

# Remote sensing for response to Nitrogen fertilizer in maize

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## ABSTRACT

Modern maize hybrids prolong the period that they photosynthesize and accumulate Nitrogen (N) out of the soil which has helped them produce more yield per unit of N fertilizer. However, the increase in post flowering activity is inversely correlated with N remobilization from the leaves. Further gains in N response could be achieved by breaking this association, but doing so requires an in-depth understanding of the temporal dynamics of maize canopy traits and plant N mobilization. Leaf nutrient samples were collected at five time points and remote sensing phenotypes were extracted from Unoccupied Aerial System (UAS) imagery (orthomosaics and point clouds). Spectral indices and point-cloud based metrics were used to investigate the relationship between changes in N storage dynamics and yield among hybrids grown in low and high N treatments. From these combined phenotypes, it is possible to dissect how rate of growth and canopy health help to describe hybrid response N and also provide clues for how to break the negative relationship between yield and N remobilization.

**Keywords:** Remote sensing, Nitrogen, Canopy, Temporal

## 1. INTRODUCTION

Plant breeding has indirectly improved maize hybrids' ability to respond to increasing N input, in pursuit of higher yield[1]. Adaptation of hybrids to higher input availability has significantly altered the way modern hybrids grow and metabolize N[2]. Modern hybrids accumulate more yield and have lower grain N%, but because yield gains have outpaced decline in grain N%, the total amount of N removed from the field at harvest has overall increased[3]. Decreased grain N% is associated with significant changes in the plant post-flowering including increased leaf N% and photosynthetic capability combined with continued N accumulation from the roots (hereafter referred to as stay-green)[4]. Thus, modern hybrids can support the resource demands of the growing grain without sacrificing the ability to continue nutrient accumulation.

Although the stay-green trait is generally beneficial it is inversely related to the ability to remobilize N out of the leaf and into the grain. The balance between stay-green and remobilization is delicate. Stay-green can come at a yield cost if it prevents the grain from receiving adequate resources in time[5]. Conversely, too much remobilization will drain the leaves of vital resources and end stay-green activity prematurely[6]. There exists significant variation for N remobilization potential among maize, although modern hybrids strongly favor post-flowering uptake[3]. Under N-limiting conditions, maize hybrids can source approximately 63% of grain N from leaf tissue but under non-limiting conditions only 50% is remobilized[7]. Breaking the inverse relationship between remobilized and reproductive N could help unlock further gains in N response by taking full advantage of the different sources of N available to the

grain (remobilized vs. acquired after flowering). It is not quite known if this association is an inescapable consequence of stay-green or if mechanisms exist that allow plants to effectively maintain both sources. It has been demonstrated that the amount of remobilized N is positively associated with the amount of N accumulated at flowering[4], so increasing the amount of N available for remobilization may be a valid strategy to improve this trait. Another strategy would likely require an in-depth understanding of the timing and regulation of senescence in plants within the context of their development.

Unoccupied aerial systems (UAS) provide an opportunity to collect more observations of the canopy and discover clues on how to break-up the remobilization conundrum. Remote sensing (RS) phenotypes can be leveraged in multiple ways to ask questions about plant biology. They can provide accurate representations of defined canopy phenotypes including height modeled from point-cloud data[8] but also more abstract measures such as plot level spectral index values. Spectral indices are calculated from image reflectance data, and can be used as a proxy for important traits like canopy stay-green[9]. UAS technology can thus be leveraged to expand on the number of time-points and subjects considered in an experiment which will increase the power to detect meaningful differences, especially for a temporally sensitive trait such as senescence. RS phenotypes were extracted from UAS imagery, and leaf and grain nutrient samples were collected concurrently to answer questions about how the temporal dynamics of maize hybrids are related to changes in N metabolism. Additionally, comparisons were made among individual hybrids to uncover insights on enhancing remobilization without sacrificing stay-green and yield.

