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# In-season Diagnosis of Rice Nitrogen Status using Crop Circle Active Canopy Sensor and UAV Remote Sensing

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***Abstract.*** *Active crop canopy sensors have been used to non-destructively estimate nitrogen (N) nutrition index (NNI) for in-season site-specific N management. However, it is time-consuming and challenging to carry the hand-held active crop sensors and walk across large paddy fields. Unmanned aerial vehicle (UAV)-based remote sensing is a promising approach to overcoming the limitations of proximal sensing. The objective of this study was to combine unmanned aerial vehicle (UAV)-based remote sensing system and Crop Circle ACS-430 to estimate rice (Oryza sativa. L.) N status for guiding topdressing N application in Northeast China. Two N rate experiments involving two different varieties were conducted in 2014 at Jiansanjiang Experiment Station of China Agricultural University, Heilongjiang Province, Northeast China. An active canopy sensor Crop Circle ACS-430 with three spectral bands (red(R), red edge (RE) and near infrared (NIR)) and an Octocopter UAV equipped with a Mini Multi-Camera Array (Mini-MCA) imaging system with five spectral bands (blue (B), green (G), R, RE and NIR) were used to collect reflectance data at the panicle initiation (PI) and stem elongation (SE) stages. The preliminary results indicated that Crop Circle ACS430-based vegetation indices (VIs) explained 79-80% and 86-87% variability of aboveground biomass (AGB) and plant N uptake (PNU), respectively, but had very poor relationship with plant N concentration (PNC) ($R^2$ = 0.16-0.21) across all stages. The N sufficiency index (NSI)* *calculated with Crop Circle ACS-430 vegetation indices (NNI-VIs) had better correlation with NNI than the original VIs, especially at SE stage and across both stages, with the best $R^2$ of 0.65 and 0.69. UAV-based remote sensing VIs could be used to estimate Crop Circle VIs and NSI-VIs very well at both growth stages. The $NSI_{VIs}$-NNI approach performed well for diagnosing rice N status. Combining UAV-based remote sensing system and Crop Circle ACS-430 had a good potential for in-season diagnosis of rice N status at PI stage, with the highest accuracy rate (90%) and kappa statistics (0.62), but did not perform well at SE stage and across both stages. More studies are needed to further evaluate these different strategies.*

***Keywords***：*Nitrogen nutrition index, Nitrogen diagnosis, Low altitude remote sensing, Crop Circle ACS 430, Vegetation index*

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## INTRODUCTION

Nondestructive estimation of crop nitrogen (N) and growth status in space and time is essential for in-season site-specific N management (Miao et al. 2011;Yao et al. 2012; Zhao et al. 2013). Active crop canopy sensor-based precision N management has been reported to increase both crop yield and N use efficiency (Yao et al. 2012; Wang et al. 2013). However, it is very time-consuming and challenging to carry the hand-held active crop sensors and walk across large paddy fields, taking into consideration the water in these fields (Huang et al. 2015). Satellite remote sensing is potentially more efficient for monitoring crop growth status across large areas, but repeat cycle and bad weather conditions often hinder satellite systems from acquiring the data when needed (Torres-Sánchez et al. 2013). Unmanned aerial vehicle (UAV)-based remote sensing is a promising approach to overcoming the limitations of proximal sensing and satellite remote sensing and has a good potential for in-season crop N status monitoring (Lu et al. 2015).

Nitrogen nutrition index (NNI) is a good index for evaluation of crop N status and can be used to guide in-season site-specific N management (Huang et al. 2015). It indicates N deficiency when NNI < 1, while NNI > 1 indicates N surplus. Moreover, crop NNI can be estimated by proximal and remote sensing technologies (Cao et al. 2013, 2015; Huang et al. 2015). For rice, four edge-based indices including the Red Edge Soil Adjusted Vegetation Index (RESAVI), the Modified RESAVI (MRESAVI), the Red Edge Difference Vegetation Index (REDVI) and the Red Edge Re-normalized Difference Vegetation Index (RERDVI) performed equally well for estimating rice NNI across growth stages ($R^2$ = 0.76) by using Crop Circle ACS-470 (Cao et al. 2013). Shen et al. (2014) using the Crop Circle ACS-430 reported that red edge-based indices, normalized difference red edge (NDRE) and red edge ratio vegetation index (RERVI) had consistently better correlations with NNI ($R^2$ = 0.71) than red light based vegetation indices (NDVI, RVI) ($R^2$ = 0.57-0.66) across different growth stages, varieties, and year.

