 show that FAI tended to increase with plant growth i.e. the plant canopy structure of cane became more erectophile as it grows. The change in canopy structure at later sampling time was more evident with HoCP 96-540 since the maximum range of FAI values during 3 WKN to 6 WKN was 0.19 while it was 0.15 for variety L 01-283 and 0.09 for variety L 99-226. This outcome is different from the current sugarcane variety classification reported by Jackson (2010). The high FAI and height in L 01-283 can assume that the biomass accumulation is toward vertical direction. On the other hand, variety L 99-226 which recorded the low FAI and height has more horizontal biomass accumulation.

Based on the effect of N on FAI and height (Tables 3.4 and 3.5), their RI values would not largely deviate from RI value of 1 (Figure 3.4 and 3.5). The range of RI_{HEIG} value was from 0.75 to 1.44 while RI_{FAI} was from 0.77 to 1.38. The RI_{HEIG} values of L 99-226, which is classified as having a droopy canopy structure, were mostly lower than 1. It indicates that the increase in biomass associated with applied N rate (Table 3.3) enhanced the non-erectness of the canopy structure. On the other hand, more erectophile varieties, their RI_{HEIG} values were generally >1. It indicates that plant become taller with N fertilization.

The RI_{FAI} values of L 99-226 being less than 1 supports the assumption made on the effect of N fertilization on RI_{HEIG} . Since lower FAI values indicate a wider opening of canopy structure, N fertilization enhanced the droopiness of this variety. For variety L 01-283 which is classified as having electrophile canopy in our study, the RI_{FAI} values were close to 1 across N rates, sampling periods, and cropping year indicating that the erect structure of L 01-283 is relatively stable than L 99-226 and HoCP96-540 regardless of N supply, size of biomass, and crop age.

Tiller Number

The number of tillers was similar across varieties and N levels (Table 3.6). In 2010, the highest tiller number was at 4 WKN for L 99-226, while highest tiller number was achieved at 6 WKN and 5 WKN for L 01-283 and HoCP 96-540, respectively. Bezuidenhout et al. (2003) discussed that decreased tiller number is often associated increase stalk senescence due to light competition. This observation was similar with our findings, where variety L 99-226 (planophile canopy) achieved canopy closure earlier than L 01-283 and HoCP 96-540 which led to the light competition at earlier stage. Therefore, the peak of tiller number was observed generally at earlier week in L 99-226. However, this observation was not detected in 2011 and 2012. Low amount of precipitation more likely restricted the active growth of tillering at vegetative stage in 2011. On the other hand, intensive accumulation of biomass due to optimum condition for sugarcane growth may result in early canopy closure with L 01-283 which induced the decrease in tiller number at 1 week earlier than other varieties in 2012.

There was no significant interaction effect (variety x N rate) on tiller number ($p>0.05$; Table 3.6) while the range of RI_{TILL} values was from 0.68 to 1.64 and the number of WKN to attain maximum N response was highly variable across variety and cropping year (Figure 3.6). In 2010, the highest RI_{TILL} was observed as early as 3 WKN for HoCP96-540 but at 6 WKN for both L 99-226 and L 01-283. Different observations were noted in 2012; maximum RI_{TILL} was archived at 6WKN for L 99-226, at 5 WKN for L 99-283, and 4 WKN for HoCP 96-540. The study conducted by Inman-Bamber (1984) also reported this inconsistent response of tiller numbers to N in sugarcane varieties. This indicates the challenge of using RI_{TILL} as a tool for early-season evaluation of cane yield response to N response.

Nitrogen Content

The %N was similar across varieties and N levels but tended to decline as the plant grew except in 2012 (Table 3.7). The highest %N (ranged from 1.33 to 1.73) was observed at 3 WKN and the lowest %N (ranged from 0.59 to 1.43) was at 6 WKN across varieties and N levels in 2010 and 2011. Generally plant N content is highest at the early growth stage and decrease due to a dilution effect caused by growth through cell differentiation (Mistele and Schmidhalter, 2008). Since rapid change in N content is associated with plant growth, it is important to consider the relationship of N content with biomass accumulation. For example, if two plant have the same N content but have different amount in biomass it does not mean the same amount of N to be fertilized. With the greater biomass production of variety L 01-283 and HoCP 96-540 (Table 3.3), it is possible to assume that these varieties have greater N uptake.

The $RI_{N\%}$ value ranged from 0.86 to 1.78 (Figure. 3.7). In 2010, %N in biomass of N fertilized cane did not show large separation from the check plots resulting in $RI_{N\%}$ close to 1. It is simply because the fallow period in cane production allows substantial amount of N turnover from mineralization thus plant cane does not commonly require large amount of N fertilizer when compared with ratoon crop (Wood, 1964; Wiedenfeld 1995; Muchovej and Newman, 2004). In the first (2011) and the second (2012) ratoon crops, increases in $RI_{N\%}$ were more evident especially for variety L 99-226 and L 01-283. Unlike the other variables measured in this study, $RI_{N\%}$ showed an increasing trend over time (WKN). Similar to RI_{BIOM} , in most cases the highest $RI_{N\%}$ was observed at 5 or 6 WKN.

Over all, the dynamic of RI across variety, N treatment and sampling time was detected in biomass, tiller number and N% but not in height and FAI. Generally, the maximum RI was observed at 5 to 6 WKN in biomass and N% which suggests that this could be the optimum

Table 3.3. Analysis of variance for the effect of variety and N rate on dry biomass (kg ha^{-1}) of cane at St. Gabriel, LA 2010-2012.

Treatment	Dry biomass kg ha^{-1}											
	2010				2011				2012			
	3WKN	5WKN	5WKN	7WKN	3WKN	4WKN	5WKN	6WKN	3WKN	4WKN	5WKN	6WKN
Variety												
L 99-226	823a [†]	2317a	2313b	3866b	1033ab	1156ab	1688ab	2156a	3085b	4204b	5713b	7988b
L 01-283	919a	2834a	3061ab	6024a	1194a	1430a	1900a	2333a	5181a	7798a	8411a	13524a
HoCP 96-540	845a	2381a	3188a	5945a	840b	850b	1255b	1438b	2816b	3951b	5077b	8341b
N Rate kg ha^{-1}												
0	834a	2298a	2316a	4651a	965a	998a	1254a	1727a	2723b	4467a	4711b	6628b
45	816a	2782a	2992a	5272a	980a	1088a	1571a	2078a	3285b	4524a	5160b	8685ab
90	790a	2582a	2933a	4961a	1029a	1195a	1789a	2028a	3858ab	5691a	7782a	12238a
135	1009a	2381a	3174a	6228a	1116a	1301a	1842a	2070a	4910a	6589a	8550a	12253a
Variety x N Rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS Not significant at $\alpha=0.05$ level

WKN-number of weeks after N fertilization

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis

*Significant at $\alpha = 0.05$ level

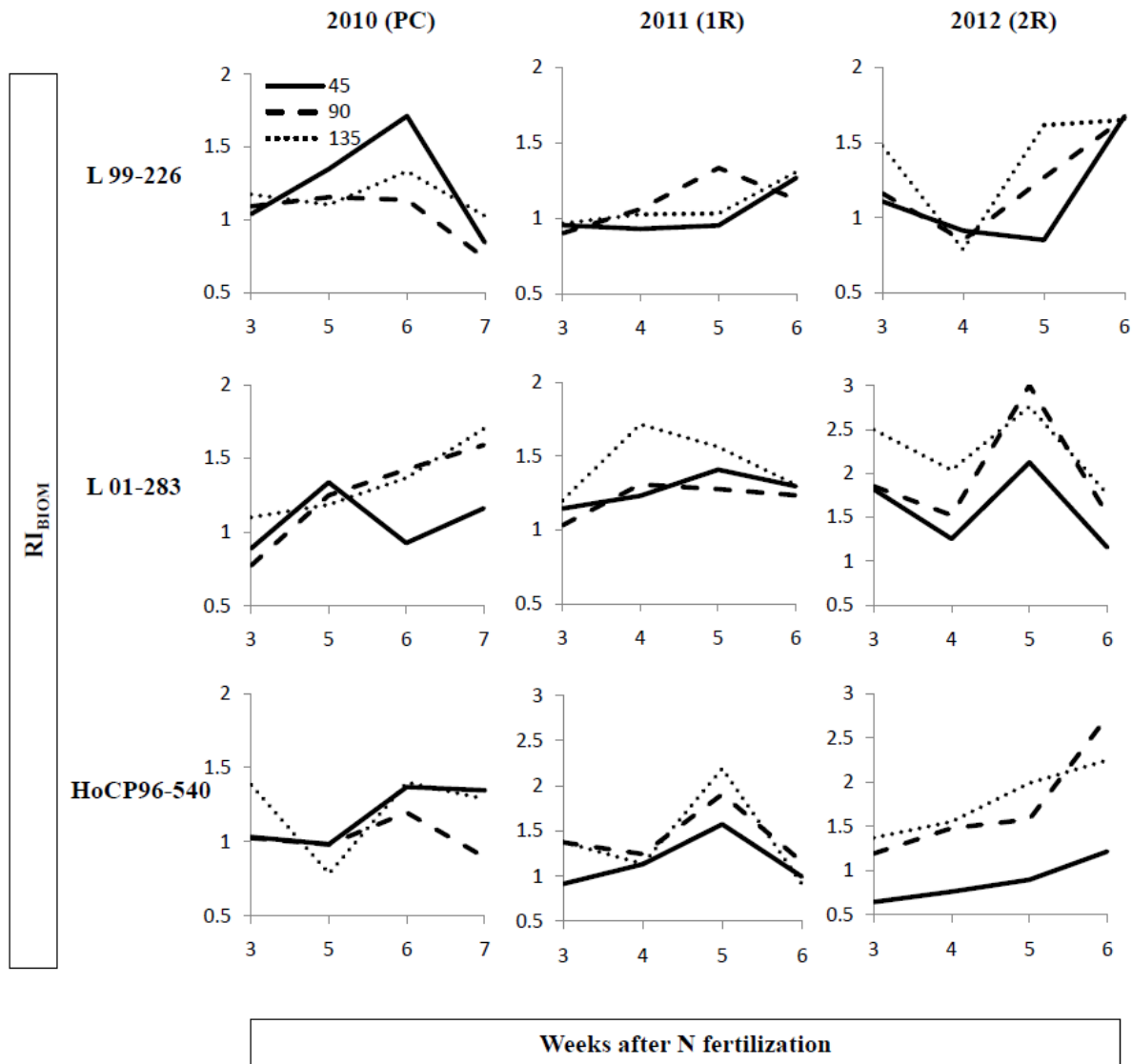


Figure 3.3. Response of biomass to each N rate where 0 kg N ha⁻¹ as base level (Response Index-RI_{BIOM}) for 3 different varieties in St. Gabriel, LA from 2010 to 2012.

Table 3.4. Analysis of variance for the effect of variety and N rate on height (cm) of cane at St. Gabriel, LA 2010-2012.

Treatment	Height cm							
	2011				2012			
	3 WKN	4 WKN	5 WKN	6 WKN	3 WKN	4 WKN	5 WKN	6 WKN
Variety								
L 99-226	59c [†]	65b	74b	89b	61b	62c	80c	92
L 01-283	78a	93a	101a	111a	92a	105a	125a	144
HoCP 96-540	67b	65b	79b	88a	70b	80b	99b	119
N Rate kg ha ⁻¹								
0	68a	73a	85a	95a	68b	82a	94b	109
45	68a	71a	81a	92a	69b	78a	97ab	112
90	68a	80	90a	101a	76ab	82a	103ab	129
135	67a	74a	84a	92a	84a	87a	111a	123
Variety x N Rate	NS	NS	NS	NS	NS	NS	NS	*

NS Not significant at $\alpha=0.05$ level

WKN-number of weeks after N fertilization

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis

*Significant at $\alpha = 0.05$ level

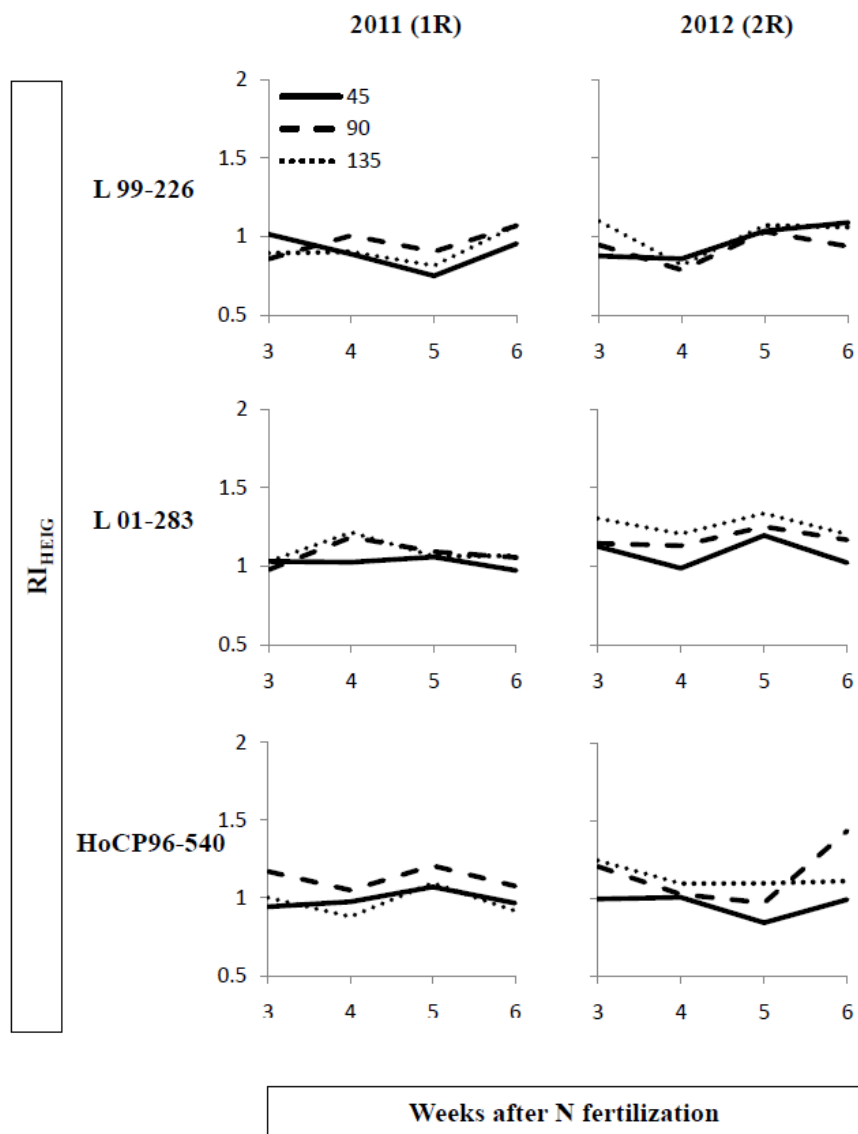


Figure 3.4. Response of plant height to each N rate where 0 kg N ha⁻¹ as base level (Response Index-RI_{HEIG}) for 3 different varieties in St. Gabriel, LA in 2011 and 2012.

Table 3.5. Analysis of variance for the effect of variety and N rate on foliar angle index (FAI) of cane at St. Gabriel, LA 2011-2012.

Treatment	Foliar Angle Index							
	2011				2012			
	3 WKN	4 WKN	5 WKN	6 WKN	3 WKN	4 WKN	5 WKN	6 WKN
Variety								
L99-226	0.31c	0.43c	0.36c	0.40c	0.51c	0.45c	0.45c	0.53c
L01-283	0.63a	0.72a	0.76a	0.78a	0.76a	0.83a	0.81a	0.86a
HoCP96-540	0.46b	0.56b	0.55b	0.65b	0.65b	0.63b	0.69b	0.75b
N Rate kg ha ⁻¹								
0	0.54a	0.57a	0.59a	0.67a	0.62a	0.63a	0.66a	0.73a
45	0.36a	0.57a	0.51a	0.59ab	0.65a	0.68a	0.66a	0.67a
90	0.46a	0.57a	0.55a	0.63ab	0.63a	0.62a	0.64a	0.74a
135	0.46a	0.57a	0.58a	0.55b	0.64a	0.62a	0.65a	0.69a
Variety x N Rate	NS	NS	NS	NS	NS	NS	NS	*

NS Not significant at $\alpha=0.05$ level

WKN-number of weeks after N fertilization

† Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis

*Significant at $\alpha = 0.05$ level

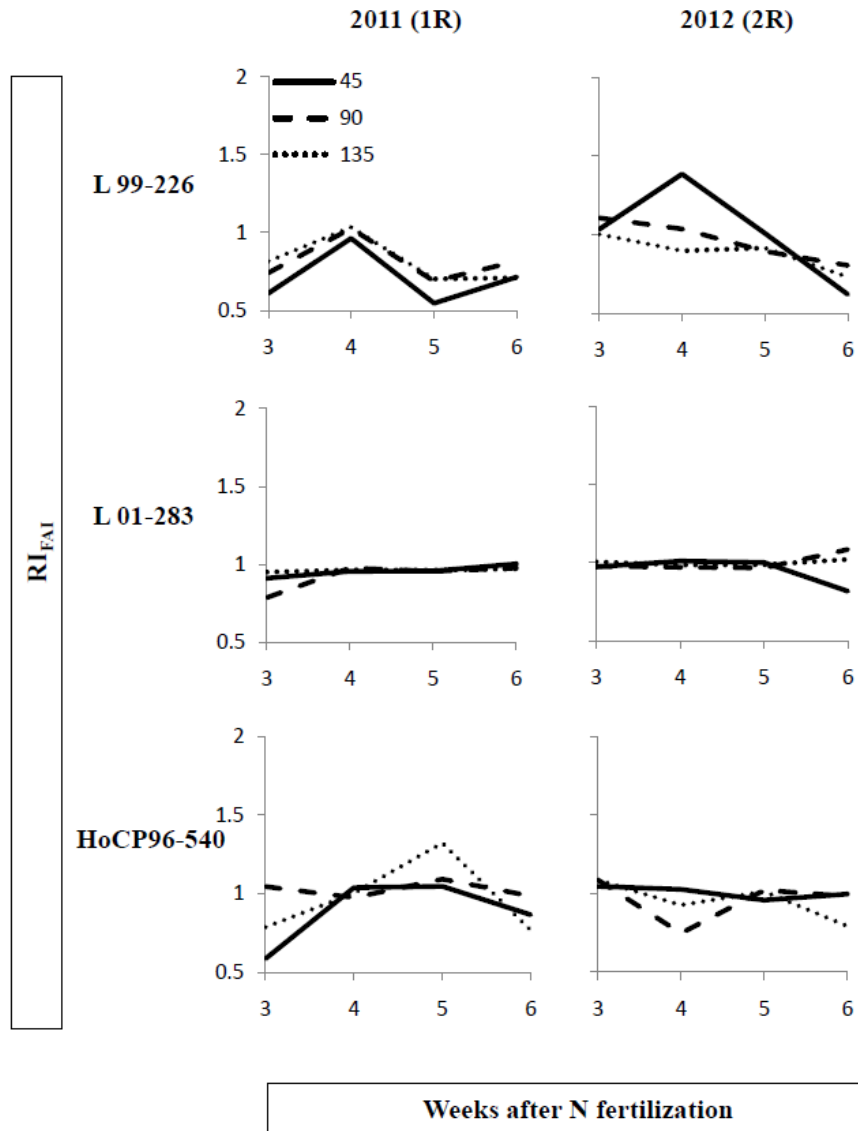


Figure 3.5. Response of foliar angle index (FAI) to each N rate where 0 kg N ha⁻¹ as base level (Response Index-RI_{FAI}) for 3 different varieties in St. Gabriel, LA in 2011 and 2012..

Table 3.6. Analysis of variance table for the effect of variety and N rate on number of tillers (1000 numbers ha⁻¹) of cane at St. Gabriel, LA 2010-2012.

