ICPA
17th INTERNATIONAL CONFERENCE ON PRECISION AGRICULTURE
ConBAP 2026
CONGRESSO BRASILEIRO DE AGRICULTURA DE PRECISÃO E DIGITAL
13-16 July 2026 | Porto Alegre, Brazil
ISPA AsBraAP

Detection of Chlorophyll A and B in Maize Using Visible Reflectance under Conventional and Variable Rate Nitrogen Management

Thiago Orlando Costa Barboza[1], Octávio Pereira da Costa[1], Raí Fernandes Alves Queiroz[1], Sérgio Henrique Godinho Silva[1], Lorena Nunes Lacerda[2], Adão Felipe dos Santos[1]

[1]Lavras School Agricultural Science (ESAL), Federal University of Lavras (UFLA)

[2]Integrative Precision Agriculture, Crop and Soil Science Department, University of Georgia

A paper from the Proceedings of the
17th International Conference on Precision Agriculture and 11th Brazilian Congress on Precision and Digital Agriculture
13-15 July 2026
Porto Alegre, Brazil

Abstract.

Plant-environmental interactions regulate physiological aspects that determine crop yield. However, measuring physiological parameters in-field remains a challenge, as sampling often requires time-consuming laboratory analysis. Therefore, this study analyzed the influence of reflectance using a handheld sensor in detecting chlorophyll A and B in different nitrogen application strategies before and after side-dressing. The experiment was conducted at a commercial farm in Campo do Meio, Minas Gerais, Brazil, during the 2025 second crop season (April–September). A split-plot design with randomized block was used, with Biofertilizer (Azospirillum brasilense – BF and without biofertilizer NBF) as the main factor, and Nitrogen Management (Conventional Farm Production – CFP and Variable Rate Nitrogen – VRN) as the secondary factor. Leaf reflectance (400–700 nm, 10 nanometers intervals) was measured using the NIX Spectra L®, Hamilton, Ontario, Canada, and chlorophyll A and B levels were determined using the ClorofiLOG® Falker, São João, Porto Alegre, Brazil at 32 days after planting (DAP) and at the Tasseling stage (VT/68 DAP). Data analysis included descriptive statistics, feature selection using the wrapper algorithm (Random Forest) via the Boruta package in RStudio and the analysis of variance performance using the AgroR package in RStudio. Results indicated a distinct temporal dynamic, where Chlorophyll B increased by approximately 72% from 32 to 68 DAP, presenting the highest values in the CFP and BF combination (18% higher than VRN/NBF). Feature selection identified specific wavelengths for Chlorophyll A and B in all dates that most appear for all treatments (530, 560, 570, 690 and 700 nm). Nevertheless, the blue region (400 nm) was the region with the lowest interaction following the Boruta analysis for all treatments and

The authors are solely responsible for the content of this paper, which is not a refereed publication. Citation of this work should state that it is from the Proceedings of the 17th International Conference on Precision Agriculture and 11th Brazilian Congress on Precision and Digital Agriculture. EXAMPLE: Last Name, A. B. & Coauthor, C. D. (2026). Title of paper. In Proceedings of the 17th International Conference on Precision Agriculture and 11th Brazilian Congress on Precision and Digital Agriculture (unpaginated, online). Monticello, IL: International Society of Precision Agriculture and Brazilian Congress on Precision and Digital Agriculture.

input parameters evaluated. The analysis of variance suggests that at 32 DAP any significant difference was observed following the Tukey's test ($p<0.05$) for 530, 560, 570, 690 and 700 nm reflectance bands and the chlorophyll A and B. On the other hand, at 68 DAP the difference was significant for subplot variable (CFP and VRN) where an increase in almost 8% was observed for 560, 570, 690 and 700 nm reflectance bands for VRN compared to CFP nitrogen treatment, while the chlorophyll B the treatment VRN present 12% less chlorophyll compared to CFP. The blue and red regions are often related to chlorophyll saturation and do not present differences in measure the chlorophyll and the light penetration is low. However, green and red edge regions, i.e. the selected bands from 530 to 570 and 690 to 700, respectively, can map the chlorophyll content, since deep light penetration presents high sensitivity to low or high chlorophyll content. As the conclusion the VRN and CFP present differences at Tasseling stage (VT/68 DAP) demonstrating the potential for VRN technology in decrease the nitrogen leaching and volatilization, mainly when associated with biofertilizer. Nevertheless, integrated spectral indices in handheld sensor to decrease the leaf thickness effect, seems to be an approach to evaluate the nitrogen and chlorophyll status of the plant offer field diagnosis using a non-destructive method.