Mini Multi-Camera Array (Mini-MCA) imaging system mounted on UAV system has near infrared (NIR), red edge (RE), red (R), green (G), and blue (B) bands, which are similar to Crop Circle ACS-470 and ACS-430 bands. It has been reported that aerial hyperspectral remote sensing and chlorophyll meter data or satellite remote sensing and ground canopy reflectance sensor data were combined to diagnose crop N status (Miao et al. 2014; Yang et al. 2008). The objective of this study was to combine unmanned aerial vehicle (UAV)-based remote sensing system and Crop Circle ACS-430 to improve estimation of rice N status for guiding topdressing N application in Northeast China.

## MATERIALS AND METHODS

### Study site description and experiment design

Two N field experiments were carried out in 2014 at Jiansanjiang Experiment Station of China Agricultural University, located in Sanjiang Plain, Heilongjiang province, Northeast China. The experimental site received five N rates (0, 40, 80, 120, and 160kg N $ha^{-1}$) with the exception of variety difference: one used an 11-leave variety (Longjing31) and the other used a 12-leave variety (Longjing21). N fertilizer was distributed in three splits: 40% as basal N before transplanting, 30% at tillering stage, and 30% at stem elongation stage. For all treatments, 50 kg $ha^{-1}$ $P_2O_5$ as triple super-phosphate was applied before transplanting and 105 kg $ha^{-1}$$K_2O$ as potassium sulfate was applied as two splits: 50% before transplanting and 50% at stem elongation stage. All field management, such as rice seedlings preparation, irrigation, weeding and pesticide applications, followed the local standard practices.

### Active canopy sensor and UAV-based remote sensing data collection

Crop Circle ACS-430 (Holland Scientific Inc., Lincoln, Nebraska, USA) is an active canopy sensor, with three fixed wavebands: red (670 nm), red-edge (730 nm), and near infrared (780 nm) (Tremblay et al. 2011). The sensor was carried 0.7-0.9 m above rice canopy to collect canopy reflectance data in each plot. An Octocopter UAV (TTA Aviation, Beijing, China) equipped with a Mini Multi-Camera Array (Mini-MCA) imaging system (Tetracam Inc., Chatsworth, CA, USA) was used in this study. The imaging system acquired data in near infrared (NIR), red edge (RE), red (R), green (G), and blue (B) bands. It has an incident light sensor (ILS), which gathers down-welling radiation at wavelengths that are identical to the up-welling reflected radiation monitored by the system's remaining channels. This enables system software to calculate precise reflectance values as a fraction of the detected incident radiation. At the panicle initiation (PI) and the stem elongation (SE) stages, Octocopter UAV-based remote sensing system was used to collect images at 150 m height for the whole two N field experiments. The imaging data was processed using the PixelWrench2 software (Tetracam Inc., Chatsworth, CA, USA) and ArcGIS. Fifteen VIs were calculated for this study (Table 1).

**Table 1. Spectral indices used in this study.**

| Index | Definition | Reference |
|---|---|---|
| Normalized difference vegetation index(NDVI) | (NIR-R)/(NIR+R) | Rouse et al. (1974) |
| Ratio vegetation index (RVI) | NIR/R | Jordan (1969) |
| Normalized difference red edge(NDRE) | (NIR-RE)/(NIR+RE) | Barnes et al. (2000) |
| Red edge ratio vegetation index (RERVI) | NIR/RE | Jasper et al. (2009) |
| Soil-adjusted vegetation index (SAVI) | 1.5*(NIR-R)/(NIR+R+0.5) | Huete (1988) |
| Optimized SAVI (OSAVI) | (1+0.16)*(NIR-R)/(NIR+R+0.16) | Rondeaux et al. (1996) |
| Modified Soil-adjusted vegetation index (MASVI) | 0.5*{2*NIR+1-SQRT[(2*NIR+1)2-8*(NIR-R)]} | Qi et al. (1994) |
| Medium Resolution Imaging Spectrometer (MERIS) | (R+NIR)/2 | Dash and Curran. (2004) |
| MERIS terrestrial chlorophyll index (MTCI) | (NIR-RE)/(RE-R) | Modified from Dash and Curran. (2004) |
| Difference vegetation index (DVI) | NIR-R | Tucker (1979) |
| Red edge difference vegetation index (REDVI) | NIR-RE | Modified from Tucker (1979) |
| Renormalized difference vegetation index (RDVI) | (NIR-R)/SQRT(NIR+R) | Roujean and Breon (1995) |
| Transformed Normalized vegetation index (TNDVI) | SQRT[(NIR-R)/(NIR+R)+0.5] | Sandham and Zietsman. (1997) |
| Normalized difference index (NDI) | (NIR-RE)/(NIR-R) | Datt (1999) |
| Simple ratio vegetation index (SR) | RE/R | Modified from McMurtrey et al. (1994) |