Treatment	1000 tiller number ha ⁻¹											
	2010				2011				2012			
	3WKN	4WKN	5WKN	6WKN	3WKN	4WKN	5WKN	6WKN	3WKN	4WKN	5WKN	6WKN
Variety												
L 99-226	114b [†]	206a	160b	189a	161a	202a	223a	216a	240a	308a	348a	368a
L 01-283	159a	175a	179ab	187a	166a	180ab	209a	182a	287a	319a	282b	290b
HoCP 96-540	140ab	163a	203a	192a	144a	152b	197a	182a	248a	283a	281b	309ab
N Rate kg ha ⁻¹												
0	140a	175a	147b	178a	143a	160b	116b	171a	247a	256b	271a	270b
45	127a	207a	203a	193a	159a	162b	220ab	195a	234a	289ab	278a	323ab
90	128a	179a	177ab	194a	159a	167ab	215ab	198a	264a	344a	341a	353a
135	156a	164a	197a	192a	167a	225a	238a	212a	289a	327ab	325a	343ab
Variety x N Rate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS Not significant at $\alpha=0.05$ level

WKN-number of weeks after N fertilization

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis

*Significant at $\alpha = 0.05$ level

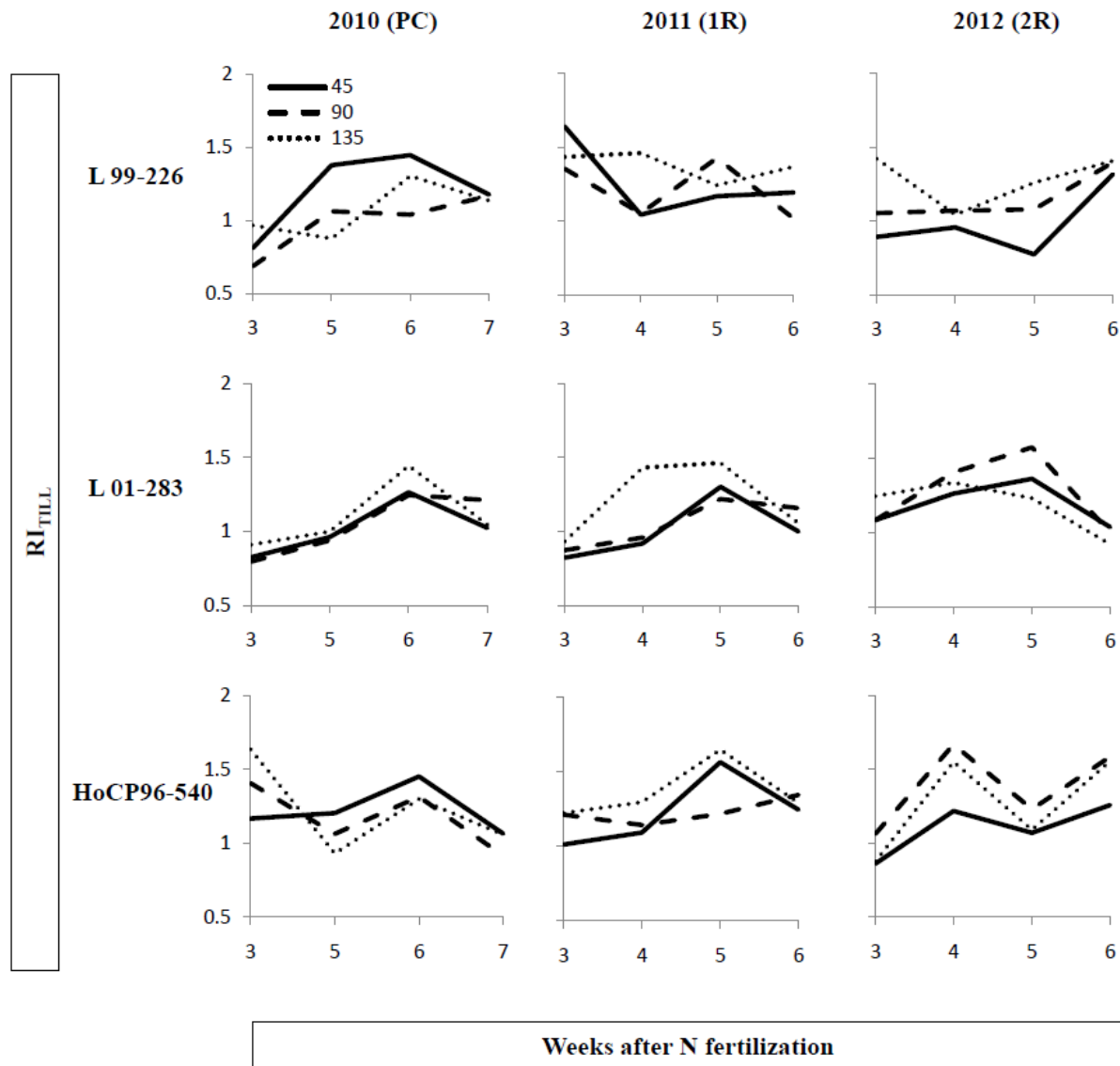


Figure 3.6. Response of tiller number to each N rate where 0 kg N ha⁻¹ as base level (Response Index- RI_{TILL}) for 3 different varieties in St. Gabriel, LA from 2010 to 2012.

Table 3.7. Analysis of variance table for the effect of variety and N rate on N content (%) of cane at St. Gabriel, LA 2010-2012.

Treatment	2010				2011				2012			
	3WKN	4WKN	5WKN	6WKN	3WKN	4WKN	5WKN	6WKN	3WKN	4WKN	5WKN	6WKN
Variety												
L 99-226	1.56b [†]	1.37b	1.49a	1.39a	1.49b	1.44a	1.29a	1.07	1.38a	1.52	1.77a	1.61a
L 01-283	1.72ab	1.54a	1.54a	1.32a	1.59ab	1.57a	1.48a	1.24	1.33a	1.47	1.51a	1.41a
HoCP 96-540	1.76a	1.48a	1.52a	1.34a	1.66a	1.56a	1.41a	1.28	1.51a	1.68	1.70a	1.62a
N Rate kg ha ⁻¹												
0	1.72a	1.34b	1.51a	1.14b	1.33b	1.33b	1.05b	0.89	1.23b	1.19	1.44b	1.13b
45	1.73a	1.49ab	1.43a	1.41ab	1.66a	1.52ab	1.57a	1.23	1.39ab	1.67	1.89a	1.58a
90	1.58a	1.43ab	1.52a	1.43a	1.61a	1.56a	1.46a	1.26	1.46a	1.63	1.59ab	1.73a
135	1.67a	1.60a	1.63a	1.43a	1.71a	1.66a	1.49a	1.41	1.52a	1.73	1.71ab	1.75a
Variety x N Rate	NS	NS	NS	NS	NS	NS	NS	*	NS	*	NS	NS

NS Not significant at $\alpha=0.05$ level

WKN-number of weeks after N fertilization

[†] Same letter within column indicate no significant differences between the treatment means based on the Turkey's post-hoc analysis

*Significant at $\alpha = 0.05$ level

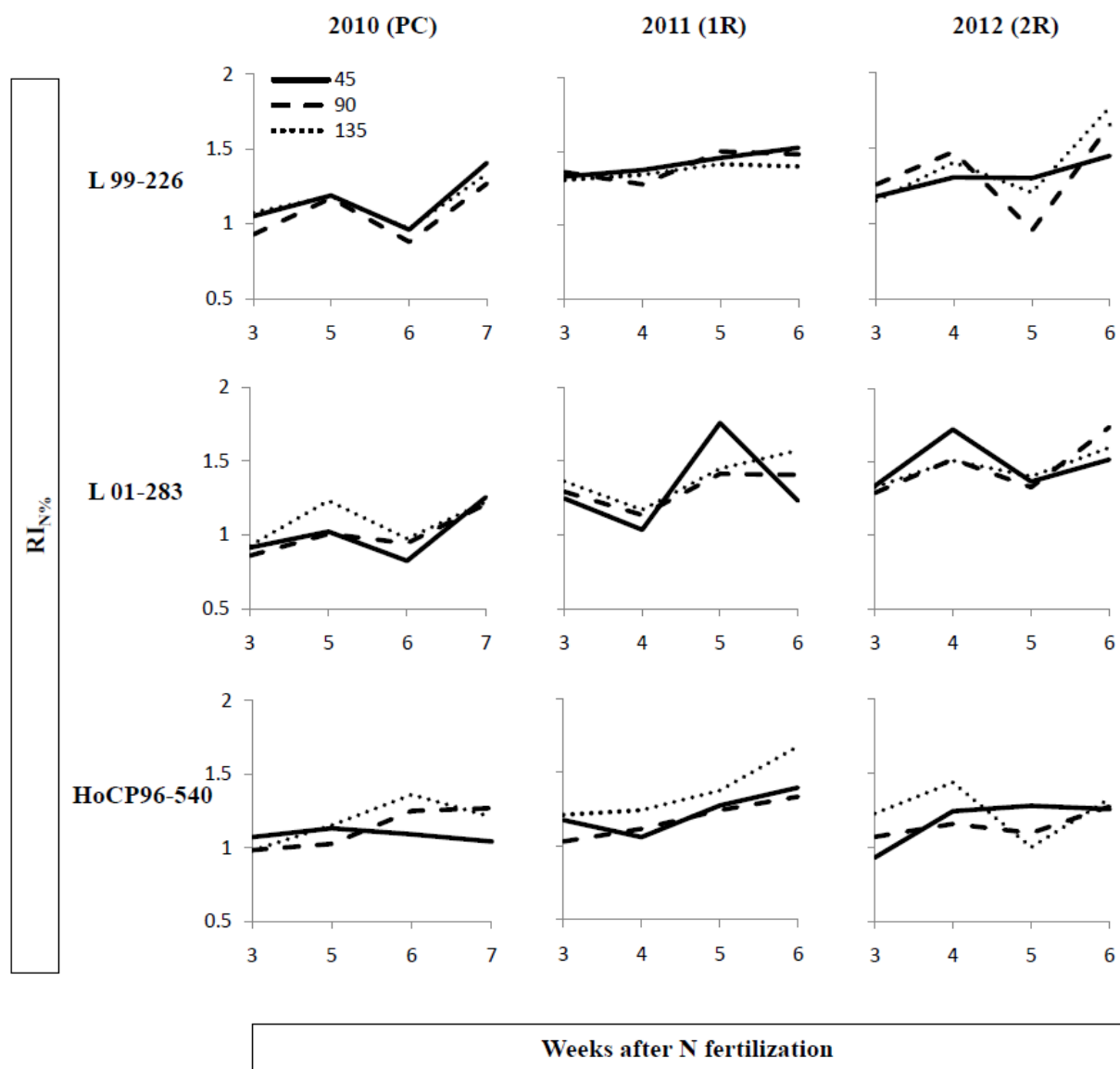


Figure 3.7. Response of N content (%) to each N rate where 0 kg N ha⁻¹ as base level (Response Index-RI_N%) for 3 different varieties in St. Gabriel, LA from 2010 to 2012.

timing of conducting early-season prediction of actual cane yield response to applied N. There was inconsistent pattern of N response across year and variety in tiller number, and low response of height and FAI to N may make difficult to use those parameters for detecting plant N response in the practical field. Nevertheless, this does not infer that these parameters are less useful to estimate actual increases in sugar yield due to N fertilization. The objective of this study was to estimate RI_{SUGAR} using mid season RI based on agronomic parameters. Therefore, simple regression analysis was performed to identify the best model (parameters) to predict RI_{SUGAR} .

3.3.4. Relationship Between RI-Sugar Yield and Agronomic Parameters

The Table 3.8 showed the correlation coefficient table for linear regression analysis to determine the relationship between RI_{SUGAR} and RI of agronomic parameters. The RI_{SUGAR} had the strongest relationship with $RI_{N\%}$; 51 and 58 % of total variation in RI_{SUGAR} were explained by $RI_{N\%}$ at 3 and 6 WKN, respectively. The RI_{BIOM} also showed good relationships with RI_{SUGAR} at 4 and 5 WKN ($r = 0.62$ and 0.68 , respectively). Similarly, RI_{TILL} had the best relationship with RI_{SUGAR} at 4 and 5 WKN. These findings are similar to the outcome of a study conducted by Lofton et al. (2012) which showed that response of normalized difference vegetation index (NDVI) to N measured using handheld remote sensor at 4 to 5 WKN had a strong correlation with the RI of sugar and cane yield.

There was a weak relationship between RI_{HEIG} and RI_{SUGAR} while RI_{FAI} had no relationship with RI_{SUGAR} at all. This made sense since neither height nor FAI showed remarkable response to N compared with other agronomical parameters such as biomass and N content. These results suggest that N response of biomass and %N measured at 4 to 5 WKN can be used to estimate N response of sugar yield at harvest.

Table 3.8. Summary of simple linear regression analysis between response of sugar yield to N and response of mid-season agronomic parameters to N at St. Gabriel, LA 2010- 2012.

Response Index	Time	Number	Mean	Correlation Coefficient	Significance
RI _{BIOM}	3WKN	36	1.15	0.50	***
	4 WKN	36	1.14	0.62	***
	5 WKN	36	1.39	0.68	***
	6 WKN	36	1.27	0.51	***
RI _{TILL}	3WKN	36	1.06	0.14	NS
	4 WKN	36	1.12	0.58	***
	5 WKN	36	1.21	0.57	**
	6 WKN	36	1.14	0.40	***
RI _{N%}	3WKN	36	1.11	0.71	***
	4 WKN	36	1.18	0.62	***
	5 WKN	36	1.17	0.54	***
	6 WKN	36	1.30	0.76	***
RI _{HEIG}	3WKN	24	0.39	0.39	NS
	4 WKN	24	0.42	0.42	*
	5 WKN	24	0.41	0.41	*
	6 WKN	24	0.40	0.40	*
RI _{FAI}	3WKN	24	-0.14	-0.14	NS
	4 WKN	24	0.09	0.09	NS
	5 WKN	24	-0.01	-0.01	NS
	6 WKN	24	-0.10	-0.10	NS

NS Not significant at $\alpha=0.05$ level

* Significant at $\alpha = 0.05$ level

** Significant at $\alpha = 0.01$ level

*** Significant at $\alpha = 0.001$ level

WKN- number of weeks after N fertilization

3.4. Conclusions

In sugarcane production in Louisiana, N fertilizer rate recommendation is established from multiple response trials then further refined base on soil texture and crop age. Earlier works introduced the concept of using early-season sensor readings to estimate cane yield potential and probability of response to N fertilizer as major steps in an attempt to improve N recommendation in sugarcane. The sensor reading expressed in the form of NDVI is related to plant variables such

as biomass and N content. Our study showed that there was significant interaction (N rates x Variety) effect on sugar yield for three years and it indicates the effect of N level on the sugar yield was not consistent across varieties. Also response of sugar yield to N is highly variable and the high N supply does not necessarily lead to high sugar yield and vice versa. The measured agronomic variables at early growth stage, i.e. biomass, tiller number, N content, height, and FAI of three cane varieties and their responses to N fertilizer were also highly variable across years. The N response was evident in biomass, tiller number and %N but not in height and FAI. The maximum N response was observed at 5 to 6 WKN in biomass and %N however the maximum N response in tiller number varied with year and variety. The sugar yield response to N determined at harvest had stronger linear relationships with N response of biomass and N content at 4 to 5 WKN. The findings of this study suggest that variables like biomass and N content can be used to estimate sugar yield N response at harvest and support the previous findings that NDVI can be used to non-destructively acquire biomass N status of cane.

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Chapter 4. Effect of Sugarcane (*Saccharum* spp.hybrid) Varieties on the Relationships Between Spectral Reflectance and Agronomic Parameters

4.1 Introduction

Nitrogen (N) is one of the most important inputs in sugarcane production. In Louisiana, generally a single N application is made in March or April and N rate recommendations ranged from 67 to 135 kg N ha⁻¹ depending on crop age and soil type (Wiedenfeld, 1997; Legendre, 2001; Johnson et al., 2008). However, crop responses to additional N are spatially and temporally variable. Remote sensing technique which has the ability to rapidly examine a large area in the absence of physical contact has a great potential to monitor variable crop status for better N management in sugarcane. The use of this technology in crop N management has been extensively studied in the past few decades for many grain crops while research related to sugarcane is still limited (Raun et al., 1999; Scharf et al., 2002; Simões et al., 2005; Tubana et al., 2011).

Although many researchers reported that crop yield is a function of mid-season biomass and biomass is a function of N content, using N content as a guideline for N management remains questionable because N content is highly variable depending on plant growth stage, age of leaf, and variety (Lemaire and Gastal, 1997; Wells et al., 1989; Gastal and Lemaire, 2002; Farruggia et al., 2004; Wang and Daun, 2004; Harrell et al., 2011). Mistele and Schmidhalter (2008) pointed out that N content is dependent on the amount of plant biomass and suggesting that N content alone does not reflect the overall health status of a crop. Other studies reported that estimating the total N uptake is the key therefore both N content and biomass amount are important information (Lukina et al., 2001; Gastal and Lemaire, 2002; Raun et al., 2002). However, most of spectral studies have focused on estimating plant N or chlorophyll content

(Filella et al, 1995; Gitelson et al., 2002; Xue et al., 2012). Therefore, it is essential to evaluate spectral reflectance over N uptake which is associated with biomass and N accumulation.

The use of normalized difference vegetation index (NDVI) to estimate N concentration, leaf area index, and biomass has been evaluated for many crops (Blackburn, 1998; Carlson and Ripley, 1997; Gitelson et al., 2003). Simple ratio (SR) computed from first derivative reflectance yielded a high r^2 with leaf N concentration of 6-month old sugarcane (Abdel-Rahman et al., 2010). The study conducted by Simões et al. (2005) reported that correlation coefficient of linear relationship between NDVI and sugarcane yield was 0.84. Satellite-based remote sensing technology can also be used to estimate sugarcane yield in fields (Krishina Rao et al., 2002; Bégué et al., 2010). However, a long period of growing season and high biomass production in sugarcane limit satellite-based assessment in obtaining detailed information of cane canopy to associate them with certain spectral signatures. Recent study by Lofton et al. (2012a) showed that NDVI measured three weeks after N fertilization can project the actual increases in cane and sugar yield due to N fertilizer. Moreover, a good relationship was established between sugarcane yield (both cane and sugar yields) and NDVI readings collected between 601-750 cumulative growing degree days (GDD) (Lofton et al., 2012b). The outcomes of these studies also highlighted the possible limitations of remote sensing in sugarcane, i.e., a narrow window of time for sensing and problems associated with NDVI saturation especially for cane varieties with planophile canopies (drooping).

The distinct difference in geometrical structure among sugarcane variety is one of the challenging aspects in identifying spectral signature. Canopy structure may alter the intensity of spectral reflectance due to different sunlight penetration. More than 10 commercial cane varieties are available from LSU Ag-Center and varietal differences exist with respect to yield potential, disease resistance, weed management program, and tillering. Variety HoCP 96-540 shows good

erectness (erectophile canopy) and moderate shading while L99-226 has poor erectness (planophile canopy) and very good shading (Tew et al., 2003; Bischoff et al., 2009). Jackson and Pinter (1986) obtained 20 % of higher reflectance in wheat with planophile canopies (non-erect) where distinct differences arising from the reflectance readings at the green (550 nm) and near infrared (NIR; 800 nm~) bands. Galvão (2005) reported similar result in sugarcane. Johnson et al. (2008) utilized leaf spectral reflectance to discriminate sugarcane varieties associated with plant pigments. With their research, NDVI and weighted NDVI (Gitelson, 2004) resulted in more than 80 % of success rate in discriminating varieties. Using multiple spectral wavebands from EO-1 Hyperion data, Galvão et al. (2005) successfully classified 5 sugarcane varieties with overall classification accuracy at 87.5 %.

The influence of different canopy structure on the relationship of spectral reflectance readings with plant biomass accumulation and N uptake is understudied. In addition, although NDVI has been reported to be useful for non-invasive characterization of sugarcane canopy N health status, the saturation problem associated with canopy closure limits its application and use at the later growth stage of sugarcane. These underscore the need to identify other spectral vegetation indices and parameters to more effectively characterize sugarcane N status. The objectives of our study were 1) to identify the wavebands from which the spectral reflectance readings and vegetation indices should be derived for biomass and N uptake estimation in sugarcane, 2) to determine the window of time where there is high association among N uptake, biomass and spectral reflectance readings and vegetation indices, and 3) to evaluate the effect of different canopy structure on the relationship of spectral reflectance and vegetation index with measured agronomic parameters.