Keywords.
NIX, Machine Learning, Reflectance, Sensors.

Introduction

Nitrogen is the nutrient that most limits corn yield, and global consumption has risen steadily to sustain production for a growing population, with the United States, China and Brazil supplying 33%, 23% and 10% of world grain corn, respectively (USDA, 2026). Nitrogen is also among the most difficult nutrients to manage. It interacts strongly with environmental conditions and is readily lost through leaching, volatilization and denitrification (Adalibieke et al., 2023), so poorly matched rates can reduce yield by up to 70% (Scharf et al., 2002) and impair nitrogen assimilation within the plant (Miflin and Lea, 1976; Xu et al., 2012). Matching nitrogen supply to crop demand is therefore central to sustainable corn production.

Because nitrogen is a structural component of the chlorophyll molecule, leaf chlorophyll content responds closely to crop nitrogen status and is widely used as an indicator to guide nitrogen management (Ustin et al., 2009). Chlorophyll also governs how efficiently the canopy captures light and sustains photosynthesis, linking nitrogen supply directly to growth. Measuring chlorophyll by conventional means, however, depends on destructive sampling and laboratory analysis, which is slow, labor-intensive and unable to capture the spatial and temporal variability of the field.

Canopy reflectance offers a non-destructive route to assessing chlorophyll over large areas, but not all wavelengths are equally informative, the relationship between spectral signature and chlorophyll content is nonlinear, and reflectance in some regions saturates or penetrates the leaf only weakly. Knowing which wavelengths track chlorophyll most reliably is therefore a prerequisite for sound spectral diagnosis. Feature-selection approaches, such as Random Forest wrappers (Kursa et al. 2010), can isolate the spectral bands most strongly associated with chlorophyll while discarding redundant or uninformative regions (Liakos et al., 2018; Ustin et al., 2009), providing an objective basis for in-field reflectance measurement.

At the same time, two strategies can reduce mineral nitrogen inputs while sustaining crop nitrogen status. The first is variable-rate application guided by remote sensing, in which vegetation indices derived from reflectance recommend nitrogen according to local demand (Holland & Schepers, 2013; Thompson et al., 2015). The second is biological, plant growth-promoting bacteria such as *Azospirillum brasilense* can enhance nitrogen acquisition and lower the dependence on chemical fertilizer. Current remote sensing recommendations, however, do not account for microbial inoculation, and it remains unclear whether a handheld reflectance sensor can track chlorophyll status across these contrasting strategies and crop growth stages.

Taken together, this suggests that handheld reflectance sensing, combined with feature selection to identify the most informative wavelengths, could monitor chlorophyll across nitrogen strategies and reveal whether variable-rate management and biological inoculation maintain crop status with reduced nitrogen. Accordingly, the objective of this study was to evaluate a handheld reflectance sensor for assessing chlorophyll a and b under different nitrogen application strategies, before and after side-dressing, and to identify the spectral bands most associated with chlorophyll content. We tested two hypotheses: (i) variable-rate nitrogen guided by remote sensing maintains chlorophyll content comparable to conventional farm management, and (ii) *Azospirillum brasilense* reduces the required nitrogen rate while maintaining leaf chlorophyll content in corn.

Material and Methods

Experimental design

The study was conducted in 2025 year in a commercial farm located in Campo do Meio, Minas Gerais, Brazil. The experimental design adopted was a split-plot with randomized block, the strip level or primary factor represent the *Azospirillum brasilense* inoculation or not (BF and NBF) and the sub-plot level or secondary factor the nitrogen fertilization strategy divided into Conventional Farm Production (CFP) and Variable Rate Nitrogen (VRN). For each one of the primary level three repetitions were use 6 strips, while for the secondary factor 9 repetition per strip were used to define the 54 sub-plots, thus we adopted the following representation for the statistical design: CFP.BF, CFP.NBF, VRN.BF and VRN.NBF.