## Plant sampling and measurement

Rice plant samples were acquired the same day as image acquisition for each growth stage (PI stage and SE stage). At the measurement dates, destructive plant samples of above ground biomass were taken by randomly clipping three hills according to the average tillering numbers in each plot. All samples were rinsed with water, and the roots were removed. The samples were separated into leaves and stems, which were put into oven for deactivation of enzymes under 105°C for half an hour, and then dried at 80°C until constant weight and weighed. Plant nitrogen concentration (PNC) was analyzed using the Kjeldahl-N method, and the plant N uptake was determined by multiplying plant N concentration with dry biomass.

## Calculation of nitrogen nutrition index

The critical N dilution curve of rice in Northeast China developed by Huang et al. (2015) was used in this study:

$$N_c = 27.7\ W^{-0.34} \qquad (1)$$

where $N_c$ is the critical N concentration expressed as g $kg^{-1}$ dry matter (DM) and W is the aboveground biomass (AGB) expressed in Mg DM $ha^{-1}$.

The NNI was calculated following Lemaire et al. (2008):

$$NNI = N_a/N_c \qquad (2)$$

where $N_a$ is the actual measured N concentration and $N_c$ is the critical N concentration as determined by (1).

### Statistical analysis

Data collected from the N rate experiments were pooled together and then randomly divided into calibration dataset (75% of the observations) and validation dataset (25% of the observations). The mean value, standard deviation (SD) and the coefficient of variation (CV, %) of rice agronomic parameters were calculated using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Correlation and stepwise multiple linear regression were performed to establish the relationships between VIs and agronomic parameters or VIs between UAV remote sensing and Crop Circle sensor using SPSS 18.0 (SPSS Inc., Chicago, Illinois, USA), and the model with the highest coefficient of determination ($R^2$) were selected. In addition to $R^2$, the performance of the model for predicating rice status indicators was also evaluated using the root mean square error (RMSE) and the relative error (REr).

Nitrogen sufficiency index (NSI) was calculated using Crop Circle ACS-430 VIs (NSI-VIs) of check plots or plots receiving different N rates divided by that of N-rich plots (plots receiving sufficient N supply). In this study, the plots of 160 kg N $ha^{-1}$ were used as N rich plots, and their average VI values were used for NSI calculation.

The N treatment plots were grouped into three classes based on NNI values: deficit (NNI < 1.0), optimal (1.0 ≤ NNI ≤ 1.1) and surplus (NNI > 1.1). Different N status diagnostic approaches were compared with areal agreement and kappa statistics (Campbell and Wynne 2011). The areal agreement is the percentage of plots that shared a common classification and the Kappa statistics provides a more robust measure of how two classifications agreed compared with a “chance” agreement and was, therefore, a more rigorous statistical indicator to compare two classifications.

## Results and Discussion

### Variability of rice nitrogen status indicators

The rice N status indicators varied greatly across different N rate treatments, growth stages and varieties (Table 2). Across growth stages, the AGB was most variable, with CV being 49%, followed by PNU (CV = 41%), PNC (CV = 16%) and NNI (CV = 14%). The validation dataset had similar variability as the calibration dataset. These results indicated that the similar variability of these parameters for calibration and validation made it a good dataset to evaluate the potential of using Crop Circle ACS-430 for estimating rice N status.

**Table 2. Descriptive statistics of rice aboveground biomass (AGB), plant N concentration (PNC), plant N uptake (PNU) and N nutrition index (NNI) for calibration and validation datasets across different N rate treatments, varieties and stages in 2014.**

| Variety | AGB (t $ha^{-1}$) | | | PNC (g $kg^{-1}$) | | | PNU (kg $ha^{-1}$) | | | NNI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | CV | Mean | SD | CV | Mean | SD | CV | Mean | SD | CV |
| Calibration dataset | | | | | | | | | | | | |
| Across both stages (n=63) | 2.78 | 1.36 | 49 | 18.2 | 2.82 | 16 | 48.7 | 19.9 | 41 | 0.89 | 0.13 | 14 |
| Validation dataset | | | | | | | | | | | | |
| Across both stages (n=21) | 2.87 | 1.36 | 47 | 18.1 | 2.81 | 16 | 47.5 | 18.3 | 38 | 0.89 | 0.12 | 14 |

SD: standard deviation of the mean; CV: coefficient of variation, CV in %.