4.2 Materials and Methods

4.2.1 Experimental Design

Data were collected from two Variety x N trials established at the LSU AgCenter Sugar Research Station located in St. Gabriel (30°15'13"N 91°06'05"W) and New Iberia Research Station in Jeanerette (29°54'59"N 91°40'21"W), Louisiana. Soils associated with each experiment were as follows: Canciene silty clay loam (Fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquept) in St. Gabriel and Baldwin silty clay loam (Fine, smectitic, hyperthermic, Chromic Vertic Epiaqualf) in Jeanerette. The experiment consisted of a complete factorial treatment structure between three varieties and four N rates in a randomized complete block design where each treatment was replicated four times. The three varieties consisted of a variety that has an erect-leaf canopy structure, HoCP96-540 (Tew et al., 2003), a variety with a droopy-leaf canopy, L 99-226 (Biscoff et al., 2009), and one intermediate variety, L 01-283 (Gravois et al., 2010). The four N application rates were 0, 45, 90, and 135 kg N ha⁻¹. The plot size was 12 m by 5.4 m containing three rows in St. Gabriel, and 7.2 by 5.4 m in Jeanerette. Bedded rows were opened wherein three whole cane stalks were placed side by side for each run; each run (three cane stalk) were overlapped with the next run of three stalks by 8 cm or including at least two mature internodes. In mid- April, liquid urea-ammonium nitrate (UAN; 32-0-0) was knifed-in near the shoulder of each bed at 15 cm depth. For weed management, metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) and atrazine (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) were applied in early spring based on LSU AgCenter recommendations. The dates of N fertilization and sampling are summarized in Table 4.1

Table 4.1. Schedule of collecting agronomic variables and spectral reflectance at St. Gabriel and Jeanerette in LA from 2010 to 2012.

	2010		2011		2012	
	Plant Cane		1st Ratoon		2nd Ratoon	
	St. Gabriel	Jeanerette	St. Gabriel	Jeanerette	St. Gabriel	Jeanerette
N fertilization	22-Apr	23-Apr	13-Apr	12-Apr	16-Apr	10-Apr
1st sampling	11-May	13-May	9-May	10-May	1-May	3-May
2nd sampling	29-May	28-May	16-May	18-May	7-May	10-May
3rd sampling	3-Jun	11-Jun	23-May	25-May	14-May	17-May
4th sampling	10-Jun	17-Jun	31-May	1-Jun	21-May	24-May

4.2.2 Sampling Area and Data Collection

The following data were collected from a 1 m section row for each sampling time: spectrometer reflectance, plant biomass, N concentration in biomass and plant height (only at St. Gabriel site). Collections of these data were made every week for four consecutive weeks from three weeks after N fertilization (WKN).

The Ocean Optics Jaz spectrometer detects continuous wavebands from 300 nm to 1100 nm with optical resolution at 1.5 nm full width half maximum. Reflectance was measured both at plant canopy and leaf levels. Before plant canopy measurement was done, both incident light (downwelling irradiance) and the outgoing light (upwelling) were determined from a 1 m² white steel plate coated with barium sulfate for correcting environmental noise interference. The distance between the fiber optic sensor and target (white plate or sugarcane canopy) was determined to make sure that the field of view covered a 1 m² area (sampling area size). The distance between the sugarcane canopy and fiber optic sensor was calculated based on the lens' field of view by using trigonometry function. The cosine corrector and Gershun tube with 28 degree field of view was attached to the fiber optic sensor (Ocean Optics, Dunedin, FL). Since the field of view was 28 degree, the height required to cover 1 m² was computed by multiplying

Tangent 14° with length of the adjacent side. Leaf spectral reflectance reading was collected in 2011 and 2012 from St. Gabriel site. The youngest fully expanded leaf was selected from each sub-plot. Several reflectance readings were taken from middle of leaf and then averaged. The light source was a tungsten- halogen lamp and reference reading was done using a diffuse reflectance standard. Dark readings were measured by covering the sensor with a cap and fabric material.

A 1 m long row of sugarcane plants were cut at the base for biomass sampling. Biomass samples were oven-dried at 60°C for 48 hours, weighed, ground and analyzed for total Carbon and N using dry combustion method (LECO Corp., St. Joseph, MI). Then total N uptake (kg/ha) was determined with multiplying the total biomass (kg/ha) by determined N concentration (%).

4.2.3 Spectral Reflectance and Its Indices

Four bands were selected for basic spectra reflectance. Reflectance of 20 nm width was averaged and used as a point value of reflectance.

$$\rho_{\text{green}} = 545 - 565\text{nm}$$

$$\rho_{\text{red}} = 665 - 685\text{nm}$$

$$\rho_{\text{red-edge}} = 715 - 735\text{nm}$$

$$\rho_{\text{near-infrared}} = 775 - 795\text{nm}$$

The following vegetation indices were computed; green simple ratio (SR_{green}), red simple ratio (SR_{red}), red edge simple ratio ($\text{SR}_{\text{rededge}}$), normalized difference vegetation index ($\text{NDVI}_{\text{green}}$, NDVI_{red} , $\text{NDVI}_{\text{rededge}}$), perpendicular vegetation index (PVI_{red} , $\text{PVI}_{\text{red-edge}}$).

$$\text{SR}_{\text{greem}} = \frac{\rho_{\text{near-infrared}}}{\rho_{\text{green}}} \quad (4.1)$$

$$SR_{red} = \frac{\rho_{near-infrared}}{\rho_{red}} \quad (4.2)$$

$$SR_{red-edge} = \frac{\rho_{near-infrared}}{\rho_{red-edge}} \quad (4.3)$$

$$NDVI_{green} = \frac{\rho_{near-infrared} - \rho_{green}}{\rho_{near-infrared} + \rho_{green}} \quad (4.4)$$

$$NDVI_{red} = \frac{\rho_{near-infrared} - \rho_{red}}{\rho_{near-infrared} + \rho_{red}} \quad (4.5)$$

$$NDVI_{rededge} = \frac{\rho_{near-infrared} - \rho_{red-edge}}{\rho_{near-infrared} + \rho_{red-edge}} \quad (4.6)$$

$$PVI_{red} = \sqrt{(\rho_{REDsoil} - \rho_{670})^2 + (\rho_{NIRsoil} - \rho_{780})^2} \quad (4.7)$$

$$PVI_{rededge} = \sqrt{(\rho_{REDsoil} - \rho_{730})^2 + (\rho_{NIRsoil} - \rho_{780})^2} \quad (4.8)$$

With the concept of derivative analysis, red-edge points (REP) were also determined by using linear extrapolation technique. The linear extrapolation technique was developed by Cho and Skidmore (2006). This method eliminates the problem from the double-peak which can be observed in high N treated plant or when chlorophyll concentration is high using the first derivative analysis. The two straight lines, one from near infrared and other from red points, were computed based on the first derivative reflectance and the intersection of those straight lines was considered as REP.

$$REP_{LE} = \frac{-(b_1 - b_2)}{(a_1 - a_2)} \quad (4.9)$$

where near infrared line: 1st derivative reflectance $\rho_{near-infrared} = a_1\lambda + b_1$

red line: 1st derivative reflectance $\rho_{red} = a_2\lambda + b_2$

To determine the near infrared lines, 725 and 750 nm were selected while lines 680 and 700 nm were selected for red line.

4.2.4 Data Analysis

Statistical analysis was performed using SAS 9.2. (SAS Institute, 2009) and R (R Developing Core Team, 2008). Principal component analysis was performed to identify varietal differences in leaf spectral reflectance. Analysis of covariance (ANCOVA) and regression analysis were performed with PROC REG procedure at each sampling period. The regression analysis was performed to determine the relationship between spectra indices and agronomic variables; biomass, N uptake and plant height.

4.3 Results

Table 4.2. The mean of biomass and N uptake (kg ha^{-1}) at St Gabriel and Jeanerete in LA from 2010 to 2012.

Year	Biomass (kg ha^{-1})		N uptake (kg ha^{-1})	
	St. Gabriel	Jeanerette	St. Gabriel	Jeanerette
2010 (PC)	2879	1841	41.23	35.45
2011 (1R)	1435	3032	19.77	35.46
2012 (2R)	6341	7281	82.5	98.61

4.3.1 Climatic Conditions

The cumulative precipitation and CGDD from 2010 to 2012 are summarized in Figure 4.1. In all three years, CGDD was higher in Jeanerette than in St. Gabriel. At 6 WKN which was the last sampling period, there was 50 to 100 units' difference in CGDD between Jeanerette and St. Gabriel and differences was evident in 2010 and 2012 but not in 2011. It was only 2012 where CGDD exceeded 200 at the week of N fertilization. The high CGDD could potentially produce more biomass. In fact, the mean biomass production across sampling periods in Jeanerette tended to be higher than in St. Gabriel (Table 4.2). Total precipitation was higher in

St. Gabriel compared to Jeanerette for all three years. The 2011 cropping was generally dry and little to no rain was received between 3WKN to 6WKN at both sites. This limited amount of precipitation reduced biomass accumulation and N uptake in St. Gabriel (Table 4.2).

4.3.2 Varietal Differences on Leaf Spectral Reflectance

Multivariate analysis is a useful technique to evaluate the effect of variables on numerous response variables of interest. Principal component analysis (PCA) is one of multivariate analysis and its goal is to reduce the dimension of data by forming principal components (PC) which in this case is a linear combination of spectral reflectance for each observation in the study. These PC are orthogonal (uncorrelated) to each other and first PC accounts for the largest variability. Absolute value of eigenvectors for each wavelength can indicate the relative weight of linear combination to establish PC. By using this technique, it is important to investigate whether there is a different behavior of leaf spectral reflectance among varieties.

In this study, only PC1 and PC2 were evaluated since these two components accounted for more than 95 % of total variability in all measurements. Figure 4.2 and 4.3 show the results of PCA in 2011 and 2012. There was no clear cluster by varieties observed; the principal components were similar at 3 WKN in 2011 and across all sampling periods in 2012. This result indicates that there were no differences in leaf spectral reflectance among varieties. On the other hand, slight differences were observed at 4, 5, and 6 WKN in 2011 (Figures 4.2. b, c, and d). Variety L01-283 and HoCP 96-540 tended to have low PC2 scores compared with L 99-226 at 4 WKN. The high absolute score of eigenvectors at 550 and 700-750 nm explains their influences on PC2; which further implies that reflectance at green and red-edge bands is different

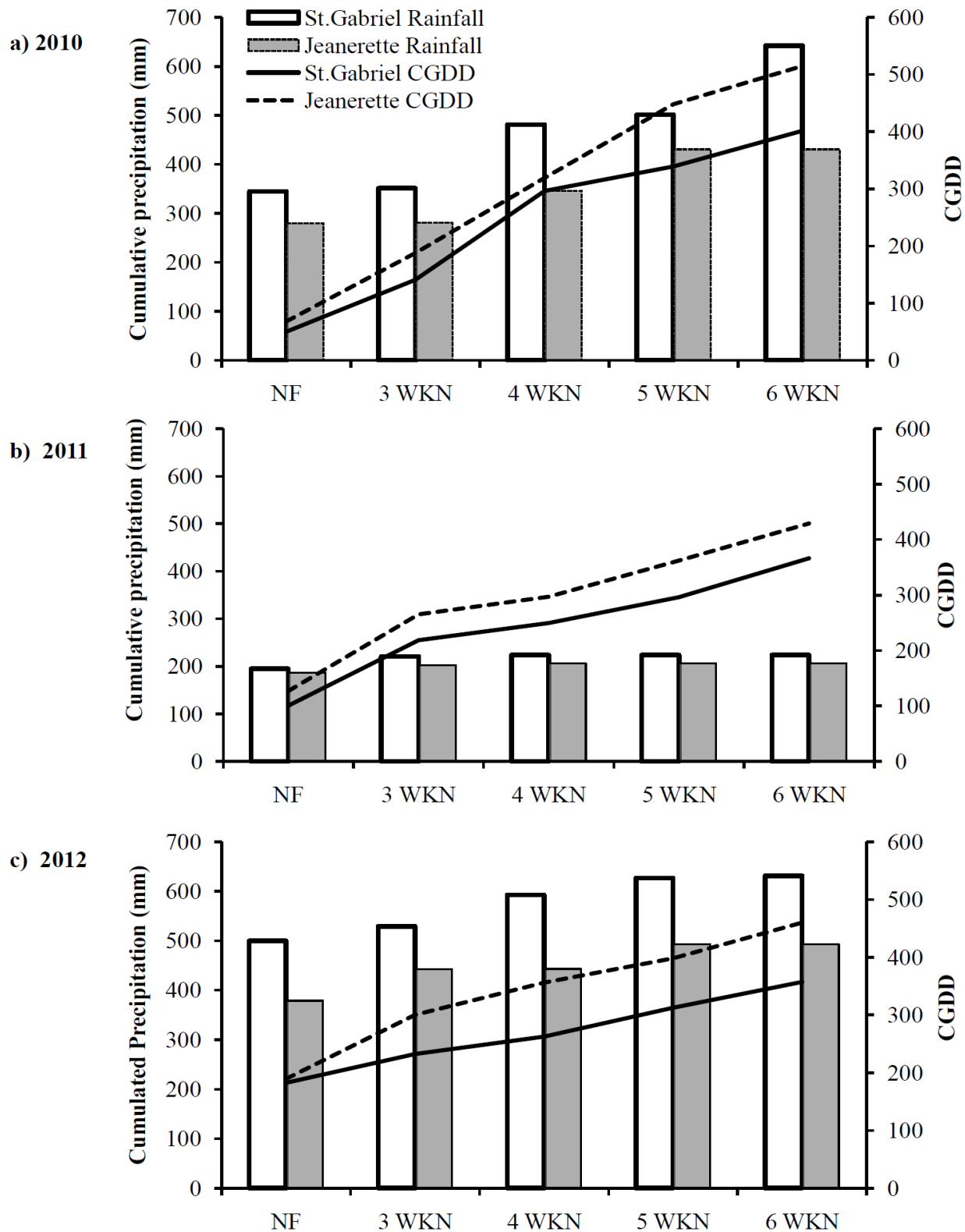


Figure 4.1. Cumulative precipitation and cumulative growing degree days (CGDD) from the beginning of year to nitrogen fertilization (FN), 3 , 4, 5 and 6 weeks after N fertilization (WKN) in St. Gabriel and Jeanerette in LA from 2010 (a) to 2012(c).

among varieties (Figure 4.4 a). These visible wavebands are related with chlorophyll contents and highly dependent on plant N status and leaf chemical compositions of different sugarcane varieties (Carter and Spiering, 2002; Johnson et al., 2008; Zhao et al., 2012). At 5 WKN, variety L 99-226 had lower PC1 and higher PC2 compared with the other two varieties (Figure 4.2). Since the absolute value of eigenvector was high between 700 to 900 nm in PC1, the varietal difference can be associated with red and near-infrared bands (Figure 4.4 a). Generally near-infrared is associated with leaf structures such as mesophyll layer or pore spaces (Gitelson et al., 2002).

The distinct varietal differences were not observed from leaf spectral reflectance based on PCA in our study while some studies showed the success of discriminating varieties using leaf spectral reflectance. Johnson et al. (2008) reported spectral reflectance from 560 to 720 nm were useful to discriminate 7 varieties; using discriminant analysis, 95-100 % correct classification was obtained. Study conducted by Zhao et al. (2012) reported that significant differences of leaf reflectance among varieties were observed from May to October. Similar to our result, their study showed that green and red-edge wavebands had strong association with high genotypic variation in leaf spectral reflectance. In the present study, PCA was performed to reduce spectral data dimension to explain maximum variability among varieties in response to different level of N supply but not to select wavebands to discriminate varieties. In addition, the influence of N supply on chlorophyll content and leaf elements was probably stronger than varietal effect thus unlike the outcomes of earlier studies, clear differences in leaf spectral reflectance among varieties were not observable in this study.

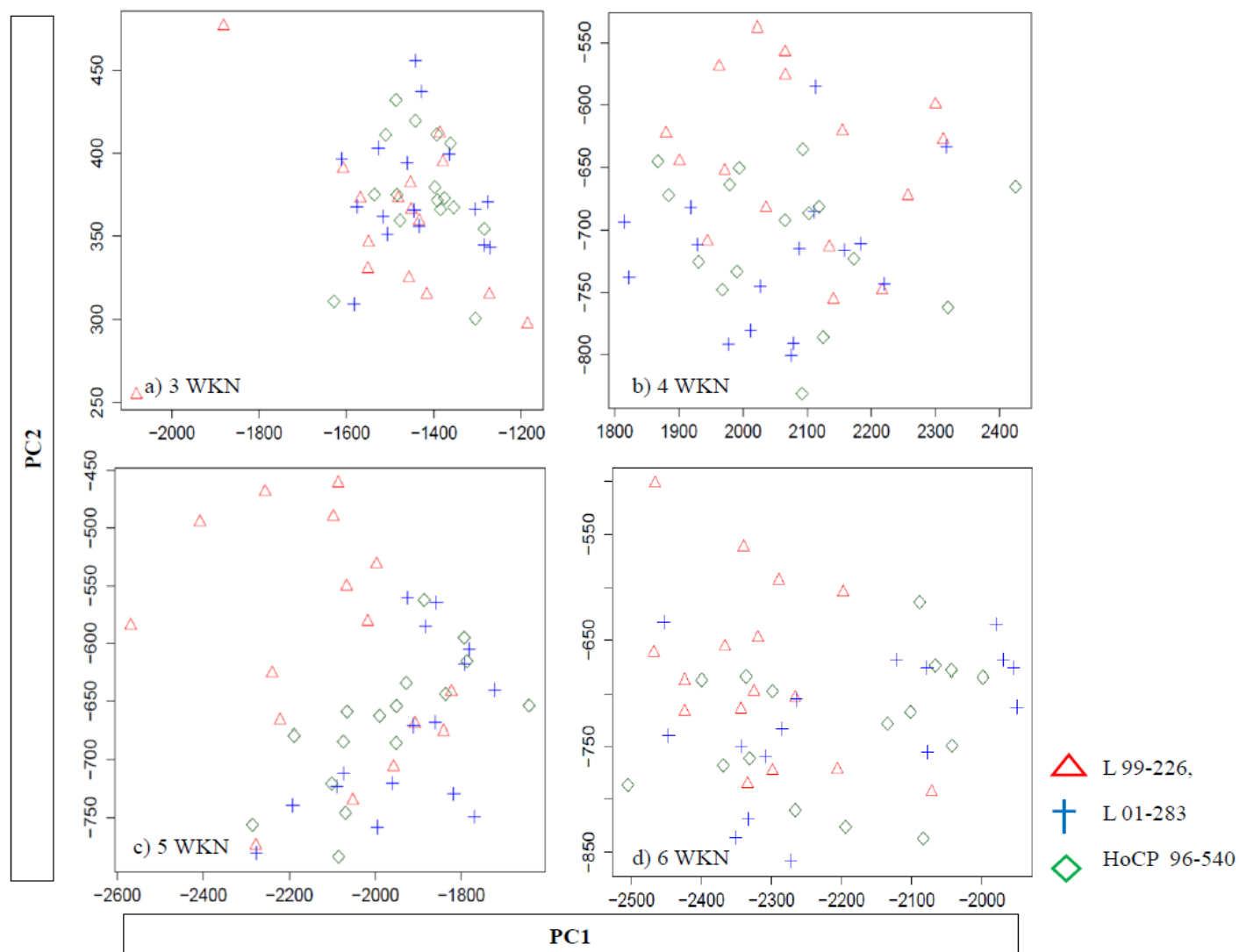


Figure 4.2. Principal component (PC) score for variety L 99-226, L 01-283, and HoCP 96-540 computed from leaf spectral reflectance at 3, 4, 5 and 6 weeks after N fertilization (WKN) at St. Gabriel in LA in 2011.