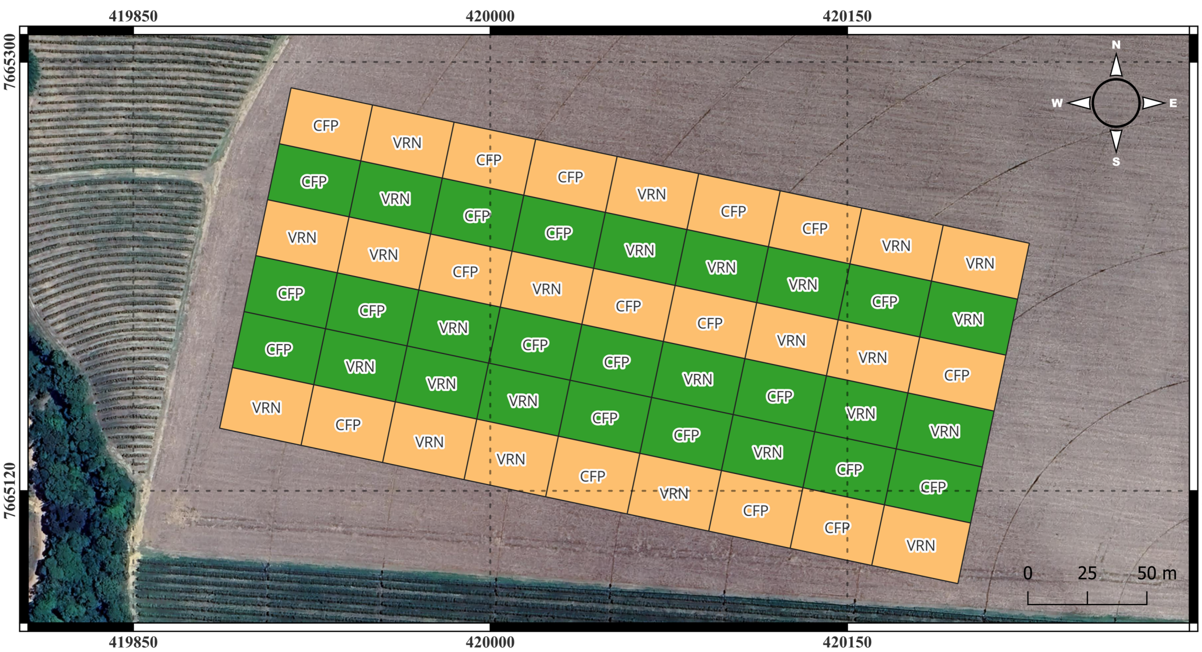

Fig 1. statistical design (split-plot) adopted for experimental study in Campo do Meio, Minas Gerais, Brazil in 2025 year. The green strips represent the inoculation of *Azospirillum brasilense* while the orange the no-inoculation. CFP – Conventional Farm Production and VRN – Variable Rate Nitrogen.

The total area for the experiment was almost 5 hectares with the dimensions of each plot of 35 meters long to 24 meters wider totalizing 840 square meters. The size of the plots was defined according to the fertilizer machine used during the side-dressing application.

Agricultural Practices

The agricultural practices such as herbicides, insecticides and fungicides application followed the farm calendar. In the sowing step the corn hybrid used was Pioneer P3440PWU with a row spacing of 0.90 meters and plant population of 74,000 plants per hectare. The fertilization used for this step was Mosaic NPK 12-16-13 with 13% of sulfur (S), 10% of sulfate-sulfur S(SO4) and 0.12% of Borum (B) and the dose of 220 kilograms per hectare (kg ha-1), which receive almost 27 kg ha^{-1} of nitrogen representing 18% of the total dose of 144 kg ha-1. This dose was applied for all plots (CFP and VRN). The *Azospirillum brasilense* product used for in-furrow application during the sowing was MBio Azo® - *Azospirillum brasilense* (AbV5 and AbV6) in the dose of 0.2 liters per hectare (L ha-1) and a spray volume of 40 L ha-1.