### Relationships between vegetation indices derived from Crop Circle ACS-430 and rice N status indicators

The top 5 indices with the highest $R^2$ among the 15 indices evaluated in this study are listed in Table 3. Across varieties and stages, the top 5 VIs performed similarly well for estimating AGB ($R^2$ = 0.79-0.80) and PNU ($R^2$ = 0.86-0.87), but very poor for PNC ($R^2$ = 0.16-0.21). The performance of VIs differed with growth stages. At the PI stage, red edge difference vegetation index (REDVI) was best for estimating AGB ($R^2$ = 0.78) and PNU ($R^2$ = 0.87), which was stronger than the best VIs estimating AGB ($R^2$ = 0.70) and PNU ($R^2$ = 0.77) at the stem elongation (SE) stages. REDVI and MERIS terrestrial chlorophyll index (MTCI) as red edge VI explained 54% and 51% of PNC at PI and SE stage, respectively.

The top 5 VIs were further evaluated with validation data across varieties and stages in 2014 (Table 4). For AGB, the models of the top 5 VIs performed better ($R^2$= 0.83-0.92, RE = 6.8-20.8%) than PNU ($R^2$= 0.58-0.81, RE = 15.8-20.8%) either at a single stage or across both stages. The top 5 VIs did not perform as well ($R^2$= 0.22-0.43, RE = 5.3-14.0%) for estimating PNC.

**Table 3. Top 5 coefficients of determination ($R^2$) for the relationships between vegetation indices calculated from Crop Circle ACS-430 and rice aboveground biomass (AGB), plant N concentration (PNC) and plant N uptake (PNU) across varieties and stages in 2014.**

| Stage | AGB ($t\ ha^{-1}$) | | | | PNC ($g\ kg^{-1}$) | | | | PNU ($kg\ ha^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Index | Model | $R^2$ | p | Index | Model | $R^2$ | p | Index | Model | $R^2$ | p |
| PI | RERVI | Q | 0.78 | *** | RERVI | Q | 0.54 | ** | RERVI | Q | 0.87 | *** |
| | REDVI | Q | 0.78 | *** | REDVI | Q | 0.53 | ** | REDVI | Q | 0.87 | *** |
| | NDRE | Q | 0.77 | *** | NDRE | Q | 0.53 | ** | NDRE | Q | 0.86 | *** |
| | DVI | Q | 0.76 | *** | DVI | Q | 0.48 | ** | DVI | Q | 0.83 | *** |
| | MSAVI | Q | 0.76 | *** | RVI | Q | 0.48 | ** | RVI | Q | 0.83 | *** |
| SE | SR | Q | 0.70 | *** | MTCI | Q | 0.51 | *** | RVI | Q | 0.77 | *** |
| | NDVI | Q | 0.63 | *** | NDI | Q | 0.50 | *** | MSAVI | Q | 0.76 | *** |
| | TNDVI | Q | 0.63 | *** | SR | Q | 0.15 | * | SAVI | Q | 0.75 | *** |
| | RVI | Q | 0.63 | *** | RVI | - | - | NS | RDVI | Q | 0.75 | *** |
| | MERIS | Q | 0.62 | *** | NDVI | - | - | NS | DVI | Q | 0.75 | *** |
| Across both stages | RVI | P | 0.80 | *** | SR | E | 0.21 | *** | DVI | E | 0.87 | *** |
| | MERIS | P | 0.80 | *** | RVI | P | 0.17 | ** | MSAVI | E | 0.87 | *** |
| | NDVI | E | 0.79 | *** | TNDVI | Q | 0.16 | ** | SAVI | E | 0.86 | *** |
| | MSAVI | E | 0.79 | *** | NDVI | Q | 0.16 | ** | RDVI | E | 0.86 | *** |
| | SR | P | 0.79 | *** | MERIS | Q | 0.16 | ** | NDRE | E | 0.86 | *** |

L, Q, E and P denote linear, quadratic, exponential and power fit.*, ** and *** mean significance at p<0.05, 0.01, and 0.001, respectively. NS means no significance at p<0.05.