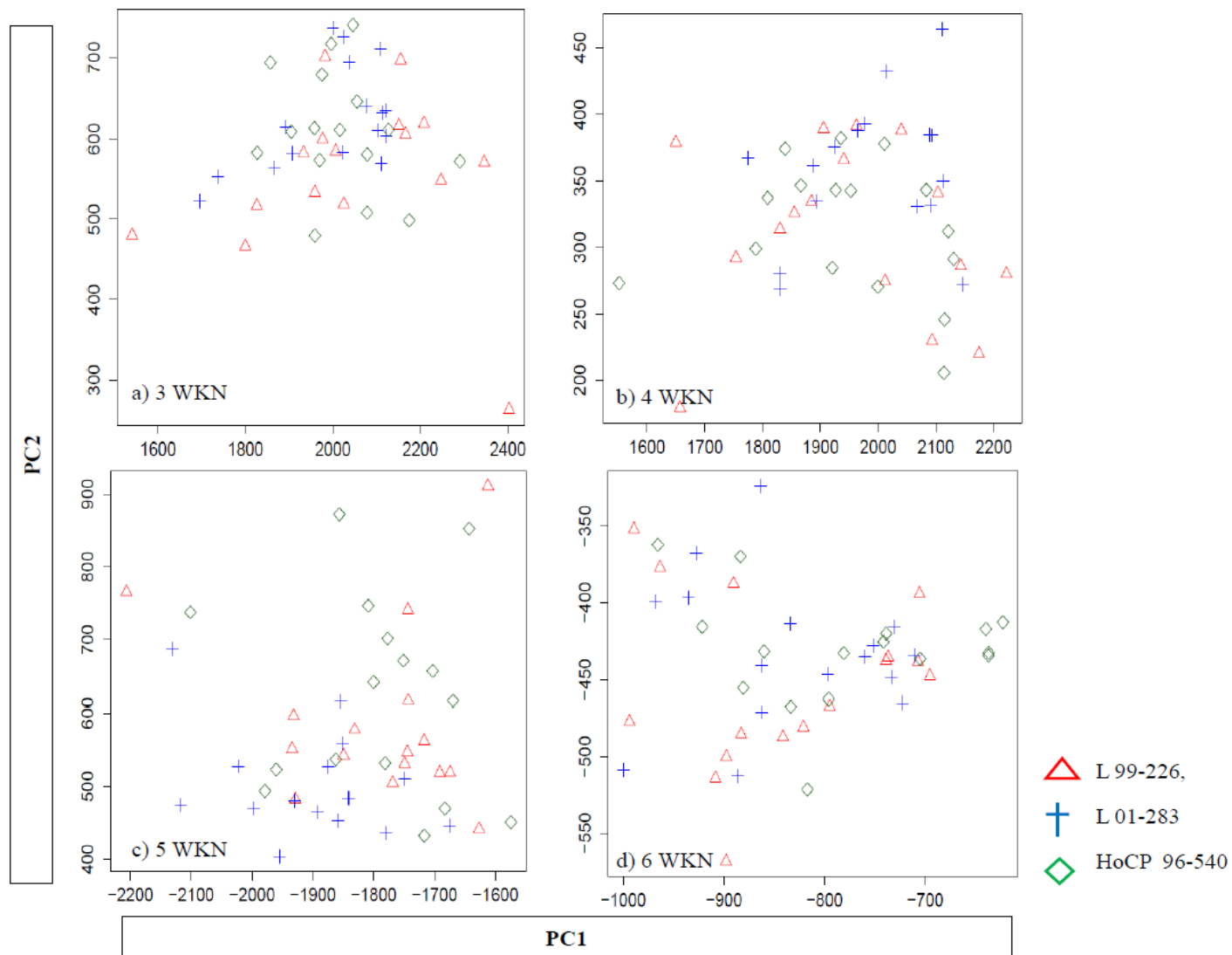


Figure 4.3. Principal component (PC) score for variety L 99-226, L 01-283, and HoCP 96-540 computed from leaf spectral reflectance at 3, 4, 5 and 6 weeks after N fertilization (WKN) at St. Gabriel, LA in 2012.

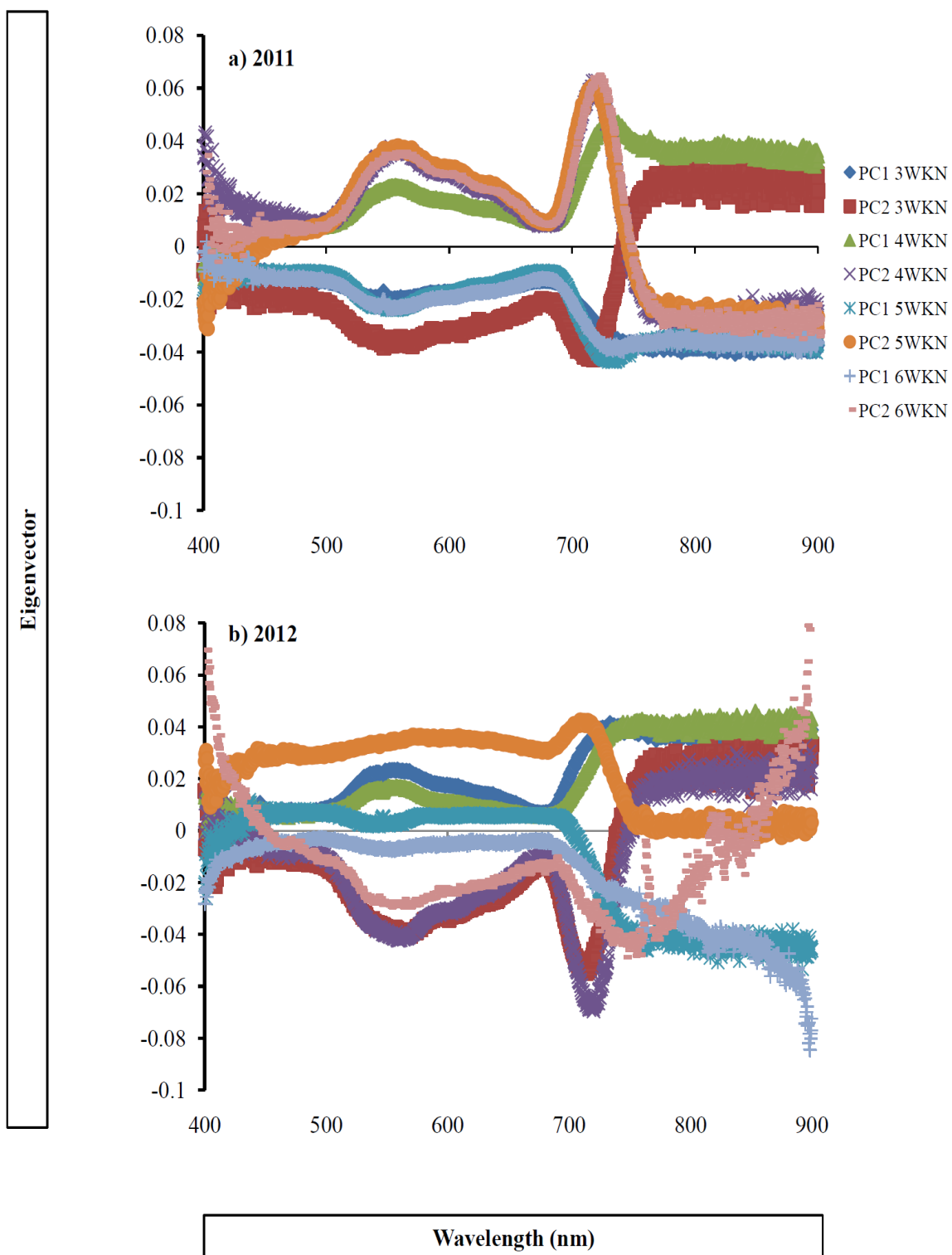


Figure 4.4. Eigenvectors of principal component (PC) 1 and 2 at 3, 4, 5, and 6 weeks after N fertilization (WKN) in 2011 (a) and 2012 (b).

4.3.3 Effect of Variety on the Relationship Between Agronomic Parameters (Biomass and N Concentrations) and Spectral Reflectance

Figure 4.5, 4.7, 4.9, 4.11, 4.13, and 4.15 show correlation coefficients determined by linear regression between spectral reflectance and agronomic parameters (biomass and N uptake) from 2010 to 2012 for each sampling period. Figure 4.6, 4.8, 4.10, 4.12, 4.14, and 4.16 show the coefficients of variety on the relationship between spectral reflectance and agronomic parameters. The middle red line indicates coefficients of variety on the relationship between spectral reflectance and agronomic parameters. The upper and bottom blue lines show the 95 % confidence interval for the coefficients. If the 95 % confidence interval includes 0 line (straight solid line), it means that there is no significant effect of variety on a relationship between spectral reflectance and agronomic parameters.

Biomass

Within visible wavebands, spectral reflectance at between 450 to 500 nm and between 650 to 700, showed higher negative correlation coefficient across sampling periods (Figure 4.5, 4.7, and 4.9). The relationships between biomass and spectral reflectance at these wavebands tended to decrease as the cane grew. At 3WKN, correlation coefficients ranged from -0.5 to -0.6 at the blue waveband and from -0.4 to -0.6 at the red band but at the later sampling time (6 WKN), correlation coefficients ranged from -0.2 to 0 and from -0.2 to -0.4 at the blue and red bands, respectively. These results are consistent with the findings of Sims and Gamon (2002). According to these authors, visible wavebands which has high association with plant pigments saturates at relatively low chlorophyll contents. In the present study, perhaps chlorophyll content did not substantially change during the sampling periods but the amount of accumulated biomass was high enough to absorb most of the light at the red band which ultimately decreased the sensitivity of the reflectance to estimate biomass. The effect of varieties on the relationship

between biomass and spectral reflectance within the visible wavelength (400 to 700 nm) were observed in 2011 but not in 2010 and 2012 (Figure 4.6, 4.8 and 4.10). At early sampling, there were only specific bands i.e., purple (400 to 450 nm), between blue and green (500-550 nm), and red-edge position (720-750 nm), that were affected by variety. However at later sampling time the varietal effect was not selective on specific bands. In other words, the coefficients of variety on the relationship between spectral reflectance and biomass were constant within the visible wavelength at 6 WKN (Figure 4.8. c and d). Although it was not significant, the results from 2012 suggest that variety has potential effect on the relationship between spectral reflectance and biomass (Figure 4.10).

It is suspected that the effect of variety was significant only in 2011 because of the drier than normal growing condition. At this given condition, the significant differences on the relationship between spectral reflectance and biomass among varieties may have come from their differences to withstand stress due to lack of moisture. Based on Jackson (2010), L01-283 is variety that performs better in heavy-textured soil while L99-226 and HoCP 96-540 prefers light-textured soil type. Based on this fact, it can be assumed that L99-226 and HoCP 96-540 had better resistance for moisture stress since it can grow at light-textured soil. Data presented in Chapter 3 Figure 3.3 shows that HoCP 96-540 had substantially high biomass responses to N in 2011 compared with the other two varieties. Also data presented in the Chapter 3 Table 2.5. shows varietal difference in foliar angle index (FAI). The FAI is a good parameter to discriminate plant canopy structure among varieties. The difference in FAI values among varieties was more evident in 2011 than in the other two years. Those differences in canopy structure and N response can result in significant varietal differences on spectral reflectance readings.

Reflectance at near-infrared wavebands had positive relationship with biomass across years and sampling periods (Figure 4.6, 4.8, and 4.10). Normally, near-infrared refers to a wide range of wavelength, 750 to 2500 nm, but band between 780 to 830 has shown to have a good relationship with biomass (Dorigo et al., 2007; Hansen and Schioerring, 2003). Similar result was obtained in our study; 750 to 800 nm showed better relation with biomass compared with other near infrared wavebands. Unlike visible wavelength, a relatively constant relationship between biomass and reflectance at near-infrared was observed across sampling periods; the correlation coefficients ranged between 0.2 to 0.4 at 3, 4, and 5 WKN, and the highest correlation coefficients ranged from 0.5 to 0.6 at 6 WKN. Clear effect of variety on the relationship between reflectance at near-infrared band and biomass was only observed at 3 and 5 WKN in 2011 (Figure 4.14. c).

Nitrogen Uptake

Reflectance at visible wavebands had strong negative relationship with N uptake across sampling periods (Figure 4.11, 4.13, and 4.15). The correlation coefficients ranged from -0.2 to -0.6 and most of the time, its value remained at -0.6. The N uptake showed constant correlation coefficients with reflectance readings at purple (400 nm), blue (450 nm), red (700 nm), and red-edge (700 to 750 nm) wavebands across sampling periods. Varietal effects on the relationships between N uptake and reflectance at visible wavebands were similar to biomass; it was only significant in 2011 at purple, blue, and red-edge bands (Figure 4.12, 4.14, and 4.16) but became more evident as the cane grew (4.14.d).

Reflectance at near-infrared band had a weak positive relationship with N uptake (Figure 4.11, 4.13, and 4.15). Except at 4 WKN in 2012, correlation coefficients were less than 0.6; it only ranged between 0.2 and 0.4 for the majority of sampling periods. This result is expected since the majority of N in leaf is incorporated in chlorophyll which is responsible for providing

the unique signatures in the visual wavebands (400 to 700 nm) but not in the near-infrared (>750 nm) (Yoder and

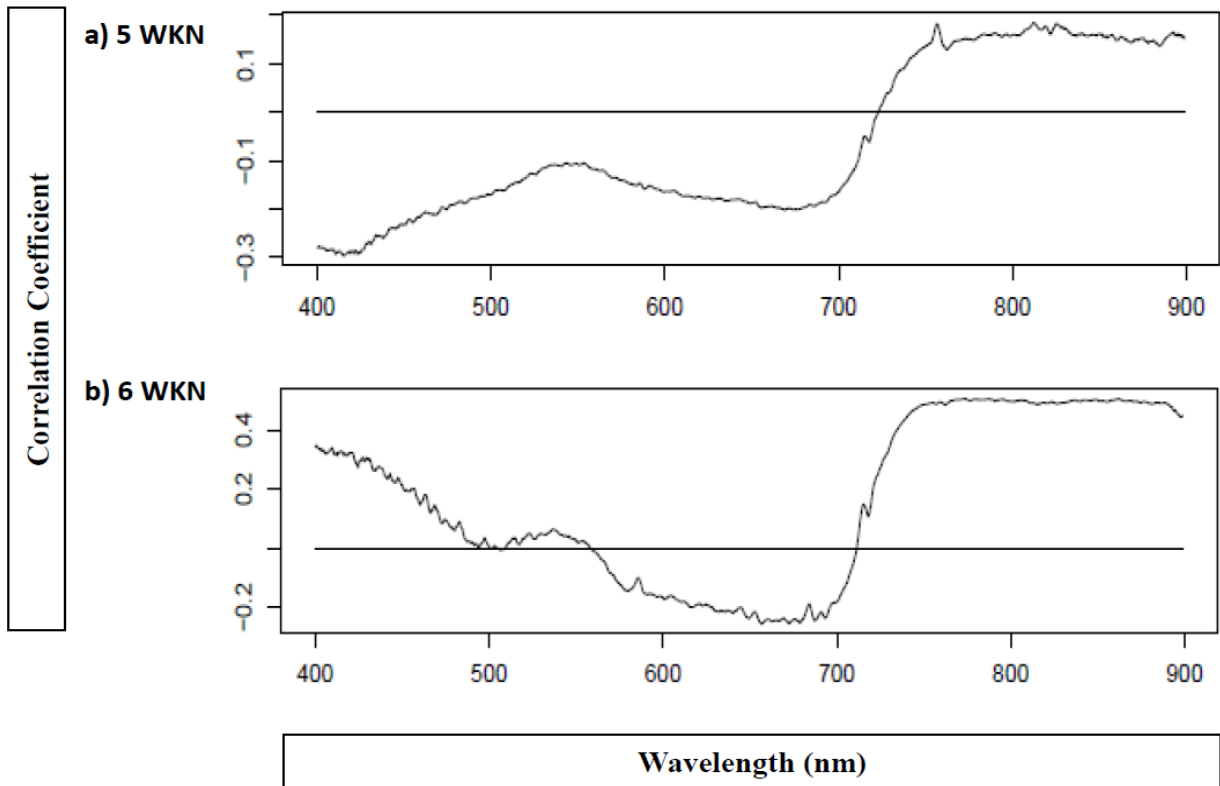


Figure 4.5. Correlation coefficients for the linear relationship between spectral reflectance and biomass at 5 (a) and 6 (b) week after N fertilization (WKN) in 2010.

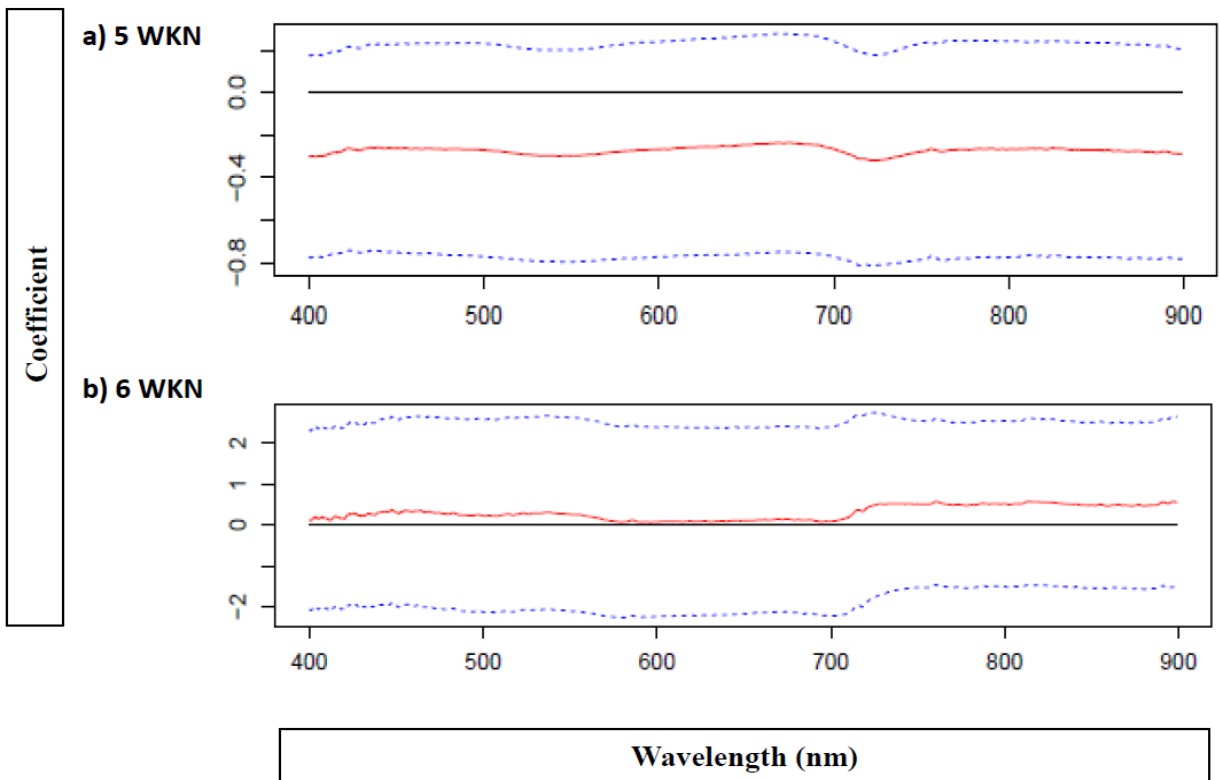


Figure 4.6. Coefficients of variety on the linear relationship between spectral reflectance and biomass at 5 (a) and 6 (b) week after N fertilization (WKN) in 2010.

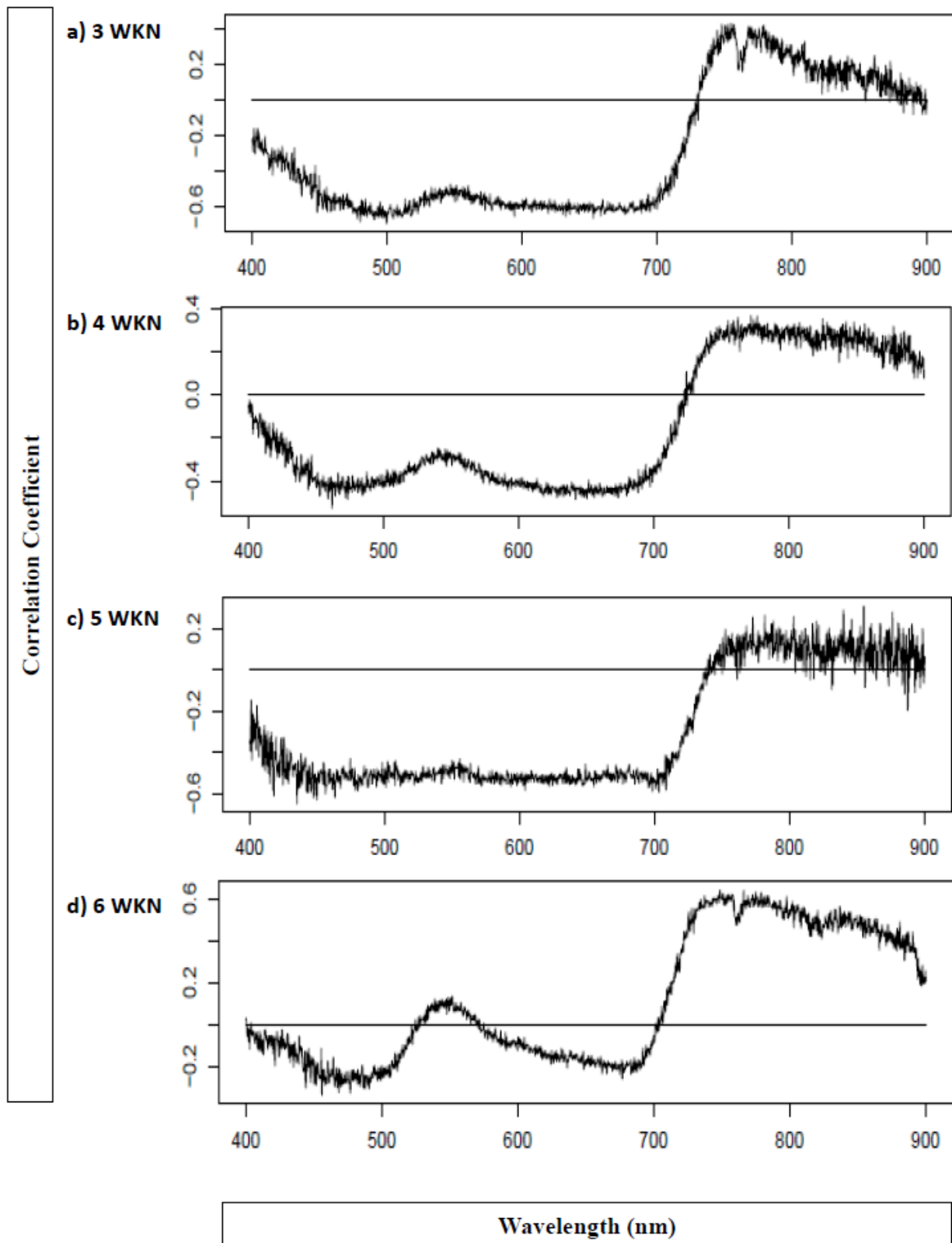


Figure 4.7. Correlation coefficients for the linear relationship between spectral reflectance and biomass at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2011.