Variable Rate Prescription

The side-dressing nitrogen doses for CFP treatment corresponding the remaining 117 kg ha^{-1} of nitrogen applied, while for VRN treatments the nitrogen doses were calculated following the Holland and Schepers (2013) equation 1.

$$Napp = (N_{OPT} - N_{PF} - N_{OM}) \times \sqrt{\frac{(1 - SI)}{\Delta SI \times (1 + 0.1 \times e^{m \times (SI_{threshold - SI})})}} \quad (1)$$

N_{app} represents the nitrogen applied, N_{opt} the maximum nitrogen prescribed by producers, N_{PF} means the pre-fertilizer nitrogen doses applied to sowing, N_{OM} the nitrogen credit from organic matter, SI is the sufficient index calculated from equation 2, ΔSI is the sufficient index difference parameter, m is the back-off rate variable (0 < m < 100) and $SI_{threshold}$ is the back-off cut-on point (Holland and Schepers, 2013).

$$SI = \frac{VI_{sensed\ crop}}{VI_{reference}} \quad (2)$$

SI represents the sufficient index, $VI_{sensed\ crop}$ represents the vegetation index normalized difference red edge (NDRE) (Sims and Gamon, 2002) calculated for the VRN plots (equation 3) and the $VI_{reference}$ the NDRE calculated for the strips (no-biofertilizer and biofertilizer), each represents the 95th percentile of the values takes from histogram. The $VI_{reference}$ varying according to each strip, thus the histogram of the values for each strip was constructed and the 95-percentile cumulative value was selected as reference (Holland and Schepers, 2013).

$$NDRE = \frac{NIR - Red\ Edge}{NIR + Red\ Edge} \quad (3)$$

NDRE means normalized difference red edge, and NIR the near infrared. NIR and Red Edge represent the bands used to calculate the vegetation index and consequently the SI values for crops and reference. The configured parameters for equation 1 following the recommendations of Holland and Schepers (2013) "m" was set as 20, $SI_{threshold}$ to 0.7, N_{opt} configurated following the CFP total doses 144 kg ha^{-1}, ΔSI configurated for 0.3 and N_{PF} 27 kg ha^{-1} of nitrogen. Despite equation 1 presenting the N_{om} parameter, the authors did not include the values, since the organic matter was not collected from the soils. The nitrogen source used for 2025 year at side-dressing was Urea 46% nitrogen.

Satellite Image Download

Planet Scope platform was used for 2025 for equation 1, 2 and 3 calculations. The sensor used to collect the images was SuperDove (PSD.SB) with 8 bands were only the bands 7 (Red Edge) and 8 (NIR) were selected for NDRE calculation. The spatial resolution of the images are 3 x 3 meters with temporal resolution of one day. Additionally, PlanetScope Surface Reflectance (SR) imagery is automatically atmospherically corrected using a radiative transfer modeling framework based on 6SV2.1 (Second Simulation of a Satellite Signal in the Solar Spectrum). This correction occurs based on the type of product selected during the final steps. Thus, the correction employs precomputed lookup tables (LUTs) derived from 6SV2.1 simulations and integrates near-real-time atmospheric parameters (aerosol optical depth, water vapor, and ozone) obtained from MODIS products. This approach converts top-of-atmosphere (TOA) reflectance into bottom-of-atmosphere (BOA) reflectance, minimizing atmospheric scattering and absorption effects. The download of the images occurred one day before the side-dressing application (04/22/2025), also the images were automatic clipped on web explorer Planet Scope without downloading the complete scene. The calculation of the NDRE was performed using the Pliman package (Olivoto, 2022) and function *image_index*.

Chlorophyll and Reflectance Measurements

The equipment used to measure the chlorophyll *a* and *b* was the Clorofilog 1030, Falker®, Porto Alegre, Rio Grande do Sul, Brazil. The equipment works by measuring the transmittance of the radiation at three specific wavelengths, been two closer to the peak of absorption of chlorophyll and one closer to the near infrared, thus the combination of the values generates the Falker Chlorophyll Index (ICF) a non-dimensional value which divides in chlorophyll *a* and *b*. The second step after using the Clorofilog was to measure the reflectance. Thus, the NIX Spectro L, Nix sensors®, Hamilton, Ontario, Canada was used and measure the reflectance values from Blue-Green-Red (RGB) starting with 400 nanometers until 700 nanometers each 10 nanometers.