**Table 4. Validation results of the top 5 vegetation indices for estimating rice aboveground biomass (AGB), plant N concentration (PNC) and plant N uptake (PNU) across varieties and stages in 2014.**

| | PI | | | | SE | | | | Across both stages | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Index | $R^2$ | RMSE | RE (%) | Index | $R^2$ | RMSE | RE (%) | Index | $R^2$ | RMSE | RE (%) |
| AGB ($t\ ha^{-1}$) | RERVI | 0.92 | 0.10 | 7.0 | SR | 0.87 | 0.43 | 11.8 | RVI | 0.92 | 0.53 | 18.5 |
| | REDVI | 0.92 | 0.10 | 6.8 | NDVI | 0.84 | 0.48 | 13.3 | MERIS | 0.91 | 0.59 | 20.4 |
| | NDRE | 0.92 | 0.10 | 6.8 | TNDVI | 0.84 | 0.48 | 13.3 | NDVI | 0.92 | 0.53 | 18.3 |
| | DVI | 0.91 | 0.10 | 7.4 | RVI | 0.85 | 0.47 | 13.0 | MSAVI | 0.91 | 0.59 | 20.5 |
| | MSAVI | 0.91 | 0.10 | 7.3 | MERIS | 0.83 | 0.49 | 13.7 | SR | 0.92 | 0.60 | 20.8 |
| PNC ($g\ kg^{-1}$) | RERVI | 0.43 | 1.09 | 5.4 | MTCI | 0.27 | 2.11 | 12.6 | SR | 0.32 | 2.36 | 13.0 |
| | REDVI | 0.43 | 1.09 | 5.4 | NDI | 0.27 | 2.12 | 12.6 | RVI | 0.28 | 2.35 | 13.0 |
| | NDRE | 0.43 | 1.08 | 5.3 | SR | 0.25 | 2.34 | 14.0 | TNDVI | 0.34 | 2.37 | 13.1 |
| | DVI | 0.30 | 1.19 | 5.9 | RVI | - | - | NS | NDVI | 0.34 | 2.37 | 13.1 |
| | RVI | 0.22 | 1.25 | 6.2 | NDVI | - | - | NS | MERIS | 0.34 | 2.37 | 13.1 |
| PNU ($kg\ ha^{-1}$) | RERVI | 0.65 | 5.84 | 19.5 | RVI | 0.65 | 9.28 | 16.5 | DVI | 0.79 | 8.16 | 17.2 |
| | REDVI | 0.66 | 5.85 | 19.5 | MSAVI | 0.63 | 9.02 | 16.0 | MSAVI | 0.81 | 7.90 | 16.6 |
| | NDRE | 0.66 | 5.87 | 19.6 | SAVI | 0.64 | 8.90 | 15.8 | SAVI | 0.81 | 7.91 | 16.7 |
| | DVI | 0.60 | 6.16 | 20.5 | RDVI | 0.64 | 8.87 | 15.8 | RDVI | 0.81 | 7.88 | 16.6 |
| | RVI | 0.59 | 5.98 | 19.9 | DVI | 0.58 | 9.41 | 16.7 | NDRE | 0.70 | 9.87 | 20.8 |

RMSE: root mean square error; REr: relative error

## Relationships between vegetation indices derived from Crop Circle ACS-430 and nitrogen nutrition index

The top 5 VIs and the NNI values calculated with Crop Circle ACS-430 (NSI-VIs) with the highest coefficients of determination ($R^2$) among the 15 indices evaluated for estimating NNI in this study are listed in Table 5. At PI stage, the top 5 original VIs had a slightly better correlation with NNI than NSI-VIs. However, the top 5 NSI-VIs were more strongly related to NNI than the original VIs ($R^2$ = 0.38-0.56) either at SE stage or across both stages, with relatively more stable performance ($R^2$ = 0.57-0.69). The validation results confirmed this observation, especially across growth stages (NSI-VIs： $R^2$ = 0.69-0.74, RE = 6.4-7.1%； VIs： $R^2$ = 0.43-0.46, RE = 9.8-10.2%) (Table 6). Well-fertilized reference plots have been commonly used to reduce the influence of other confounding factors on sensor-based N status diagnosis and eliminate the need to develop site-specific calibrations (Samborski et al. 2009).