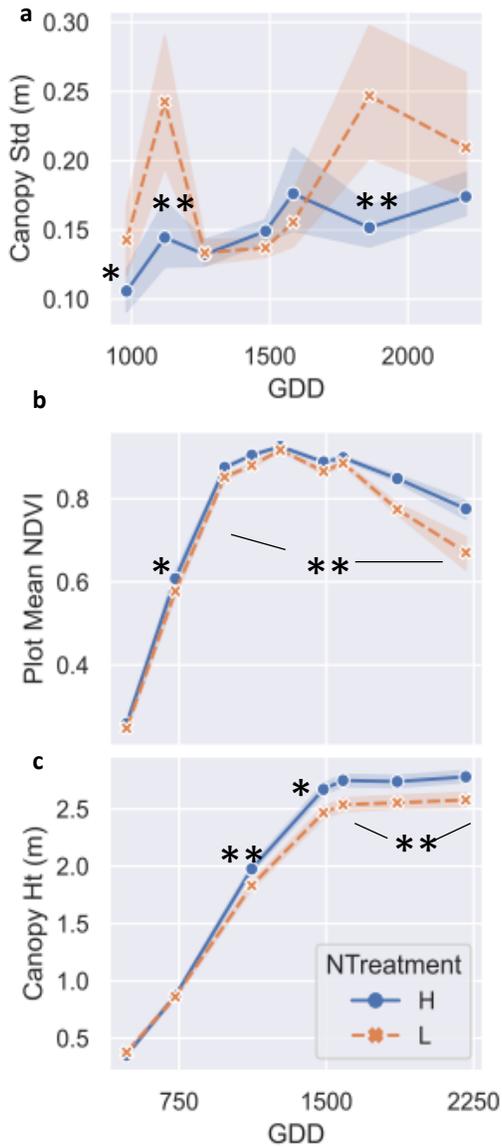
## **2. MATERIALS AND METHODS**

Sixteen maize hybrids were grown in a field located in Michigan State University, 2020. The field was planted on 25 May. All plants received 26 pounds of N per acre as a pre-plant application. Half of the plots received an additional 122 pounds N/acre 24 days after sowing (DAS) as side-dress. For 5 hybrids, the ear leaf from 2 plants was collected, dried, and analyzed for dry weight nutrient content at 56, 64, 71, and 85 DAS. Two ears were collected from each plot 140 DAS for grain nutrient analysis. All plots were harvested with a combine at 154 DAS (2570 GDD) and the yield was moisture adjusted to 15.5%. Leaf N remobilization (NRemo) was calculated as the percent decline in ear leaf N between 1585 GDD and 1861 GDD (approximately the R3 to R4 development stages).

Simultaneously with leaf N collection, 5-band MS reflectance orthomosaics and RGB derived point-cloud data were collected via UAS in addition to a later season flight 104 DAS. Raw UAS imagery was processed into orthorectified rasters and las files by the RS&GIS department at MSU. Shape files to describe plot boundaries were created with the R library UAStools[10], and fine-tuned manually in ArcGIS. Plot level reflectance and point-cloud traits were extracted from each date using automated scripts in ArcPy[11]. Briefly, vegetative pixels were segmented from the soil based on normalized difference vegetation index (ndvi) and then spectral values extracted. For height and canopy standard deviation (Std), a digital elevation model (dem) of the field was constructed from early season point-cloud data; at subsequent time points, plant height and std were calculated from the difference between ground dem and plant dem at a resolution of 15x15 cm.

### 3. RESULTS AND DISCUSSION

#### 3.1 Remote sensing phenotypes are sensitive to N treatment

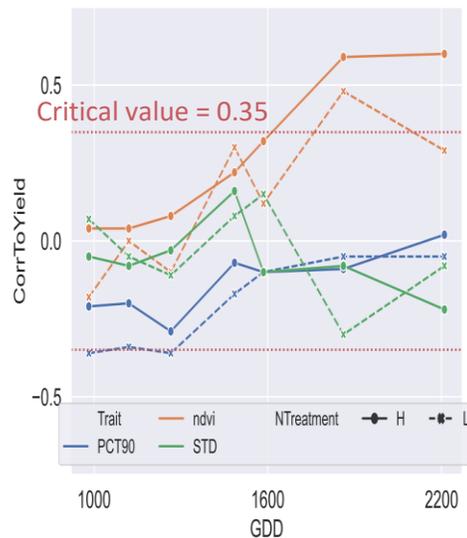


**Figure 1.** Std dev of canopy height, plot mean ndvi, and canopy height changes plotted over total growing degree days (GDD). Significance between N treatments from full 2-way ANOVA: \*  $p < 0.$ , \*\*  $p < 0.001$

#### 3.2 RS phenotypes provide clues for productive N remobilization

There was significant variation among the hybrids for Grain N %, yield, and NRemo (Table 1). NRemo was indeed negatively correlated with yield in both H and L treatments (Pearson  $r$  -0.36 and -0.31 respectively). One hybrid did exhibit desirable traits for both yield and NRemo. The PHB47xPHN82 hybrid (PxP) was exceptional as the highest yielder and ranked third for N remobilization in H (Fig 3).

The RS phenotypes exhibit a strong connection with N treatment. Both height and ndvi exhibit noticeable differences between treatments (Fig 1). Perhaps even more intriguing is the consideration of canopy std. This value represents the average distance at which a canopy dem pixel is detected from the mean canopy height. One might anticipate that a uniform canopy would be optimal for efficient light collection, and it does appear that N treatment influences the uniformity of canopy std (Fig 1). To investigate the relationship between these sensitive phenotypes and yield within each respective N treatment, the correlation coefficients for each were plotted over GDD. Each trait displays a similar trend, whether measured in low (L) or high (H) conditions. Height shows the strongest association early in the season,