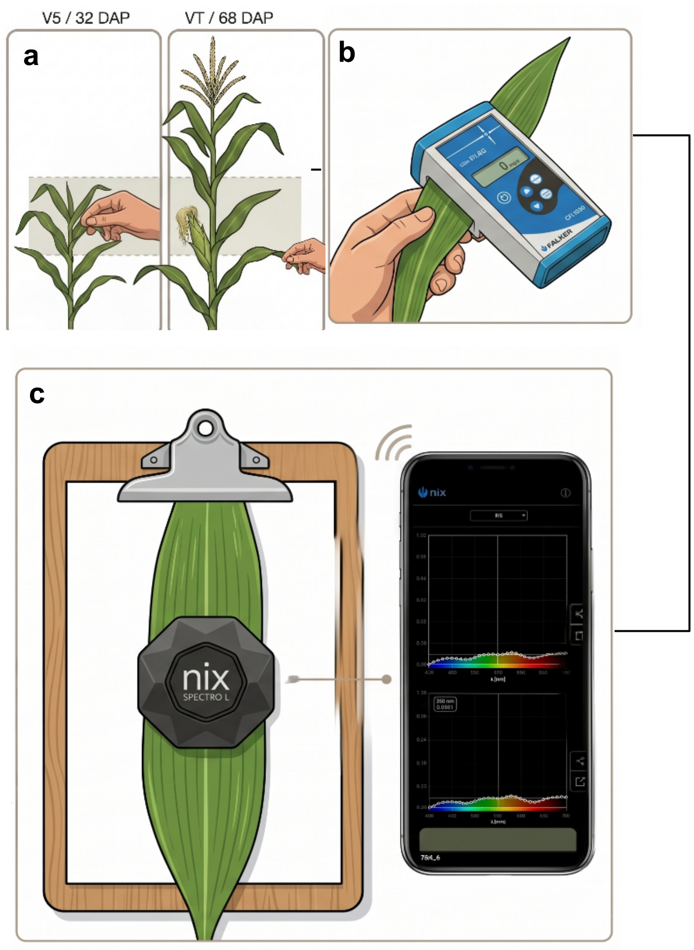

Fig 2. representation for the chlorophyll and reflectance measurement for corn leaves at 32 and 68 days after planting.

In the present study 6 leaves close to the middle part (Figure 2a) of the 6 corn plants per plot were used to measure the Chlorophyll a and b though Clorofilog (Figure 2b). These 6 leaves were then collected and using a small piece of wood as a support the leaves were measured using the NIX Spectro L (Figure 2c). The values were registered in the NIX Toolkit and export as .csv dataset.

Feature Selection and Statistical Analysis

The initial analysis represents an exploratory data analysis composed by boxplot for Chlorophyll *a* and *b* values followed by line graph using the reflectance values measured by the NIX spectra L. The next step involved the use of the Boruta package which is an all-relevant feature-selection method built around the random forest algorithm (Kursa et al., 2010). It creates randomized ("shadow") copies of each predictor by permuting their values, merges them with the original variables and repeatedly fits a random forest to the extended set. A predictor is retained only when its importance is consistently and significantly higher than the best score achieved by the shadow variables, and discarded when it is significantly lower, with the decision based on a statistical test rather than an arbitrary threshold. Because the shadow variables are random by construction, this comparison separates wavelengths that genuinely track chlorophyll from those that appear informative only through chance correlations, providing an objective basis for selecting the most relevant spectral bands.

The spectral bands retained by the Boruta selection were then submitted to analysis of variance (ANOVA) using the AgroR package (Shimizu et al., 2021). The split-plot design was analysed with the PSUBDBC function, which accommodates the randomized complete block structure with biofertilizer strips assigned to the main plots and nitrogen strategies to the subplots. The function first verifies the ANOVA assumptions and then, for significant treatment effects, performs mean comparisons using Tukey's test at the 5% significance level. Each treatment combination was evaluated for chlorophyll *a* and *b* at 32 and 68 days after planting in R 4.4.3.