**Table 5. Top 5 coefficients of determination ($R^2$) for the relationships between VIs and NSI-VIs calculated from Crop Circle ACS-430 and N nutrition index across varieties and stages in 2014.**

| Stage | Index | Model | $R^2$ | p | Index | Model | $R^2$ | p |
|---|---|---|---|---|---|---|---|---|
| PI | RERVI | Q | 0.80 | *** | NSI-REDVI | Q | 0.72 | *** |
| | REDVI | Q | 0.80 | *** | NSI-NDRE | Q | 0.72 | *** |
| | NDRE | Q | 0.79 | *** | NSI-RERVI | Q | 0.72 | *** |
| | DVI | Q | 0.74 | *** | NSI-DVI | Q | 0.70 | *** |
| | MSAVI | Q | 0.73 | *** | NSI-MSAVI | Q | 0.69 | *** |
| SE | RERVI | Q | 0.56 | *** | NSI-RERVI | Q | 0.65 | *** |
| | REDVI | Q | 0.56 | *** | NSI-REDVI | Q | 0.65 | *** |
| | NDRE | Q | 0.56 | *** | NSI-NDRE | Q | 0.64 | *** |
| | MTCI | Q | 0.53 | *** | NSI-RVI | Q | 0.58 | *** |
| | NDI | Q | 0.53 | *** | NSI-DVI | Q | 0.57 | *** |
| Across both stages | RERVI | E | 0.48 | *** | NSI-NDRE | Q | 0.69 | *** |
| | REDVI | E | 0.48 | *** | NSI-REDVI | Q | 0.68 | *** |
| | NDRE | E | 0.48 | *** | NSI-RERVI | Q | 0.66 | *** |
| | DVI | E | 0.41 | *** | NSI-DVI | Q | 0.64 | *** |
| | MSAVI | P | 0.38 | *** | NSI-MSAVI | Q | 0.63 | *** |

L, Q, E and P denote linear, quadratic, exponential and power fit.*, ** and *** mean significance at $p < 0.05$, 0.01, 0.001, respectively. NS means no significance at $p < 0.05$.

**Table 6. Validation results of the top 5 VIs and NSI-VIs calculated from Crop Circle ACS-430 and N nutrition index (NNI) across varieties and stages in 2014.**

| Stage | Index | $R^2$ | RMSE | RE (%) | Index | $R^2$ | RMSE | RE (%) |
|---|---|---|---|---|---|---|---|---|
| PI | RERVI | 0.93 | 0.04 | 5.4 | NSI-REDVI | 0.92 | 0.04 | 5.2 |
| | REDVI | 0.93 | 0.04 | 5.3 | NSI-NDRE | 0.92 | 0.04 | 5.3 |
| | NDRE | 0.93 | 0.04 | 5.3 | NSI-RERVI | 0.92 | 0.04 | 5.3 |
| | DVI | 0.89 | 0.05 | 6.4 | NSI-DVI | 0.90 | 0.05 | 5.7 |
| | MSAVI | 0.88 | 0.06 | 6.8 | NSI-MSAVI | 0.90 | 0.05 | 6.2 |
| SE | RERVI | 0.33 | 0.08 | 8.4 | NSI-RERVI | 0.48 | 0.06 | 6.6 |
| | REDVI | 0.33 | 0.08 | 8.4 | NSI-REDVI | 0.46 | 0.06 | 6.7 |
| | NDRE | 0.33 | 0.08 | 8.4 | NSI-NDRE | 0.45 | 0.06 | 6.7 |
| | MTCI | 0.35 | 0.08 | 8.5 | NSI-RVI | 0.40 | 0.06 | 7.2 |
| | NDI | 0.35 | 0.08 | 8.5 | NSI-DVI | 0.45 | 0.06 | 6.7 |
| Across both stages | RERVI | 0.46 | 0.09 | 9.8 | NSI-NDRE | 0.74 | 0.06 | 6.5 |
| | REDVI | 0.46 | 0.09 | 9.9 | NSI-REDVI | 0.74 | 0.06 | 6.4 |
| | NDRE | 0.46 | 0.09 | 10.0 | NSI-RERVI | 0.72 | 0.06 | 6.8 |
| | DVI | 0.44 | 0.09 | 10.0 | NSI-DVI | 0.70 | 0.06 | 7.0 |
| | MSAVI | 0.43 | 0.09 | 10.2 | NSI-MSAVI | 0.69 | 0.06 | 7.1 |

RMSE: root mean square error; RE: relative error

## Relationships between Crop Circle VIs or NSI-VIs and UAV remote sensing

Using stepwise multiple linear regression and UAV remote sensing reflectance, 66-82% of VIs or NNI-VIs derived from Crop Circle ACS-430 at PI stage could be explained. At SE stage, 70-78% of the best VIs or NNI-VIs variability could be explained.