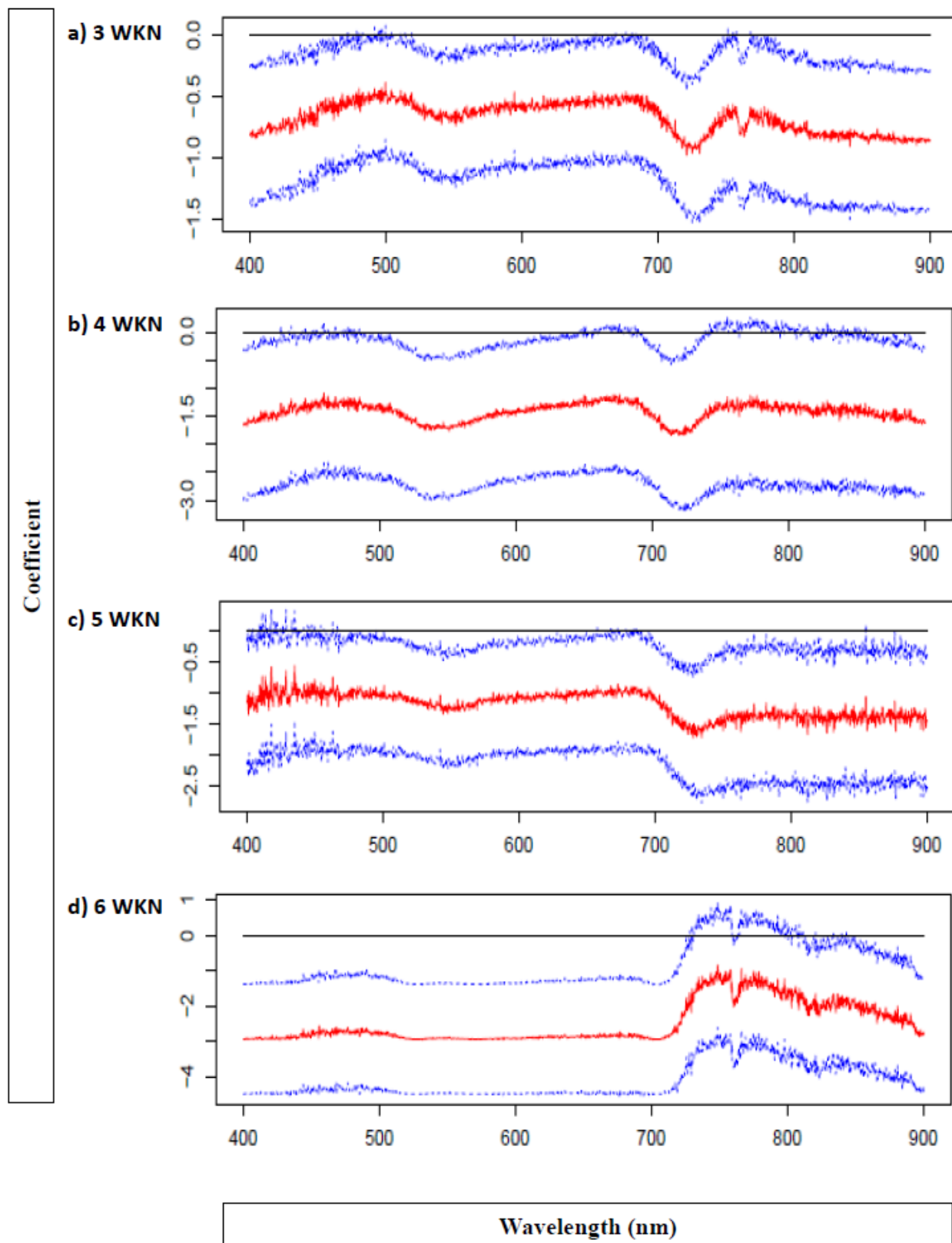


Figure 4.8. Coefficients of variety on the linear relationship between spectral reflectance and biomass at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2011.

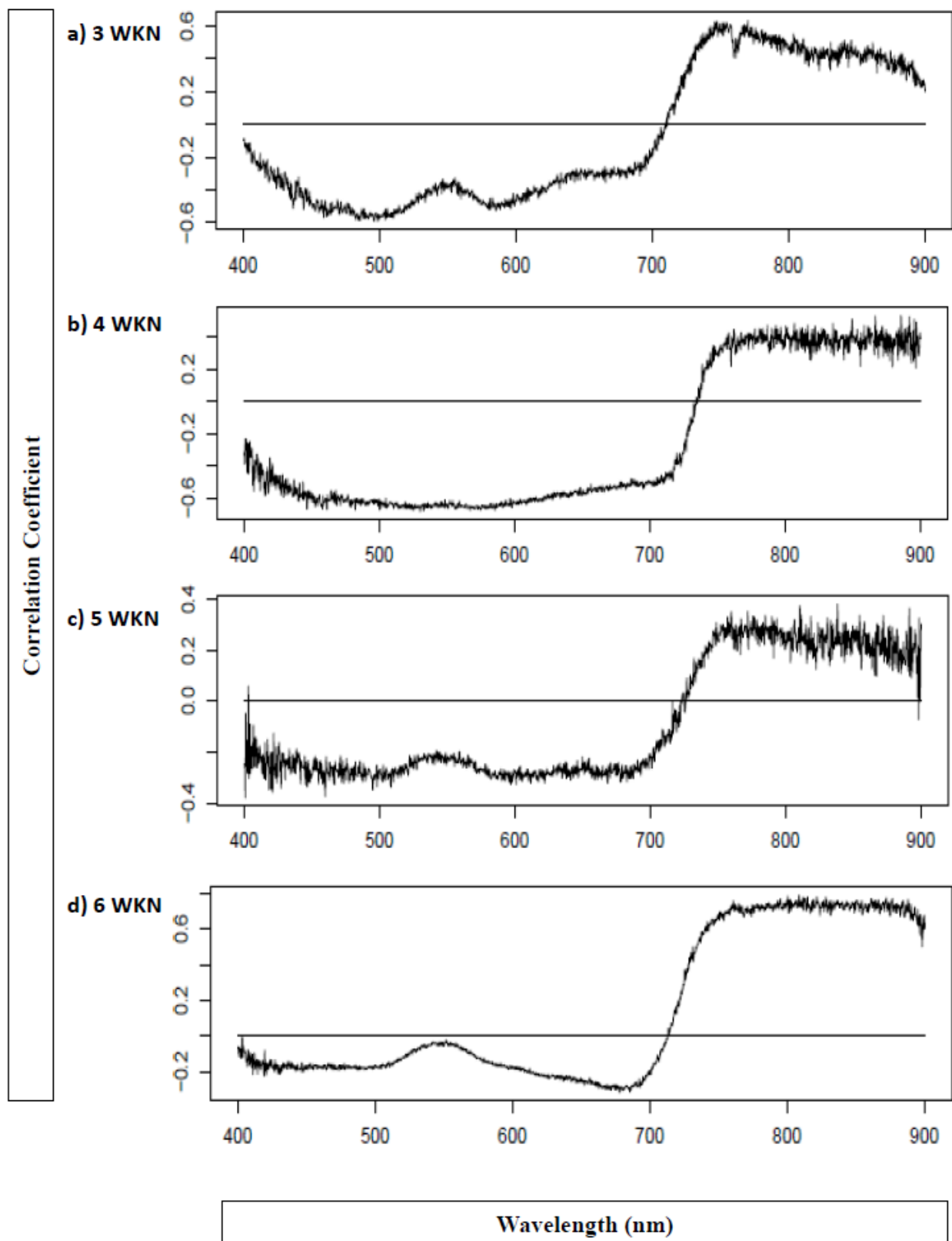


Figure 4.9. Correlation coefficients for the linear relationship between spectral reflectance and biomass at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2012.

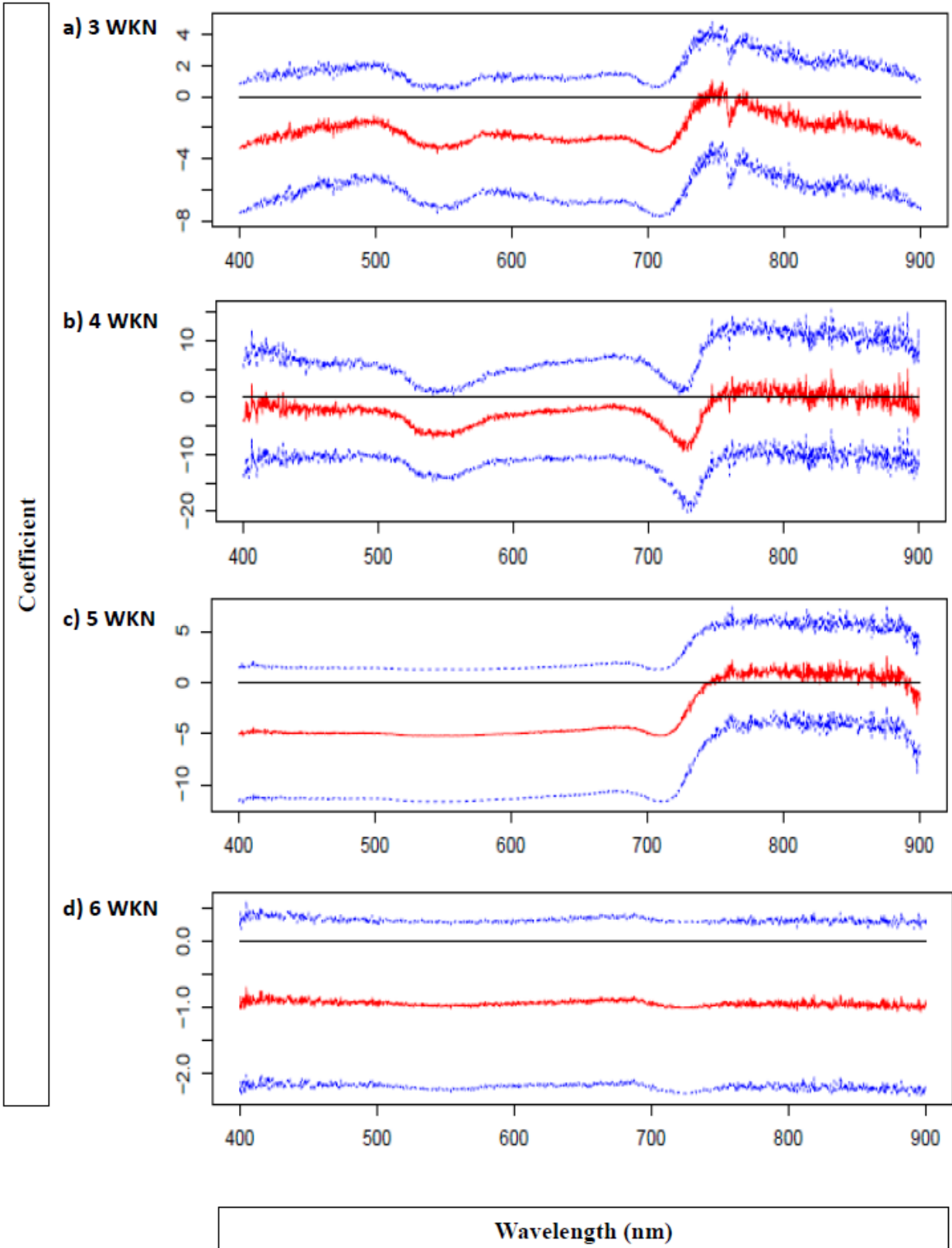


Figure 4.10. Coefficients of variety on the linear relationship between spectral reflectance and biomass at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2012.

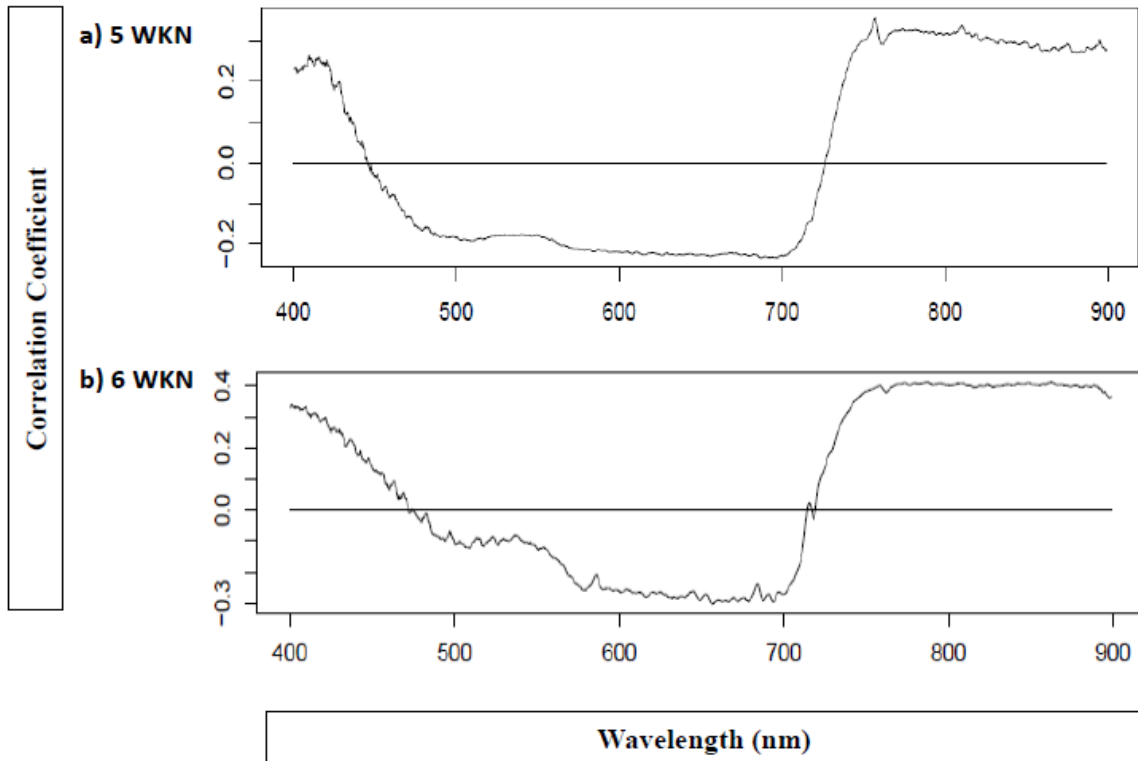


Figure 4.11. Correlation coefficients for the linear relationship between spectral reflectance and N uptake at 5 (a) and 6 (b) week after N fertilization (WKN) in 2010.

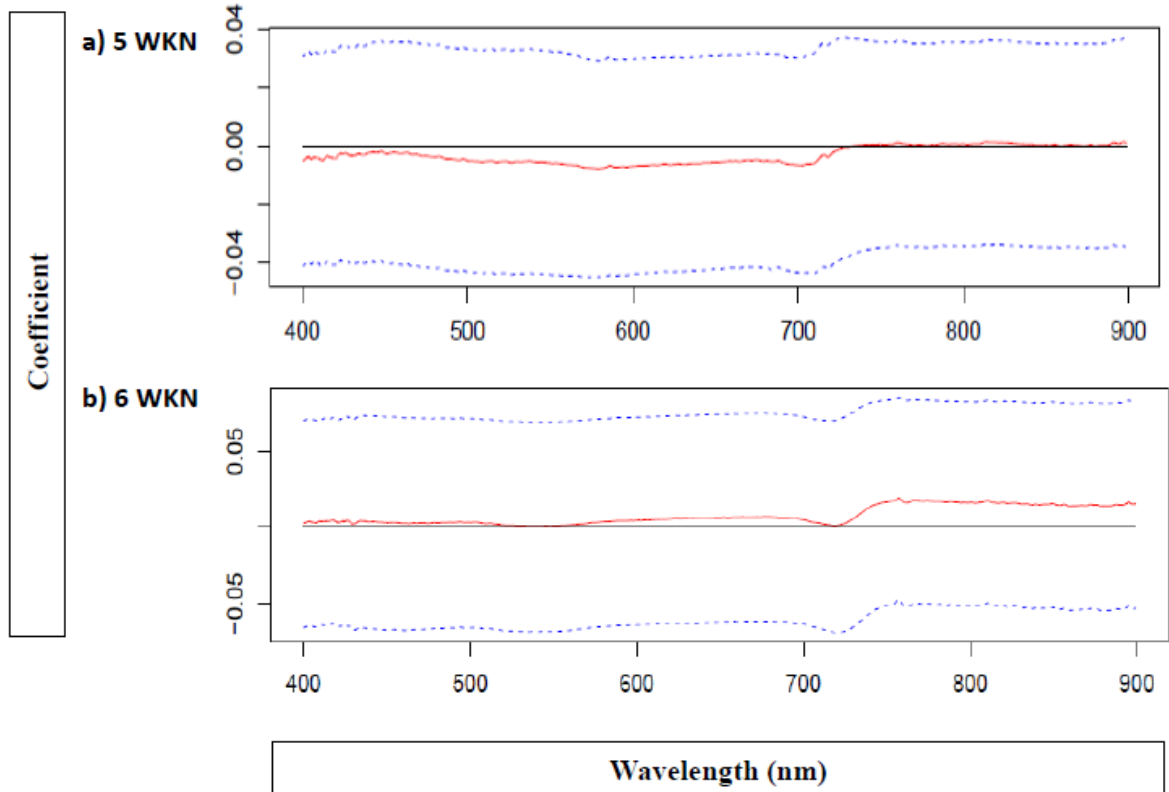


Figure 4.12 Coefficients of variety on the linear relationship between spectral reflectance and N uptake at 5 (a) and 6 (b) week after N fertilization (WKN) in 2010.