Results and Discussion

The descriptive analysis (Figure 3) revealed a marked temporal contrast between the two pigments. Chlorophyll *a* changed little between 32 and 68 DAP, whereas chlorophyll b increased by almost 72% over the same interval. As a result, the chlorophyll a:b ratio fell from approximately 4.1 to 2.5, moving toward the lower end of the 2.56–3.45 range reported for higher plants (Pareek et al., 2018). This shift is informative because the two pigments occupy different functional pools. Chlorophyll a is present in both the reaction centers and the light-harvesting antenna, whereas chlorophyll b is largely confined to the light-harvesting complexes (Pareek et al., 2018). A disproportionate accumulation of chlorophyll b therefore indicates an expansion of the light-harvesting antenna relative to the reaction centers. This is consistent with canopy development between the two sampling dates: by the tasseling stage (VT/68 DAP) the canopy was fully closed and mutual shading had increased, conditions that typically promote larger light-harvesting antennae and thus a lower a:b ratio. Because chlorophyll b is synthesized from chlorophyll a, its accumulation also reflects the maturation of the photosynthetic apparatus as leaves developed, rather than an independent pathway.

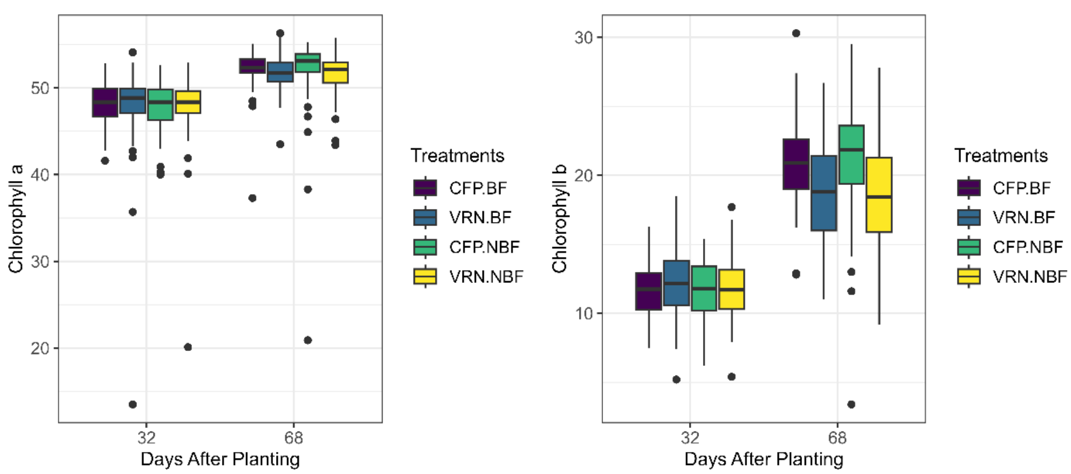

Fig 3. descriptive analysis using the boxplot for chlorophyll *a* and *b* across 32 and 68 days after planting. CFP – conventional farm production, VRN – variable rate nitrogen, BF – *Azospirillum brasilense* inoculation and NBF – non-inoculation.

The treatment effects on canopy reflectance are shown in Figure 4. Differences were most pronounced in the green region (510–580 nm) at 68 DAP. Because chlorophylls absorb strongly in this region, lower reflectance indicates higher chlorophyll content. Accordingly, the CFP.NBF combination, which showed the lowest reflectance, corresponds to the highest chlorophyll levels observed in the descriptive analysis (Figure 3), while VRN.NBF showed the highest reflectance. At 32 DAP the curves overlapped closely, and no differences between the treatment was observed, consistent with the absence of differences at this early stage.

These contrasts are ultimately driven by nitrogen availability, since nitrogen is a structural component of the chlorophyll molecule, in which a central magnesium ion is coordinated by four nitrogen atoms (Pareek et al., 2018). Under tropical conditions, however, higher nitrogen rates do not translate fully into crop uptake, because urea is rapidly hydrolyzed by urease and lost as ammonia through volatilization (Santos et al., 2023). Ma et al. (2010) reported NH_3 losses of about 4 kg N ha^{-1} at rates above 90 kg N ha^{-1} under pre-plant and side-dress application, and Santos et al. (2023) measured losses of up to 21 kg N ha^{-1} within 2.7 days of applying prilled urea under no-till conditions. The higher rate applied in CFP therefore supplied more nitrogen but was also more exposed to these losses, whereas the reduced rate under VRN (44 kg N ha^{-1}) limited the nitrogen available for chlorophyll synthesis while minimizing volatilization.