**Table 7. Stepwise multiple linear regression models based on Mini-MCA bands for estimating the top VIs and NNI-VIs of Crop Circle ACS-430 (CC430) at the panicle initiation (PI) and stem elongation (SE) stages across varieties in 2014.**

| Stage | Index for CC430 | Regression equation by Mini-MCA bands | $R^2$ | $R_{adj}^2$ | SE |
|---|---|---|---|---|---|
| PI | RERVI | 1.288+1.451*NIR-2.129*G | 0.82 | 0.80 | 0.04 |
| | RVI | -0.090+10.558*NIR-9.928*G | 0.75 | 0.73 | 0.41 |
| | SR | -0.425+4.315*NIR | 0.66 | 0.65 | 0.23 |
| | DVI | 0.093+0.472*NIR-0.536*G | 0.74 | 0.72 | 0.02 |
| | NSI-REDVI | 0.407+3.826*NIR-5.340*RE | 0.76 | 0.74 | 0.07 |
| | NSI-NDRE | 0.454+3.512*NIR-4.895*RE | 0.75 | 0.73 | 0.07 |
| SE | SR | 0.271+7.289*NIR-9.966*R | 0.72 | 0.71 | 0.23 |
| | MTCI | 0.550+2.958*NIR+17.657*B-11.466*G | 0.72 | 0.71 | 0.06 |
| | RVI | -0.342+16.093*NIR-27.475*R | 0.75 | 0.74 | 0.49 |
| | RERVI | 1.071+3.089*NIR-3.010*RE | 0.75 | 0.74 | 0.05 |
| | DVI | 0.072+0.691*NIR-0.412*RE | 0.70 | 0.69 | 0.02 |
| | NSI-RERVI | 0.366+2.290*NIR-2.814*RE+2.870*B | 0.78 | 0.77 | 0.03 |

| | | | | |
|---|---|---|---|---|
| NSI-NDRE | -0.075+3.803*NIR-4.539*RE+4.686*B | 0.77 | 0.76 | 0.05 |

SE: standard error of the estimate

### Evaluating different nitrogen status diagnostic approaches by Crop Circle ACS-430 and UAV remote sensing

Different approaches can be taken to non-destructively estimate NNI with Crop Circle ACS-430 and UAV remote sensing. There are four approaches to estimate NNI only using Crop Circle ACS-430. The first one is to estimate AGB and PNC using the best performance VIs from Crop Circle ACS-430, and from the estimated biomass, $N_c$ can be determined using the established critical N dilution curve, and NNI can then be calculated (CC-PNC-NNI). The second approach is to use the best performing VIs to estimate biomass and PNU. Using the estimated biomass, $N_c$ can be calculated. The product of biomass and $N_c$ is critical PNU ($PNU_c$), and NNI can be calculated as the ratio of PNU and $PNU_c$ (CC-PNU-NNI). The third approach is to estimate NNI directly using the best performing VIs (CC-NNI). The fourth approach is to use best NSI-VIs to estimate NNI directly (CC-NSI-NNI). There are also another four approaches to estimate NNI by combining Crop Circle ACS-430 and UAV remote sensing. By estimating the best performing VIs and NSI-VIs of Crop Circle ACS-430 by 5 band Mini-MCA image data to further estimate AGB, PNC, PNU and NNI indirectly, NNI can be calculated in the same way as the previous four approaches (UAV&CC-PNC-NNI, UAV&CC-PNU-NNI, UAV&CC-NNI and UAV&CC-NSI-NNI), respectively.

To evaluate the diagnosis accuracy of these different approaches, the experimental plots were divided into three classes: N deficient, N optimal and N surplus based on destructively measured NNI and the threshold values proposed in this study. The diagnosis results of different approaches were compared with the results based on measured NNI. According to Landis and Koch (1977), the strength of the agreement was fair, moderate and substantial if the Kappa statistics was 0.21 - 0.40, 0.41 - 0.60 and 0.61 - 0.80, respectively. At PI stage, the approaches by combining Crop Circle ACS-430 and Mini-MCA performed even better than using Crop Circle ACS-430 directly (Table 8). However, at SE stage and across both stages, the Crop Circle ACS-430-based approaches were better.