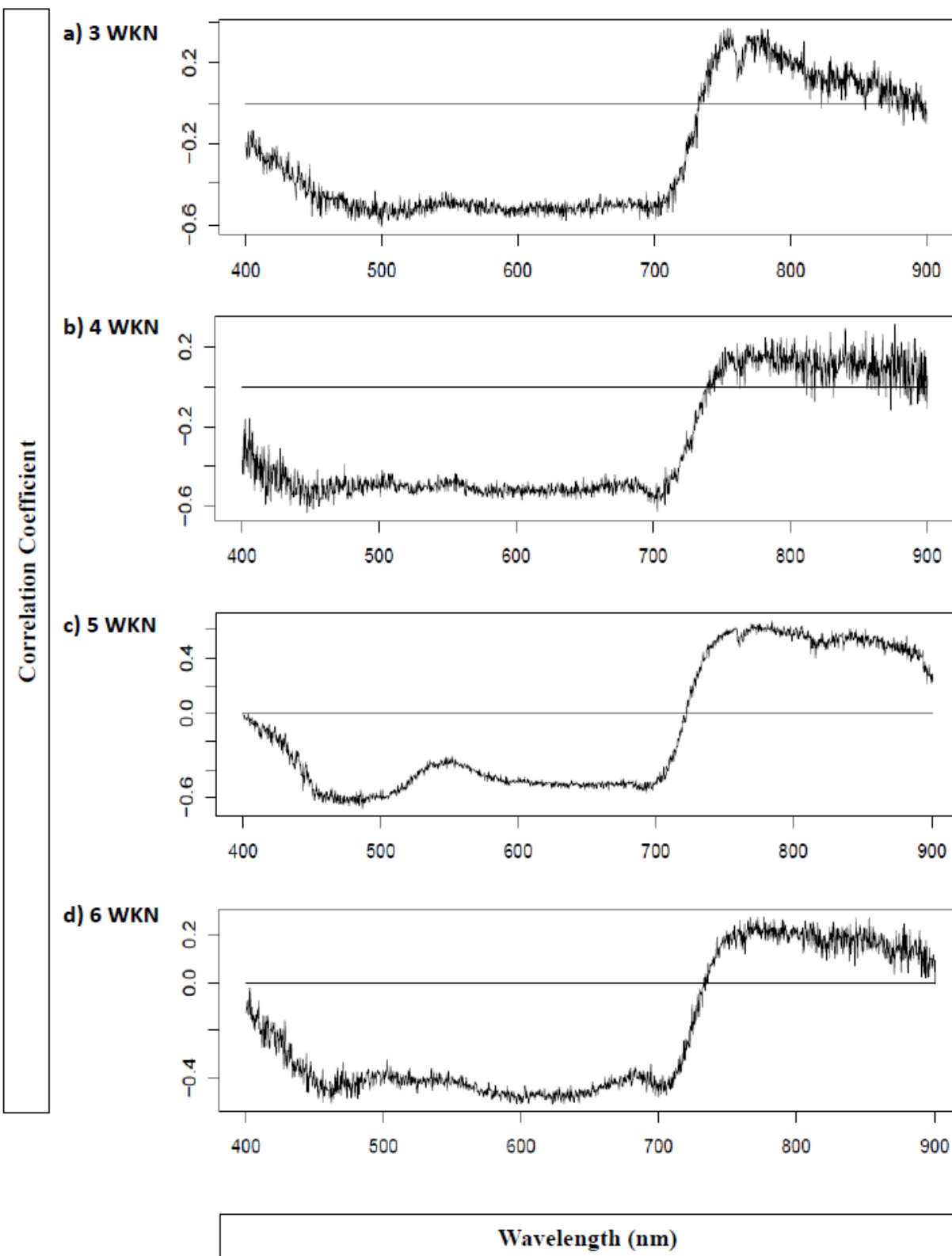


Figure 4.13. Correlation coefficients for the linear relationship between spectral reflectance and N uptake at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2011

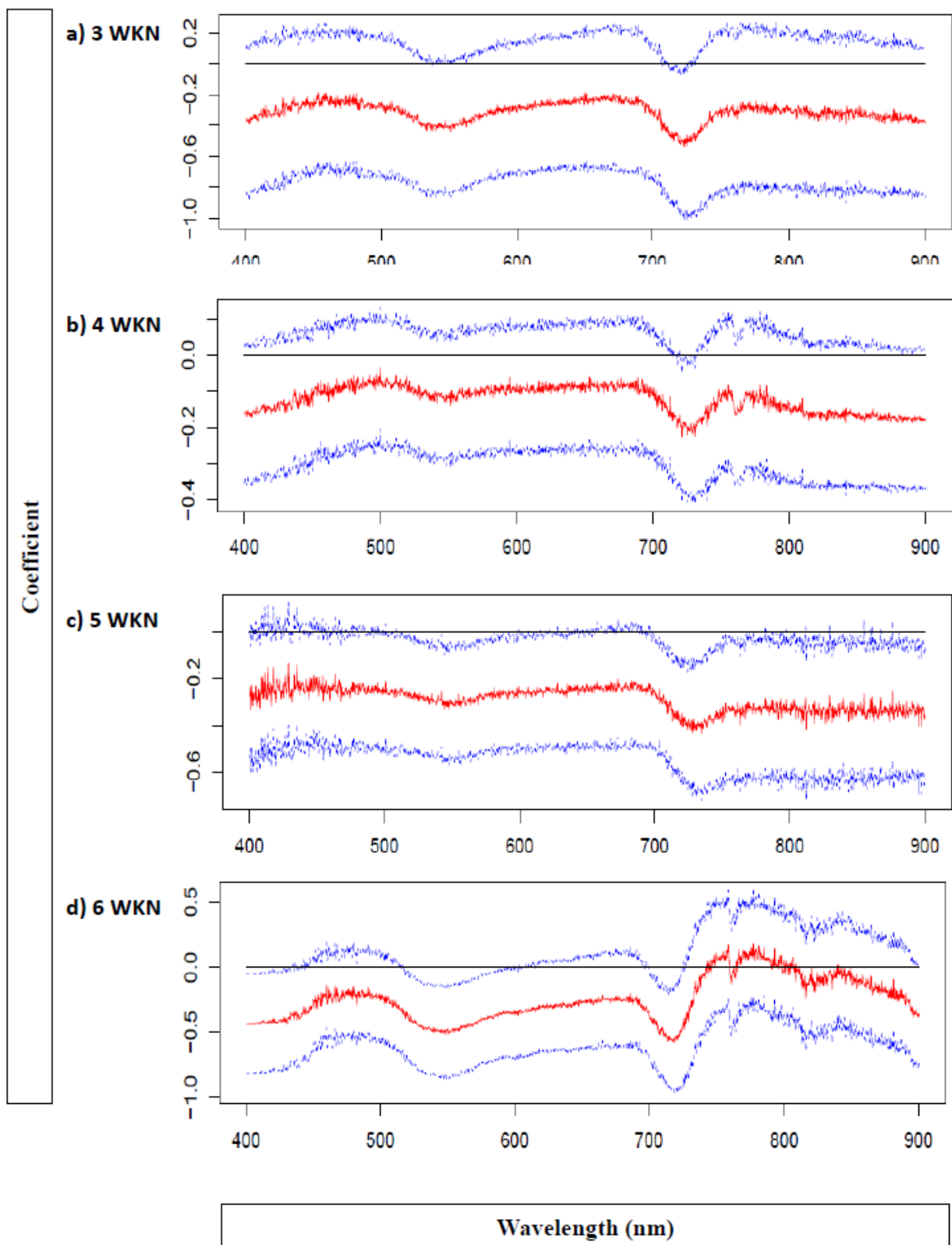


Figure 4.14. Coefficients of variety on the linear relationship between spectral reflectance and N uptake at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2011.

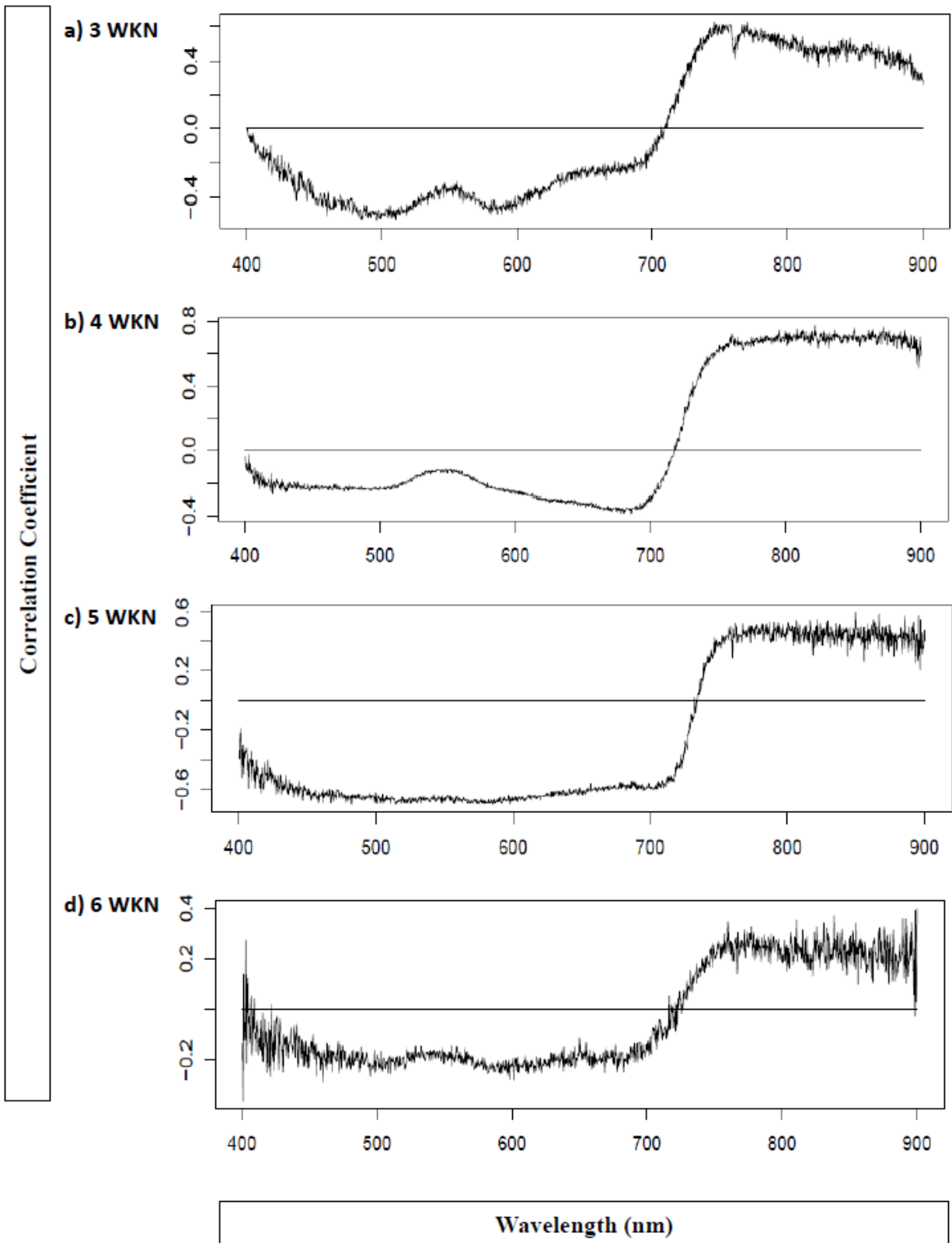


Figure 4.15. Correlation coefficients for the linear relationship between spectral reflectance and N uptake at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2012.

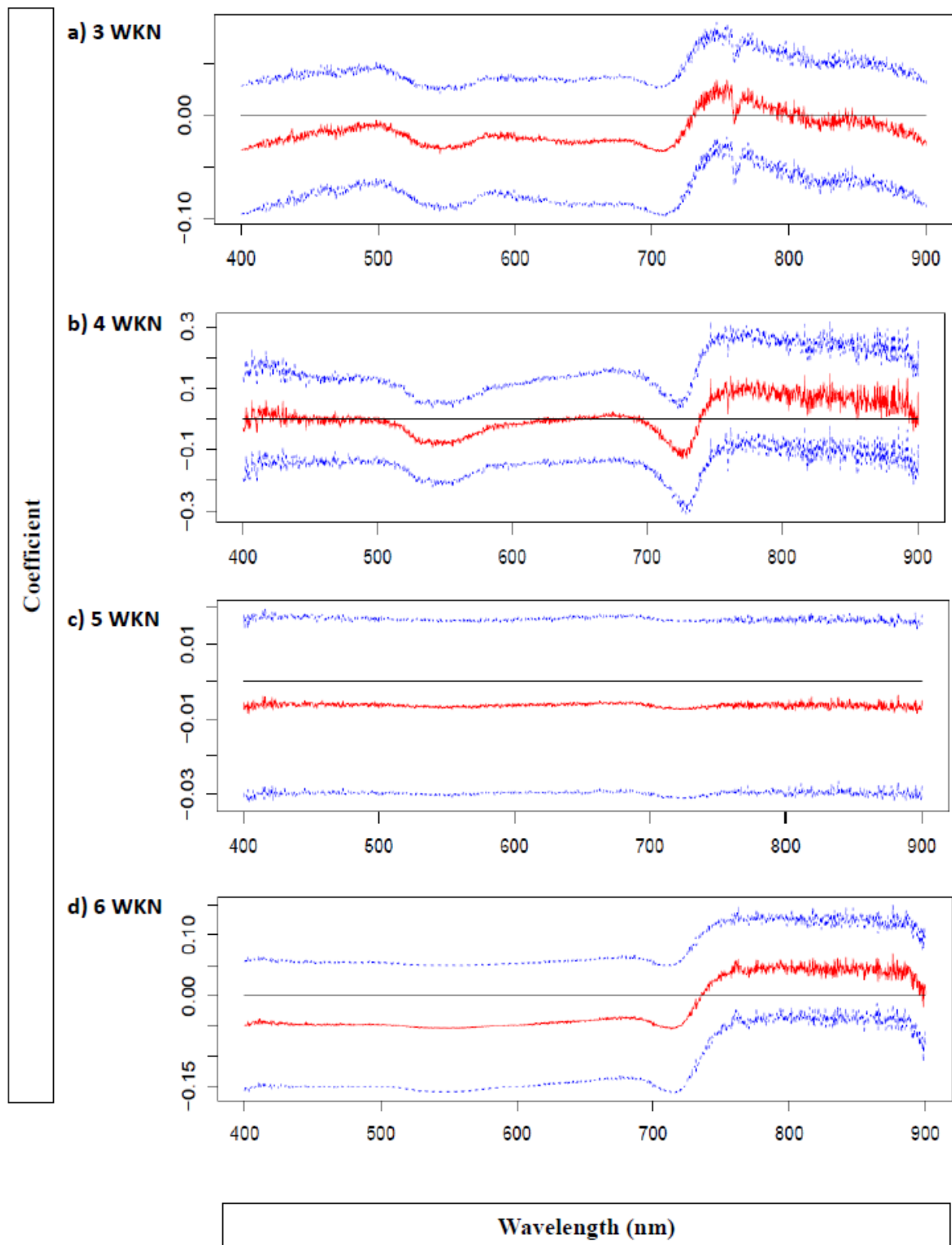


Figure 4.16. Coefficients of variety on the linear relationship between spectral reflectance and N uptake at 3 (a), 4 (b), 5 (c), and 6 (d) week after N fertilization (WKN) in 2012.

Pettigrew-Croby, 1995). Corresponding observations were reported in multiple crops (Daughtry et al., 2000; Sikuku et al., 2010; Huang et al., 2010). The effect of variety was only observed at 5 WKN in 2011 (Figure 4.14 c) indicating that there should be concern on varietal effect when using reflectance readings at near-infrared band for cane N uptake estimation.

4.3.4 Effect of Varieties on the Relationship Between Agronomic Parameters (Biomass and N Uptake) and Vegetation Indices

Simple linear regression

Table 4.3 lists the components of simple linear relationships between vegetation index and biomass for each sampling periods. Except for PVI_{red} and REP , the highest coefficient of determination ($r^2 > 0.40$) across sampling dates was consistently obtained at 3WKN. Only few of these vegetation indices maintained $r^2 > 0.40$ until the later sampling dates; SR_{red} until 5 WKN, and $SR_{red-edge}$, $NDVI_{red}$ and $NDVI_{red-edge}$ until 4 WKN. This result suggests that biomass produced within 3 to 4 WKN can be non-destructively estimated using these vegetation indices. The linear relationship between biomass and vegetation indices tended to decrease with cane growth e.g. r^2 values in SR_{red} were 0.46, 0.41, 0.40 and 0.25 at 3, 4, 5, and 6 WKN, respectively. Only SR_{red} , maintained relatively constant r^2 values across sampling periods. The linear relationship between REP and measured biomass was weak ($r^2 < 0.15$).

The components of simple linear relationships between vegetation index and N uptake for each sampling periods are listed on Table 4.4. Except for SR_{green} , the highest coefficient of determination was observed at 4 WKN. Both $SR_{red-edge}$ and $NDVI_{red-edge}$ explained more than 50 % of the total variability in N uptake from 4 to 6 WKN and maintained relative constant r^2 values across sampling periods. Red-based vegetation indices also accounted for high total variability in N uptake but it tended to decrease with cane growth e.g. r^2 values in $NDVI_{red}$ were 0.33, 0.55, 0.42, and 0.38 at 3, 4, 5, and 6 WKN, respectively. Only SR_{green} had its highest

coefficient of determination at 6 WKN. A major difference of SR_{green} from other vegetation indices is the utilization of reflectance reading at green waveband which high level of reflectance readings. This spectral aspect may alter the best timing of N uptake estimation using SR_{green} . Gitelson et al. (1996) reported green-based NDVI had better sensitivity in detecting high chlorophyll content compared with red-based NDVI. Further study conducted by Gitelson et al. (2003) showed that green-based vegetation index was not only a function of chlorophyll content but also LAI. At 6 WKN, N uptake associated biomass accumulation was relatively high and at this condition using SR_{green} would be advantageous. Similar to biomass, REP maintained relatively weak relationships with N uptake across sampling periods.

Previous studies showed that REP has the potential to address the decreasing sensitivity of NDVI derived from red and near-infrared reflectance as plant approaches canopy closure (Mutanga and Skidmore; 2004; Cho and Skidmore; 2006). Based on our study in rice (Chapter 2), the use of reflectance at REP also improved biomass and yield estimation compared to $NDVI_{red}$ or SR_{red} . Computation of REP generally required continuous spectral reflectance reading due to the analysis of first derivative reflectance. Adams and Gillespie (2006) mentioned the advantages of ratios in spectroscopy since spectral ratio computation allows offsetting influences of the illumination source, the illumination geometry, and detector systems. Unlike the ratio of spectral reflectance such as NDVI or SR, REP cannot be normalized nor standardized because REP is the identification of one point on narrow spectral wavebands (700 to 740 nm). This perceptive character can make it difficult to compute accurate REP in sugarcane field. In addition, with the tall stature and loose canopy of sugarcane the presence of wind can easily trigger in-canopy turbulence causing leaves movement eventually disrupting the amount of REP captured by the sensor. Vanderbilt et al. (1988) reported the altering canopy geometry by wind caused a red edge shift and therefore, REP should be carefully examined.

Analysis of covariate to evaluate varietal effect

Analysis of covariance (ANCOV) is a useful technique to evaluate the effect of categorical variables on a linear relationship of continuous variables. In this study, the following formula was evaluated;

$$Y = b_0 + b_1 V_{283} + b_2 V_{540} + b_3 VI + b_4 (VI \times V_{283}) + b_5 (VI \times V_{540}) + \epsilon$$

where Y= measured agronomic parameter

V_{283} =1 when variety is L 01-283, 0 otherwise

V_{540} =1 when variety is HoCP 96-540, 0 otherwise

VI=vegetation index

b=coefficients.

The sampling periods where the highest correlation between vegetation indices (SR_{red} , $SR_{red-edge}$, $NDVI_{red}$, and $NDVI_{red-edge}$) and biomass was obtained, were selected for ANCOVA to determine the effect of variety on biomass estimation. The same set of vegetation indices for all sampling periods was selected to evaluate varietal effect on N uptake estimation. There were 4 different models generated: (1) Model 1 having the same intercepts and slopes for all varieties, had no effect of variety on the relationship between vegetation index and measured agronomic parameter (Figure 4.17 a); (2) Model 2 required different intercept for cane variety L 01-283 while variety L 99-226 and HoCP 96-540 were explained by the same model (Figure 4.17 b); (3) Model 3 required different intercept for each variety which indicated that there was varietal effect on the relationship between vegetation index and agronomic parameter (Figure 4.18 a); and (4) Model 4 had different components for each variety, i.e. both slopes and intercepts (Figure 4.18 b).

The effect of variety was evident on the relationship between biomass and vegetation index computed from the reflectance at the red and near-infrared bands, i.e. SR_{red} and $NDVI_{red}$

(Table 4.5). Compared to simple linear regression, the addition of varietal parameters into linear regression model improved the correlation of determination from 0.46 to 0.51 and 0.41 to 0.46 at 3 and 4 WKN using SR_{red} , respectively. This means that about 50 % of total variability in biomass was associated with variety. The intercept coefficient for variety L 01-283 was higher than other varieties. This is consistent with the report presented in Chapter 2 where L 01-283 produced significantly higher biomass across sampling periods. The study conducted by Lofton et al. (2012b) also suggested using different models for variety with different canopy structure to estimate sugar yield using NDVI. Contrary to vegetation index derived from reflectance at red band, inclusion of variable variety into the model did not improve biomass relationship with red-edge based vegetation index.

The relationship of red-based vegetation index (SR_{red} and $NDVI_{red}$) with N uptake improved when data was separated by variety (Table 4.6). With plant growth, larger separation on the intercept and slope values among the models were observed (Figure 4.17 and 4.18). The improvement in r^2 was small when varietal effect was considered at 3 and 4 WKN. On the other hand, at 5 and 6 WKN, r^2 was improved from 0.46 to 0.64 and from 0.40 to 0.64, respectively using SR_{red} . Similar improvement was obtained for $NDVI_{red}$ where r^2 values were increased from 0.42 to 0.59 and from 0.38 to 0.55 at 5 and 6, respectively. Except at 6 WKN, variety had no effect on the relationship of N uptake and vegetation index derived from red-edge reflectance ($SR_{red-edge}$, and $NDVI_{red-edge}$) across sampling dates. Compared to red-based vegetation indices, red-edge based vegetation indices had markedly higher r^2 value across sampling periods (Table 4.4). The r^2 values after incorporating varietal effect using red-based vegetation indices and its values of simple regression in red-edge based index were similar (Table 4.4 and 4.6).