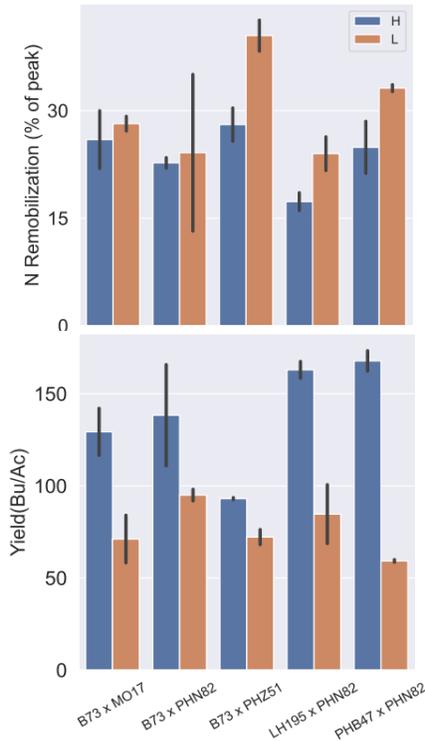
**Figure 2.** Std dev of canopy height, plot mean ndvi, and canopy height(PCT90) Pearson's correlation plotted over GDD. Critical value for sig. association at 30 degrees of freedom and  $\alpha = 0.05$ .

while ndvi exhibits the highest correlation in the late season (Fig 2). A negative  $r$  is expected if canopy uniformity increased light collection. While std never passed the critical value, it did approach the threshold late in the season (Fig 2).

PxP shared a similar habit as the hybrid with the 2<sup>nd</sup> highest yield, LH195xPHN82 (LxP), but they differed tremendously in their capacity for NRemo (Fig 3).

**Table 1.** ANOVA p-value for various traits among 5 hybrids.

|            | Grain N% | R2 N%   | R3 N%   | NReMo   | Yield   |
|------------|----------|---------|---------|---------|---------|
| Hybrid     | < 0.001  | 0.163   | 0.155   | < 0.001 | < 0.001 |
| Ntreatment | < 0.001  | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| H*N        | < 0.001  | 0.134   | 0.551   | < 0.001 | < 0.001 |

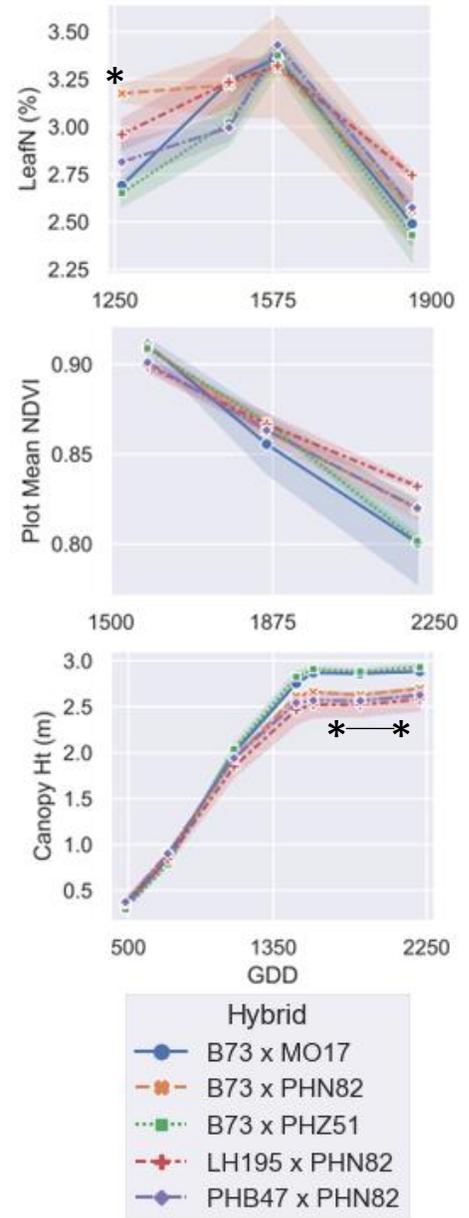


**Figure 3.** NRemo and yield among hybrids with leaf N.

Both hybrids grew to a shorter total height (Fig 4) and accumulated the most Grain N (not shown). However, LxP maintained higher ndvi in its canopy past the R3 stage when compared to PxP, but importantly, ndvi in PxP did not decline as rapidly as other hybrids (Fig 4). Further, between the 2<sup>nd</sup> and 3<sup>rd</sup> time-points of Leaf N, the week after flowering, PxP rapidly accumulates N in the ear leaf when compared to LxP. Following peak, the N in PxP exits with haste while in LxP it slowly attenuates, matching the relative slope of ndvi decline (Fig 4). In total, the key steps for PxP appear to be to halt growth early, build up a large bank of Leaf N, and then to remobilize it without sacrificing canopy health as measured by ndvi.

#### 4. CONCLUSIONS

This study illustrates how RS phenotypes can be combined with traditional measures to answer difficult biological questions. Coordination of growth, N metabolism, and senescence appear as key factors that help PHB47xPHN82 achieve active N remobilization without sacrificing yield. The response to N is a complex trait that is significantly affected by the environment so this study could be further empowered by the addition of multiple year/site locations.



**Figure 4.** Ear leaf N (%), plot mean ndvi, and canopy height plotted over different scales of GDD. \* sig for hybrid effect in H: \* p < 0.05

## 5. DATA AVAILABILIT STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

## 6. ACKNOWLEDGEMENTS

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