The results indicated that the $NSI_{VIs}$-NNI approach performed better than using VIs. At PI stage, the UAV&CC-$NSI_{VIs}$-NNI performed the best, with the highest accuracy rate (90%) and kappa statistics (0.62), while the CC-$NSI_{VIs}$-NNI performed the best at SE stage and across both stages, with the accuracy rate of 81% and 79% and kappa statistics of 0.61 and 0.48, respectively. The correlation between the bands of Crop Circle ACS-430 or UAV images and NNI at SE stage were much weaker than those at PI stage (Table 9). More studies are needed to further evaluate these different approaches.

## CONCLUSION

Crop Circle ACS-430-based VIs explained 79-80% and 86-87% variability of AGB and PNU, respectively, but had very poor relationship with PNC ($R^2$ = 0.16-0.21) across both stages. The N sufficiency indices calculated with Crop Circle ACS 430 (NSI-VIs) had better correlation with NNI than the original VIs, especially at SE stage and across both stages, the $R^2$ of the best NSI-VIs were 0.65 and 0.69. UAV-based remote sensing system could be used to estimate Crop Circle VIs and NSI-VIs well at PI and SE stage. The $NSI_{VIs}$-NNI approach worked well for diagnosing rice N status. Combining UAV-based remote sensing and Crop Circle ACS 430 had a good potential for in-season diagnosis of rice N status at PI stage, with the highest accuracy rate (90%) and kappa statistics (0.62) among the approaches tested, but did not perform well at SE stage and across both stages. More studies are needed to further evaluate these approaches and improve their performances.

**Table 8. Areal agreement and kappa statistics for different N status diagnostic approaches across varieties and stages in 2014.**

| Growth Stage | Approach | Number | Areal agreement (%) | Kappa statistics |
|---|---|---|---|---|
| PI | CC-PNC-NNI | 30 | 83 | 0.43 |
| | CC-PNU-NNI | | 83 | 0.43 |
| | CC-NNI | | 83 | 0.43 |
| | CC-$NSI_{VIs}$-NNI | | 80 | 0.40 |
| | UAV&CC-PNC-NNI | | 87 | 0.54 |
| | UAV&CC-PNU-NNI | | 87 | 0.54 |
| | UAV&CC-NNI | | 87 | 0.54 |
| | UAV&CC-$NSI_{VIs}$-NNI | | 90 | 0.62 |
| SE | CC-PNC-NNI | 54 | 72 | 0.45 |
| | CC-PNU-NNI | | 69 | 0.27 |
| | CC-NNI | | 72 | 0.40 |
| | CC-$NSI_{VIs}$-NNI | | 81 | 0.61 |
| | UAV&CC-PNC-NNI | | 67 | 0.34 |
| | UAV&CC-PNU-NNI | | 56 | -0.09 |
| | UAV&CC430-NNI | | 65 | 0.22 |
| | UAV&CC-$NSI_{VIs}$-NNI | | 65 | 0.24 |
| Across both stages | CC-PNC-NNI | 84 | 74 | 0.42 |
| | CC-PNU-NNI | | 74 | 0.35 |
| | CC-NNI | | 76 | 0.42 |
| | CC-$NSI_{VIs}$-NNI | | 79 | 0.48 |
| | UAV&CC-PNC-NNI | | 65 | 0.13 |
| | UAV&CC-PNU-NNI | | 67 | 0.29 |
| | UAV&CC-NNI | | 71 | 0.27 |
| | UAV&CC-$NSI_{VIs}$-NNI | | 76 | 0.38 |

**Table 9.Coefficients of determination ($R^2$) for the relationships between reflectance of Crop Circle ACS-430 bands and Mini-MCA (UAV) and NNI across varieties and stages in 2014.**

| Stage | Bands | CC430 | | UAV | |
|---|---|---|---|---|---|
| | | $R^2$ | p | $R^2$ | p |
| PI | NIR | 0.82 | *** | 0.78 | *** |
| | RE | 0.83 | *** | 0.69 | *** |
| | R | 0.71 | *** | 0.47 | *** |
| | G | | | - | NS |
| | B | | | 0.35 | ** |
| SE | NIR | 0.52 | *** | - | NS |
| | RE | 0.53 | *** | - | NS |
| | R | 0.15 | * | 0.19 | ** |
| | G | | | 0.17 | * |
| | B | | | 0.18 | ** |

L, Q, E and P denote linear, quadratic, exponential and power fit.*, ** and *** mean significance at $p < 0.05$, 0.01, 0.001, respectively. NS means no significance at $p < 0.05$.


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