Table 4.3. Summary of the linear regression analysis between biomass and vegetation index at St. Gabriel and Jeanerette in LA from 2010 to 2012.

	SR _{green}				SR _{red}				SR _{red-edge}			
	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value
3WKN	-4381	3544	0.49	<0.01	-1377	1988	0.46	<0.01	-17594	17636	0.44	<0.01
4WKN	-2100	3174	0.13	<0.01	-4472	3570	0.41	<0.01	-29399	25794	0.42	<0.01
5WKN	-5031	4760	0.13	<0.01	-6087	4512	0.4	<0.01	-16079	18585	0.12	<0.01
6WKN	-5535	5449	0.28	<0.01	-1450	3032	0.25	<0.01	-18678	20835	0.17	<0.01

	NDVI _{green}				NDVI _{red}				NDVI _{red-edge}			
	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value
3WKN	-3145	18273	0.43	<0.01	-1607	12885	0.41	<0.01	-408	43835	0.46	<0.01
4WKN	-2325	19280	0.1	<0.01	-6814	26737	0.43	<0.01	-5751	76497	0.47	<0.01
5WKN	-3719	24684	0.14	<0.01	-4948	24264	0.35	<0.01	2273	43764	0.13	<0.01
6WKN	-5220	31686	0.26	<0.01	-4400	24888	0.25	<0.01	1620	52358	0.16	<0.01

	PVI _{red}				PVI _{red-edge}				REP			
	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value
3WKN	2867	-244	0.15	<0.01	-1936	343	0.49	<0.01	-386049	539	0.15	<0.01
4WKN	3381	321	0.18	<0.01	-993	336	0.38	<0.01	-574251	801	0.11	<0.01
5WKN	-39	5586	0	NS	-1058	410	0.26	<0.01	-110565	160	0.1	NS
6WKN	6524	205	0	NS	844	357	0.1	<0.01	-255015	363	0.03	NS

WKN - week after nitrogen fertilization

SR - simple ratio

NDVI - normalized difference vegetation index

PVI - perpendicular vegetation index

REP - red edge position

NS- the linear model was not significant at a 0.05 level.

Table 4.4. Summary of the linear regression analysis between N uptake and vegetation index at St. Gabriel and Jeanerette in LA from 2010 to 2012.

	SR _{green}				SR _{red}				SR _{red-edge}			
	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value
3WKN	-47	43	0.42	<0.01	-8.79	23.38	0.37	<0.01	-213	219	0.39	<0.01
4WKN	-90.64	70	0.24	<0.01	-69	51	0.53	<0.01	-434	376	0.54	<0.01
5WKN	-62	54	0.25	<0.01	-44	39	0.46	<0.01	-216	236	0.53	<0.01
6WKN	-264	142	0.66	<0.01	-96	63	0.4	<0.01	-791	688	0.55	<0.01

	NDVI _{green}				NDVI _{red}				NDVI _{red-edge}			
	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value
3WKN	-33	223	0.37	<0.01	-12	151	0.33	<0.01	-0.25	544	0.41	<0.01
4WKN	-87	404	0.24	<0.01	-102	385	0.55	<0.01	-95	1152	0.61	<0.01
5WKN	-45	275	0.25	<0.01	-37	219	0.42	<0.01	21	1354	0.51	<0.01
6WKN	-259	838	0.59	<0.01	-148	500	0.38	<0.01	-123	1759	0.54	<0.01

	PVI _{red}				PVI _{red-edge}				REP			
	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value	intercept	slope	r^2	<i>P</i> value
3WKN	37	3.519	0.13	<0.01	-17	4	0.44	<0.01	-5635	7.8	0.19	<0.01
4WKN	42	4.82	0.24	<0.01	-9.63	4.27	0.38	<0.01	-9372	13.05	0.17	<0.01
5WKN	55	0.37	0	NS	-11	2.87	0.18	<0.01	-4477	6.28	0.11	<0.01
6WKN	67	5.78	0	NS	-15	6.1	0.1	<0.01	-22329	30	0.39	<0.01

WKN - week after nitrogen fertilization

SR - simple ratio

NDVI - normalized difference vegetation index

PVI - perpendicular vegetation index

REP - red edge position

NS- the linear model was not significant at a 0.05 level.

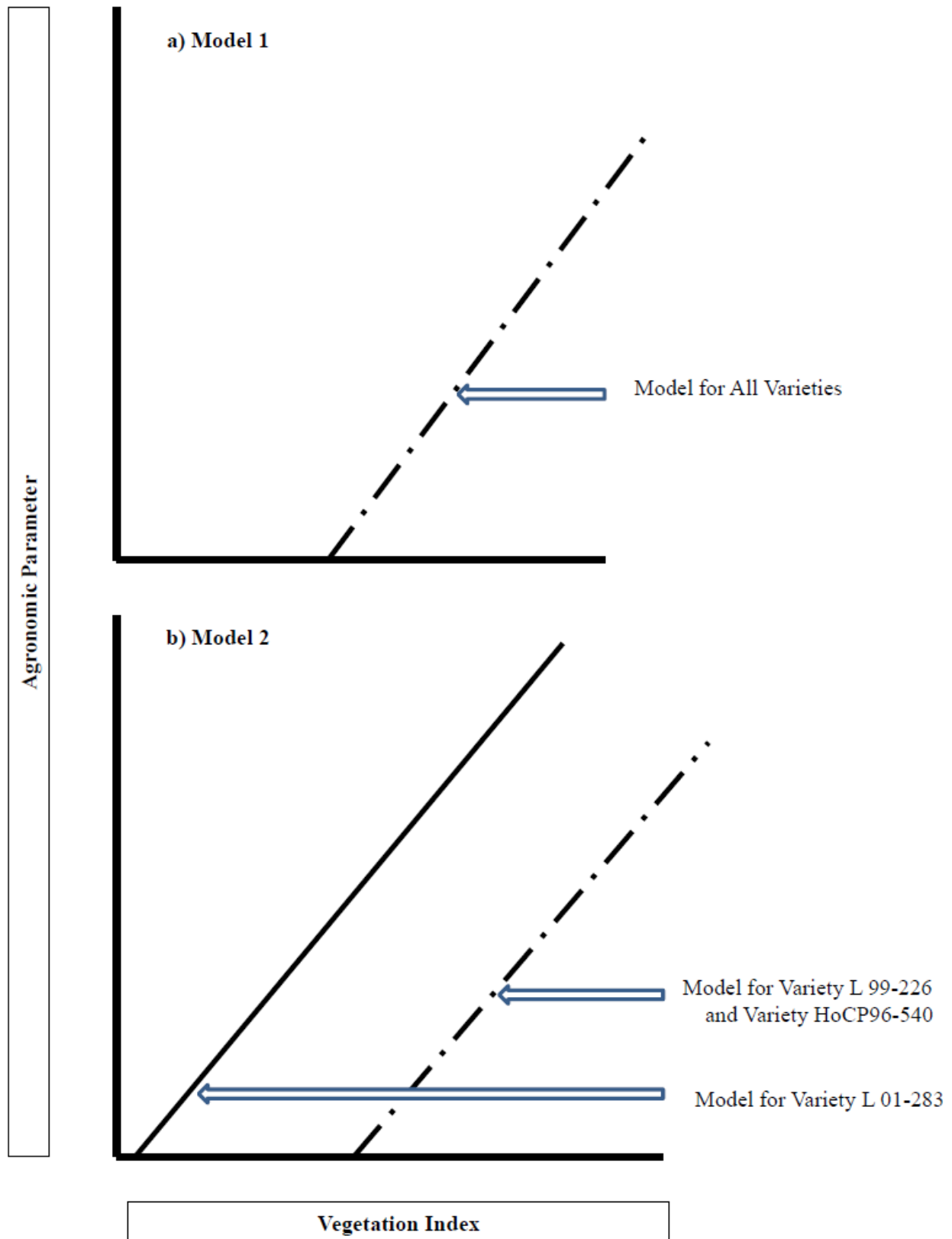


Figure 4.17. Analysis of covariance Model 1 (a) and Model 2 (b).

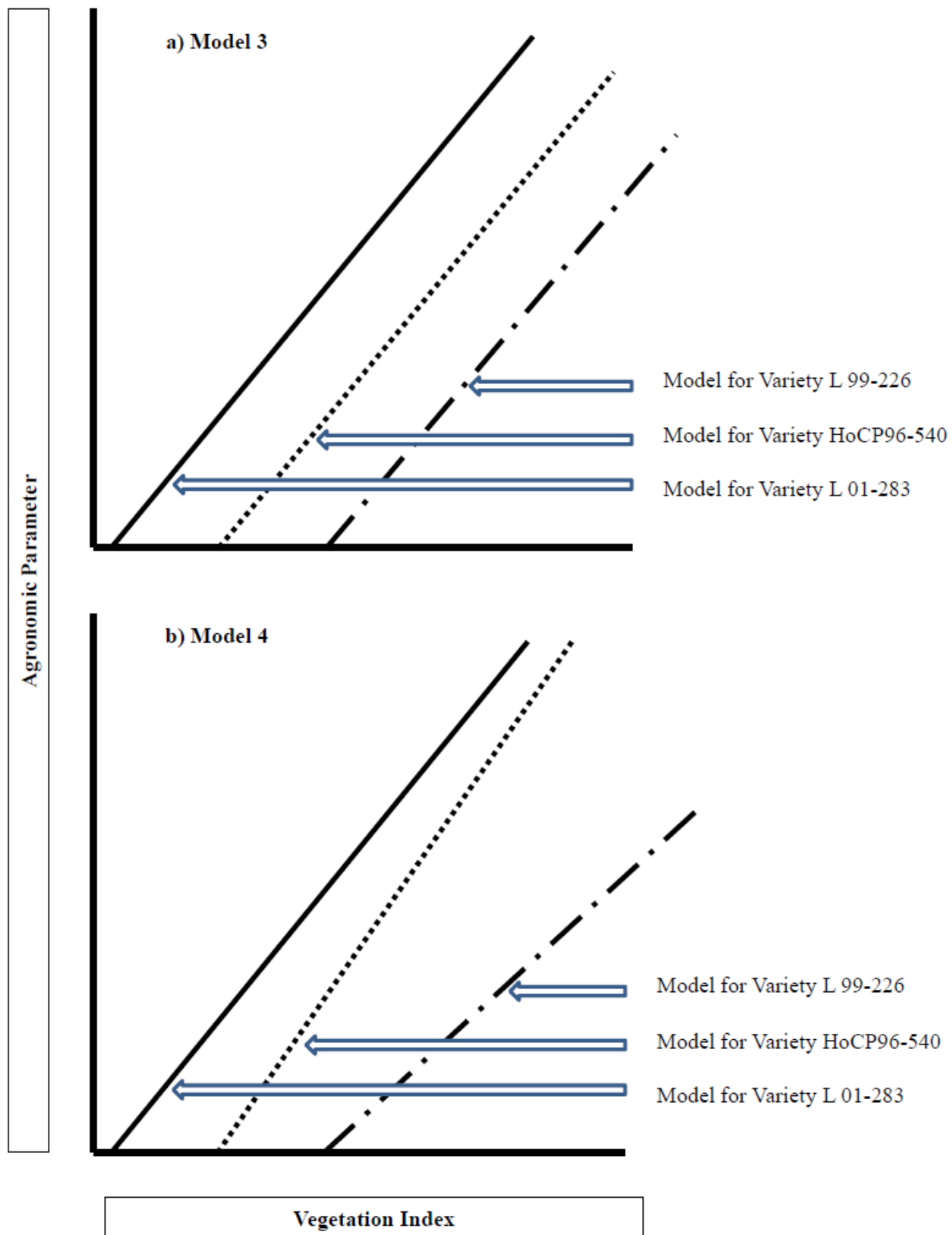


Figure 4.18. Analysis of covariance Model 3 (a) and Model 4 (b).

Table 4.5. The summary of analysis of covariance to evaluate the effect of variety on the relationship between vegetation index and biomass at St. Gabriel and Jeanerette in LA from 2010 to 2012.

	Effects				r^2	Coefficient				Model†
	Time	VI	Variety	VI*Variety		VI	L 99-226	L 01-238	HoCP 96-540	
SR _{red}	3WKN	<0.01	<0.01	NS	0.51	1899	-1496	1064	-1496	2
	4WKN	<0.01	0.02	NS	0.46	3424	-4504	1309	-4504	2
SR _{red-edge}	3WKN	<0.01	NS	NS						1
	4WKN	<0.01	NS	NS						1
NDVI _{red}	3WKN	<0.01	0.0019	NS	0.47	12217	-1673	1052	-1673	2
	4WKN	<0.01	<0.01	NS	0.51	26464	-7136	1448	-7136	2
NDVI _{red-edge}	3WKN	<0.01	NS	NS						1
	4WKN	<0.01	NS	NS						1

†If model is '1' the equation is same as Table 4.3, hence no r^2 , slope and intercept are listed.

WKN - week after nitrogen fertilization

SR - simple ratio

NDVI - normalized difference vegetation index

VI-vegetation index

NS- the effect was not significant for linear model at a 0.05 level.

Table 4.6. The summary of analysis of covariance to evaluate the effect of variety on the relationship between vegetation index and N uptake at St. Gabriel and Jeanerette in LA from 2010 to 2012.

	Effects				r^2	Coefficient				Model†
	Time	VI	Variety	VI*Variety		VI	L 99-226	L 01-238	HoCP 96-540	
SR _{red}	3WKN	<0.01	<0.01	NS	0.42	22	-10	12	-10	2
	4WKN	<0.01	<0.01	NS	0.57	51	-81	20	14	3
	5WKN	<0.01	<0.01	0.02	0.64	21, 25, 34Δ	-10	-43	-68	4
	6WKN	<0.01	<0.01	0.03	0.64	56, 49, 38	-116	-55	-58	4
SR _{red-edge}	3WKN	<0.01	NS	NS						1
	4WKN	<0.01	NS	NS						1
	5WKN	<0.01	NS	NS						1
	6WKN	<0.01	<0.01	NS	0.6	698	-825	37	-825	2
NDVI _{red}	3WKN	<0.01	<0.01	NS	0.37	143	-12	12	-12	2
	4WKN	<0.01	<0.01	NS	0.61	390	-118	24	16	3
	5WKN	<0.01	<0.01	NS	0.59	256	-69	27	22	3
	6WKN	<0.01	<0.01	NS	0.55	637	-265	81	66	3
NDVI _{red-edge}	3WKN	<0.01	NS	NS						1
	4WKN	<0.01	NS	NS						1
	5WKN	<0.01	NS	NS						1
	6WKN	<0.01	<0.01	NS	0.58	1781	-148	38	-148	2

†If model is '1' the equation is same as Table 4.4, hence no r^2 , slope and intercept are listed.

WKN - week after nitrogen fertilization

SR - simple ratio

NDVI - normalized difference vegetation index

VI-vegetation index

NS-the effect was not significant for the linear model at a 0.05 level.

These results suggest that red-edge based vegetation index can estimate N uptake without including varietal effect in the model and can explain similar level of variability in N uptake when red based indices was used with varietal effect in the model.

The influence of variety on the relationship of biomass and N uptake with vegetation index may have resulted from the differences on their canopy structures. Variety L 01-283 has an erectophile canopy structure while variety L 99-226 has a planophile canopy structure (Chapter 3). Canopy closure is attained at an earlier stage of growth for variety with a planophile canopy structure; in such case decrease in sensitivity of vegetation index to estimate agronomic parameters has always been a limitation due to saturation absorption at near-infrared and visual bands (Galvão et al., 2005; Jackson and Pinter; 1986). Second, the rate of biomass accumulation per unit area was different among the varieties; L 01-283 produced the highest biomass compared with the other varieties. Finally, timing and degree of plant N response are highly variable among varieties (Chapter 3). These are the factors which separate these varieties from one another eventually affecting the relationship of biomass and N uptake to vegetation indices. In the present study, the most affected (by variety) vegetation indices are those derived from the reflectance readings at the red spectral band. The large difference between red and red-edge bands arise from the amount the proportion of absorbed and reflected light. Pigments in leaves absorb red light; therefore spectral reflectance is smaller for plants with more leaves or biomass. Clear varietal effect on the amount of reflected light within the red waveband was reported due to different pigment compositions (Johnson et al., 2008). On the other hand, reflectance at red-edge waveband is a transition between the light absorbed by pigments and light reflected by leaf internal scattering at near infrared wavebands (Dawson and Curran, 1998). The study conducted by Galvão et al. (2005) showed that the best visible spectral bands to discriminate sugarcane variety were green and red wavebands; red was slightly better than red-edge band. Based on

these results, it is more likely that reflectance at the red waveband can be affected by variety more than the reflectance at the red-edge band.

4.3.5 Improvement of Biomass Estimation Using Height and Vegetation Indices

The use of NDVI as predictor of biomass, N concentration or N uptake becomes limited once plant canopy completely covers the ground (Hobbs, 1995; Gao et al., 2000; Thenkabail et al., 2000; Mutanga and Skidmore, 2004). Reflectance at the REP was reported to more be sensitive than NDVI (Mutanga and Skidmore, 2004). However, our study did not show any advantage of using reflectance at REP for estimating biomass or N uptake over red-based vegetation index. As mentioned in the previous section, it is likely due to the tall stature and loose canopy of sugarcane making it difficult to maintain a stable and homogeneous canopy. Therefore, an addition of plant height into biomass or N uptake estimation model was evaluated.

The variability in biomass and N uptake can be explained better if both vegetative indices and height were introduced in the model (Table 4.7 and 4.8). The r^2 values increased and the effect of height was significant at all sampling data for both biomass and N uptake. All vegetation indices combined with height measured at 4WKN obtained the highest association with biomass with r^2 values of 0.78, 0.74, 0.77 and 0.74 for SR_{red} , $SR_{red-edge}$, $NDVI_{red}$, and $NDVI_{red-edge}$, respectively. Similar results were obtained in N uptake. Across the sampling periods, r^2 values for both biomass and N uptake were >0.55 and >0.47 , respectively; these r^2 values are higher than what was obtained from using vegetative index alone. This implies that, between 3 to 6 weeks after N application, both biomass and N uptake can be non-destructively quantified using spectral reflectance vegetation indices and height. It is important to note that conditional index of $SR_{red-edge}$ was more than 30 for both biomass and N uptake at all sampling periods; therefore even though height effect was significant at $\alpha=0.05$ level, it might not be the

Table 4.7. The summary of multiple regression to improve biomass estimation using plant height at St. Gabriel in LA from 2011 to 2012.

		Effect			r^2	Conditional index	Coefficient					Model
		VI	Height	Variety			VI	Height	L 99-226	L 01-238	HoCP 96-540	
SR _{red}	3WKN	<0.01	<0.01	NS	0.61	16.07	681	20	-1170	-1170	-1170	1
	4WKN	<0.01	<0.01	NS	0.78	16.92	995	32	-2621	-2621	-2621	1
	5WKN	<0.01	<0.01	NS	0.73	14.36	1392	36	-3664	-3664	-3664	1
	6WKN	<0.01	<0.01	NS	0.59	15.98	1098	34	-2837	-2837	-2837	1
SR _{red-edge}	3WKN	<0.01	<0.01	<.001	0.64	62.21	2927	18	-3138	-300	-3138	2
	4WKN	<0.01	<0.01	NS	0.74	42.33	5450	27	-7007	-7007	-7007	1
	5WKN	<0.01	<0.01	NS	0.72	34.85	7999	27	-9870	-9870	-9870	1
	6WKN	<0.01	<0.01	NS	0.55	37.72	4606	24	-4957	-878	-4957	1
NDVI _{red}	3WKN	<0.01	<0.01	NS	0.61	15.77	4038	19	-1129	-1129	-1129	1
	4WKN	<0.01	<0.01	NS	0.77	15.38	5304	31	-2343	-2343	-2343	1
	5WKN	<0.01	<0.01	NS	0.71	13.97	7940	35	-3362	-3362	-3362	1
	6WKN	<0.01	<0.01	NS	0.57	16.15	7562	33	-3178	-3178	-3178	1
NDVI _{red-edge}	3WKN	<0.01	<0.01	<0.01	0.63	16.34	7745	18	-350	-300	-150	3
	4WKN	<0.01	<0.01	NS	0.74	13.75	14186	27	-1768	-1768	-1768	1
	5WKN	<0.01	<0.01	NS	0.72	12.55	21053	27	-2212	-2212	-2212	1
	6WKN	<0.01	<0.01	<0.01	0.55	18.13	13055	24	-686	-885	-686	2

WKN - week after nitrogen fertilization

SR - simpler ratio

NDVI - normalized difference vegetation index

VI-vegetation index

NS- the effect was not significant for the linear model at a 0.05 level.

Table 4.8. The summary of multiple regression to improve N uptake estimation using plant height at St. Gabriel in LA from 2011 to 2012.

		Effect			r^2	Conditional index	Coefficient					Model
		VI	Height	Variety			VI	Height	L 99-226	L 01-238	HoCP 96-540	
SR _{red}	3WKN	<0.01	<0.01	NS	0.47	16.08	9.22	0.35	-16.96	-16.96	-16.96	1
	4WKN	<0.01	<0.01	NS	0.7	16.92	14.84	0.52	-41.88	-41.88	-41.88	1
	5WKN	<0.01	<0.01	NS	0.71	14.36	17.15	0.55	-48.35	-48.35	-48.35	1
	6WKN	<0.01	<0.01	<0.01	0.62	19.53	15	0.62	-60.49	1.56	-60.49	2
SR _{red-edge}	3WKN	<0.01	<0.01	NS	0.58	47.12	77	0.22	-87	-87	-87	1
	4WKN	<0.01	<0.01	NS	0.74	45.83	102.39	0.42	-132.41	-132.41	-132.41	1
	5WKN	<0.01	<0.01	NS	0.71	36.22	126	0.39	-157	-157	-157	1
	6WKN	<0.01	<0.01	NS	0.69	37.11	114.99	0.47	-164.76	-164.76	-164.76	1
NDVI _{red}	3WKN	<0.01	<0.01	NS	0.56	14.26	74.88	0.28	-20.19	-20.19	-20.19	1
	4WKN	<0.01	<0.01	NS	0.7	15.38	78.68	0.52	-37.58	-37.58	-37.58	1
	5WKN	<0.01	<0.01	NS	0.56	16.65	118.74	0.49	-51	-51	-51	1
	6WKN	<0.01	<0.01	<0.01	0.67	25.12	154.07	0.21	-59.03	18.91	10.04	3
NDVI _{red-edge}	3WKN	<0.01	<0.01	NS	0.57	14.68	205.11	0.23	-14.39	-14.39	-14.39	1
	4WKN	<0.01	<0.01	NS	0.74	13.75	265.96	0.42	-33.95	-33.95	-33.95	1
	5WKN	<0.01	<0.01	NS	0.71	15.62	334.07	0.4	-36.61	-36.61	-36.61	1
	6WKN	<0.01	<0.01	NS	0.68	15.92	325.13	0.48	-58.31	-58.31	-58.31	1

WKN - week after nitrogen fertilization

SR - simpler ratio

NDVI - normalized difference vegetation index

VI-vegetation index

NS-the effect was not significant for the linear model at a 0.05 level.

best model due to the potential of multicollinearity problem. Other than this index, there was no violation due to multicollinearity in establishing these models.

Substantial improvement by introducing height into the model relating agronomic parameters and vegetation index can be because of increasing data dimension by adding vertical reading (height). Remote sensing provides more likely surface evaluation of plant canopy and leaf elements (horizontal information) thus maintaining soil background scene component in the sensor field of view is essential for effective spectral reflectance discrimination. However, the inclusion of height (plant vertical information) in the models added another dimension, expanding both the area and perspective for evaluation of plant N status. In addition, the lack of varietal effect when using both vegetation index and height as predictive variables for almost all the sampling period, is an added value (Table 4.7 and 4.8). Since there are numerous sugarcane varieties in Louisiana and practically it is difficult to calibrate spectral reflectance data for each of these varieties. If varietal effect is insignificant, it is possible to use generalized biomass and N uptake predictive models for all cane varieties. Freeman et al. (2007) reported that the NDVI combined with plant height had higher correlation with corn grain yield than using NDVI alone. Turner et al. (2008) tested indirect measurements of plant height using sonar sensor in corn, cotton, sorghum, and wheat. The outcome of the study showed the potential use of sonar sensor for rapid plant height measurement. Therefore, there is a possibility for concurrent measurement of spectral reflectance and sonar readings allowing us to monitor cane N health status on-the-go.

4.4 Conclusions

At the leaf-level, there was no distinct varietal differences in spectral reflectance at both visible and near-infrared wavebands while at the canopy level, wavebands at blue (450 to 500 nm), red (650 to 700 nm) and near infrared (780-830 nm) obtained high correlation coefficient with agronomic parameters across sampling periods. The varietal effect on the relationship between spectral reflectance and agronomic parameters were observed only in 2011 at purple (400 to 450 nm), between blue and green (550 nm), and red-edge position (720-750 nm). Vegetation indices that can be potentially used for predicting biomass and N uptake were identified: SR_{red} , $SR_{red-edge}$, $NDVI_{red}$ and $NDVI_{red-edge}$. The highest coefficient of determination values of the linear relationship between agronomic parameters and vegetation indices were observed at 3 and 4 WKN. Varietal effect on the model was significant only when red-based vegetation indices were used. As cane grows, different intercepts and slopes had to be used for each variety to maintain the linear relationship between vegetation indices and measured agronomic variables. Using plant height and vegetation indices, better linear relationships were established with biomass and N uptake estimation while diminishing the effect of variety. The findings of this study suggest that SR and NDVI measured at 3 to 4 WKN can be used to estimate biomass and N uptake in sugarcane.

4.5 References

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Chapter 5. Conclusions

Improvement of N management based on remote sensing technology by monitoring mid-season plant N status has been shown in several crops such as wheat and corn. Having water background component in the sensor field of view is a unique feature of the use and application of remote sensing in rice. Previous study conducted in Louisiana in predicting rice biomass and yield did not examine this effect. Also, commonly used vegetation index, normalized difference vegetation index (NDVI), has reported to decrease its sensitivity at dense plant canopy. Therefore effect of water background on spectral reflectance and alternative vegetation indices using red edge bands were examined to predict biomass and rice grain yield. Based on our study, water background did not alter spectral reflectance at panicle differentiation (PD), one week after panicle differentiation (PD+1wk) and 50 % heading (50% HD). Water depth slightly influenced reflectance at red wavebands but this effect was not carried over when reflectance readings were transformed to vegetation indices. The use of red-edge based vegetation indices improved biomass and grain yield predictions as compared with red-based indices. There was a varietal impact on the relationships between yield and vegetation indices (NDVI and simple ratio -SR) derived from reflectance readings at near infrared bands but none on derivative red-edge based indices (REP). The difference caused by variety may be challenging when this technology is implemented into practical fields since one need to consider which variety is evaluated. However, the model with or without including varietal effect had similar level of variability to explain grain yield. Therefore, one generalized model can be sufficient to build N algorithm for mid-season N fertilization.

Earlier works introduced the concept of using early-season readings to estimate cane yield potential and probability of response to N fertilizer in sugarcane. However, there has no documentation on the pattern of cane varieties response to N nor how agronomic variables i.e. biomass, tiller number, N content, foliar angle index (FAI) and plant height, measured early in the season relate to sugar yield response to N. Our study showed that measured agronomic variables' responses to N fertilizer were highly variable across year and variety. The high variability in sugarcane response to N suggests N management based on N response which is evaluated in growing season. The high N responsiveness in biomass and %N showed good linear relationships with sugar yield response to N at 4 to 5 WKN. These findings suggest that biomass and N content can be used to estimate sugar yield N response and therefore suggest N application only when a sugar yield gets benefit from additional N fertilizer.

However, it is time consuming and labor intensive to collect those agronomic variables from fields. Therefore, the study to estimate these variables using spectral reflectance was also conducted. The influence of distinct difference in geometrical structure among sugarcane variety was also important component in this study to evaluate the relationship between spectral reflectance and agronomic variables. At leaf-level, there was no distinct varietal difference at both visible and near-infrared wavebands while at canopy level, wavebands at blue (450 to 500 nm), red (650 to 700 nm) and near infrared (780-830 nm) showed high correlation coefficient with agronomic parameters across sampling periods. The varietal effect on the relationship between spectral reflectance and agronomic parameters were observed only in 2011 at purple (400 to 450 nm), between blue and green (550 nm), and at red-edge position (720-750 nm). Potential vegetation

indices for predicting biomass and N uptake were SR_{red} , $SR_{red-edge}$, $NDVI_{red}$ and $NDVI_{red-edge}$ which were measured at 3 and 4 WKN. Varietal effect on the model was only observed when red-based vegetation indices were used. This resulted from decrease in red reflectance sensitivity associated with difference in canopy structures among varieties. The use of both vegetation indices and plant height improved in the precision of the biomass and N uptake predictive models without varietal effect. Therefore, measurement of both spectral reflectance and plant height using sonar readings can be another approach for monitoring mid-season agronomic variables to evaluate N crop requirement.

In summary, this quick non-destructive monitoring method is promising tool to monitor biomass and yield accounting for spatial and temporal visibilities and to improve N management in rice and sugarcane.

Appendix A: SAS Code

A1. Rice

```
dm 'log; clear; output; clear';
option pageno=1
      nodate
      rightmargin=.5in
      leftmargin=.5in
      topmargin=1in
      bottommargin=.5in;
title1 'Rice';

libname rice 'C:\Documents and Settings\ykankel\Desktop\Rice
Manuscript\Rice.xlsx';
ods rtf file='C:\Documents and Settings\ykankel\Desktop\Rice
Manuscript\Rice.rtf';
ods graphics on;

data work.yield;
  set rice.'r2011$'n;
  Where samplingT=2;
  if VAR='No' then delete;
  if VAR='CL152' then VAR1=1;
  if Var='CL261' then VAR1=0;
  if trt=7 then trt=1;
  else if trt=8 then trt=2;
  else if trt=9 then trt=3;
  else if trt=10 then trt=4;
  else if trt=11 then trt=5;
  int=Var1*REPLE;

run;

proc mixed data=work.yield;
  class Var;
  model Yield=SR1e Var Var*SR1e /htype=1 3 solution ;
run;

proc reg data=work.yield;
  model Yield=VAR1 SR1e;
run;

proc mixed data=work.yield;
  class REP Nrate;
  model Biomass=Nrate;
  random REP;
  lsmeans Nrate / adjust=tukey;
  ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;

proc mixed data=work.yield;
  class REP Var Nrate;
  model Nuptake=Var Nrate Var*Nrate;
  random REP;
  lsmeans Var Nrate / adjust=tukey;
  ods output diffs=ppp lsmeans=mmm;
```

```

ods listing exclude diffs lsmeans;
run;

%include 'C:\Documents and Settings\ykankel\Desktop\Research
2011\Rice\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

ods csv close;

ods graphics off;
ods rtf close;
libname rice clear;

```

A2. Sugarcane

```

dm 'log; clear; output; clear';
option pageno=1
      nodate
      rightmargin=.5in
      leftmargin=.5in
      topmargin=1in
      bottommargin=.5in;

libname sugar 'C:\Documents and Settings\ykankel\Desktop\Sugarcane
Manuscript\Sugarcane1\Sugar1.xlsx';
ods rtf file='C:\Documents and Settings\ykankel\Desktop\Sugarcane
Manuscript\Sugarcane1\Sugar1.RI.rtf';
ods csv file='C:\Documents and Settings\ykankel\Desktop\Sugarcane
Manuscript\Sugarcane1\Sugar1.RI.csv';
ods graphics on;

data work.sugar1;
  set sugar.'RIMLS'n;
  where year = 2011 and 2012;
run;

data work.sugar1;
  set sugar.'all$n';
  where year =2012 and var=540;
run;

proc mixed data=work.sugar1;
  class REP TRT ;
  model SugarMh= trt;
  random REP;
  lsmeans trt / adjust=tukey;
  ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;

proc reg data=work.sugar1;
  model RIy=RIb;
run;
%include 'C:\Documents and Settings\ykankel\Desktop\Research
2011\Rice\pdmix800.sas';

```

```
%pdmix800(ppp,mmm,alpha=.05,sort=yes);  
run;  
ods csv close;  
ods graphics off;  
ods rtf close;  
libname sugar clear;
```

Appendix B: R Code

B1: Rice

```
#Import data and data cleaning
loc <- "C:/Documents and Settings/ykankel/Desktop/Research
2012/Rice/Rice.Water.Depth.3rd.txt"
tmp <- read.table(loc,header=T,sep="\t")
dim(tmp) #dimension of the data

biomass <- tmp[1453,-1]
mode(biomass) <- "numeric"
biomass <- as.vector(biomass)

wback <-tmp[1452,-1]
mode(wback) <- "numeric"
wback <- as.vector(wback)

data <- as.matrix(tmp[1:1451,-1]) #spectrum
wl <- as.vector(tmp[1:1451,1]) #wavelength
mode(wl) <- "numeric"

#linear regression (biomass + wback)
p <- nrow(data) #number of wavelengths
n <- ncol(data) #sample size
beta.w <- matrix(0,p,2) #beta for water depth
beta.b <- matrix(0,p,2) #beta for biomass
for (j in 1:p){
  fit <- lm(data[j,]~wback+biomass)
  beta.w[j,1] <- summary(fit)$coef[2,1]
  beta.w[j,2] <- summary(fit)$coef[2,2]
  beta.b[j,1] <- summary(fit)$coef[3,1]
  beta.b[j,2] <- summary(fit)$coef[3,2]
}

#36 is the df for error
qt(df=75,0.975) #1.992

#Lower and upper limit of beta
ul <- beta.w[,1]+1.992*beta.w[,2]
ll <- beta.w[,1]-1.992*beta.w[,2]

#CI plot
loc<-"C:/Documents and Settings/ykankel/Desktop/Research
2012/Rice/Ricebackdepth3rd.pdf"
pdf(loc,height=4, width=8)
par(mar=c(4,5,2,4),cex.lab=1.4, cex.axis=1.2, cex.main=1.5)

plot(wl,beta.w[,1],xlab="wave length",ylab="Coefficient",
      ylim=range(c(ul,ll)),type="l",lwd=1)
lines(wl,ul,col="red",lty=2)
lines(wl,ll,col="red",lty=2)
lines(range(wl),rep(0,2),col="blue")

dev.off()
```

```
#output the results
out <- cbind(wl,ul,ll,beta.w)
dimnames(out)[[2]]<-c("Wavelength","upper","lower","beta.w")
write.table(out,file="C:/Documents and Settings/ykankel/Desktop/Research
2012/Rice/outputwaterdepth1st.txt",
            sep="\t",row.names =F)#Import data and data cleaning
loc <- "C:/Documents and Settings/ykankel/Desktop/Research
2012/Rice/Rice.waterdepth70%.txt"
tmp <- read.table(loc,header=T,sep="\t")
dim(tmp) #dimension of the data

biomass <- tmp[1452,-1]
mode(biomass) <- "numeric"
```

B2: Sugarcane ‘Principal Component Analysis’

```
loc <- "C:/Documents and Settings/ykankel/Desktop/Sugarcane
Manuscript/Sugarcane2/R/Leaf/2012.4.2.txt"
tmp <- read.table(loc,header=T,sep="\t")
biomass <- tmp[,2]
variety <- tmp[,3]
wavebands <- as.matrix(tmp[,4:1454])
tmp1 <- dimnames(tmp)[[2]][4:1454]
wavelength <- rep(0,length(tmp1))
for (i in 1:length(tmp1)){
  wavelength[i] <- as.numeric(substr(tmp1[i],2,nchar(tmp1[i])))
}

##### PCA #####

pca <- prcomp(wavebands, retx=T, center=T, scale.=F)

summary(pca) #The first two PCs contains about 94% of the total variation
pca$ro

#Importance of components:
#
#          PC1          PC2          PC3          PC4          PC5          PC6
#Standard deviation    671.1708  92.00056  65.67717  56.60381  35.55033  33.26432
#Proportion of Variance   0.9216   0.01732   0.00883   0.00656   0.00259   0.00226
#Cumulative Proportion   0.9216   0.93894   0.94776   0.95432   0.95690   0.95917

#output the results
out <- cbind(pca$ro)
write.table(out,file="C:/Documents and Settings/ykankel/Desktop/Sugarcane
Manuscript/Sugarcane2/R/Leaf/r2012.4.2.txt",
            sep="\t",row.names =F)

pc1 <- as.vector(wavebands%%pca$ro[,1])
pc2 <- as.vector(wavebands%%pca$ro[,2])

pdf("C:/Documents and Settings/ykankel/Desktop/Sugarcane
Manuscript/Sugarcane2/R/Leaf/r2012.4.2.pdf",
    height=6, width=7)
par(mar=c(4,5,2,2), cex.lab=1.5, cex.axis=1.3, cex.main=1.5)
plot(pc1,pc2,xlab="PC1",ylab="PC2",type="n")
cx <- 1.5
points(pc1[variety==226],pc2[variety==226],col="red",pch=2,cex=cx)
points(pc1[variety==283],pc2[variety==283],col="blue",pch=3,cex=cx)
points(pc1[variety==540],pc2[variety==540],col="darkgreen",pch=5,cex=cx)
```

```

legend(500,-1030,legend=c("226","283","540"),pch=c(2,3,5),
      col=c("red","blue","darkgreen"),cex=1.3)
dev.off()

```

B3: Sugarcane ‘Effect of Variety on the Relationship Between Spectral Reflectance and Agronomic Variables’

```

"C:/Documents and Settings/ykanke1/Desktop/Sugarcane
Manuscript/Sugarcane2/R/bio1.txt"
tmp <- read.table(loc,header=T,sep="\t")
biomass <- tmp[,2]
variety <- tmp[,3]
wavebands <- as.matrix(tmp[,4:1454])
tmp1 <- dimnames(tmp)[[2]][4:1454]
wavelength <- rep(0,length(tmp1))
for (i in 1:length(tmp1)){
  wavelength[i] <- as.numeric(substr(tmp1[i],2,nchar(tmp1[i])))
}

FUN1 <- function(x){
  cor(x,biomass)
}

#corr.coef is the vector of correlation coefficients
corr.coef <- apply(wavebands,2,FUN1)
plot(wavelength,corr.coef,type="l",xlab="Wave length",
      ylab="Correlation coefficient")
lines(range(wavelength),rep(0,2))

FUN2 <- function(x){
  fit <- lm(biomass~variety+x)
  c(fit$coef[2],confint(fit,"variety"))
}

#try contains 3 rows and 1780 columns
#1st row is the coefficient for variety,
#2nd and 3rd rows are the Confidence interval (CI)
#for the coefficient for variety
try <- apply(wavebands,2,FUN2)

#Since the CI holds zero, it indicates the effect of variety
#is not significant
plot(wavelength,try[1,],ylim=range(try),xlab="Wave length",
      ylab="Coefficient for VARIETY",type="l",col="red")
lines(wavelength,try[2,],col="blue",lty=2)
lines(wavelength,try[3,],col="blue",lty=2)
lines(range(wavelength),rep(0,2))

#Combine two plots into one figure
pdf("C:/Documents and Settings/ykanke1/Desktop/Sugarcane
Manuscript/Sugarcane2/R/bio1.pdf",
    height=6, width=9)
par(mfrow=c(2,1),mar=c(4,5,2,2), cex.lab=1.2, cex.axis=1.1, cex.main=1.5)
plot(wavelength,corr.coef,type="l",xlab="Wave length",
      ylab="Correlation coefficient")
lines(range(wavelength),rep(0,2))

```



```
plot(wavelength,try[1,],ylim=range(try),xlab="Wave length",
      ylab="Coefficient for VARIETY",type="l",col="red")
lines(wavelength,try[2,],col="blue",lty=2)
lines(wavelength,try[3,],col="blue",lty=2)
lines(range(wavelength),rep(0,2))
dev.off()
```

Vita

Yumiko Kanke was born in July 1984, in Osaka, Japan. She attended Oklahoma State University at Stillwater, Oklahoma, where she received her Bachelor of Science degree in Plant and Soil Science in 2008. Upon completion, she continued at Oklahoma State University and graduated in 2009 with a Master of Science in Plant and Soil Science under the guidance of Dr. Bill Raun. In June of 2010 she was admitted into the doctoral program in the School of Plant, Environmental, and Soil Science at Louisiana State University Agricultural and Mechanical College. She has since been under the guidance of Dr. Brenda Tubana working in improvement of N management using sensor technology in sugarcane and rice production in Louisiana. The title of her dissertation is “Optimizing yield and crop nitrogen response characterization by integrating spectral reflectance and agronomic properties in sugarcane and rice”.