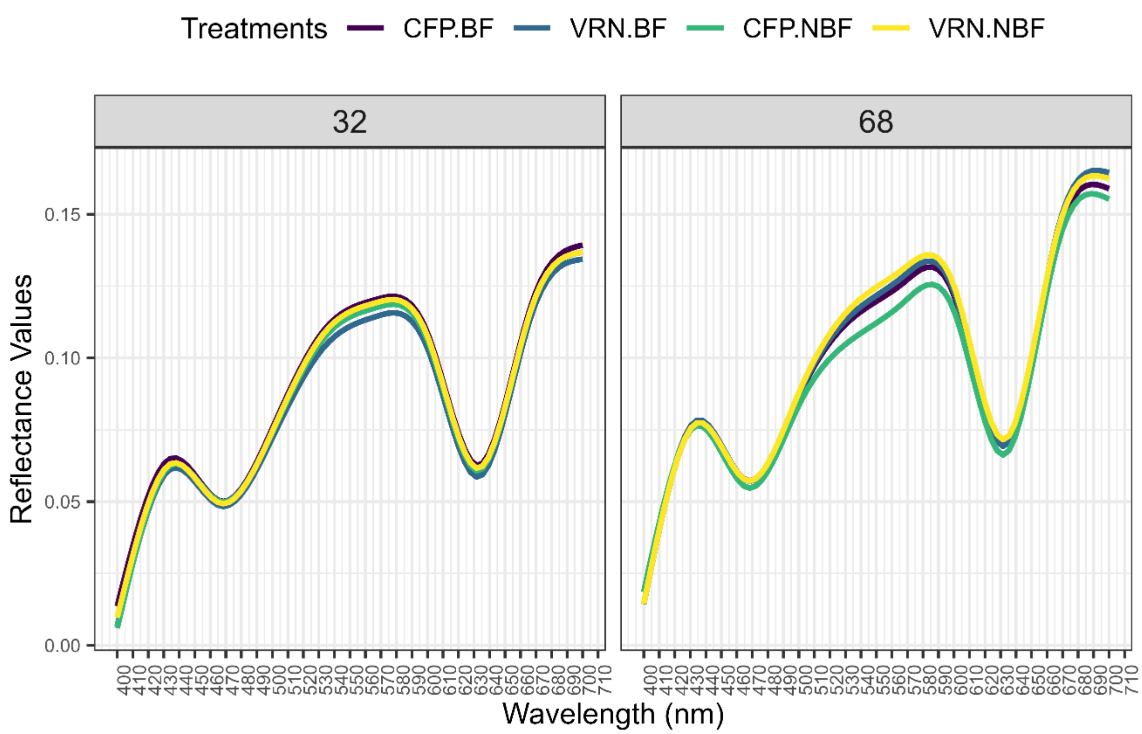

Fig 4. reflectance values from 400 to 700 each 10 nanometers collect using the NIX spectra L for 32 and 68 days after planting. CFP – conventional farm production, VRN – variable rate nitrogen, BF – *Azospirillum brasilense* inoculation and NBF – non-inoculation.

Following the descriptive analysis, the Boruta method was applied to identify the most relevant reflectance bands for chlorophyll a (Figure 5). At 32 DAP, band confirmation was generally weak and inconsistent across treatments. VRN.NBF confirmed only four bands in the green region (530, 540, 560 and 580 nm), whereas VRN.BF confirmed nine bands spanning the green and red-edge regions (430, 520, 530, 540, 550, 560, 570, 580 and 660 nm). In contrast, CFP.BF yielded no confirmed bands, indicating that the model could not isolate relevant wavelengths, and CFP.NBF confirmed only two bands in the blue region (440 and 450 nm). Across treatments, bands in the 620–700 nm range frequently fell below the shadowMin threshold and were rejected, because shadowMin represents the lowest importance reached by the randomized shadow variables, bands below it carry no more information than noise. This region coincides with the chlorophyll absorption peak, where reflectance saturates and no longer discriminates differences in pigment content.

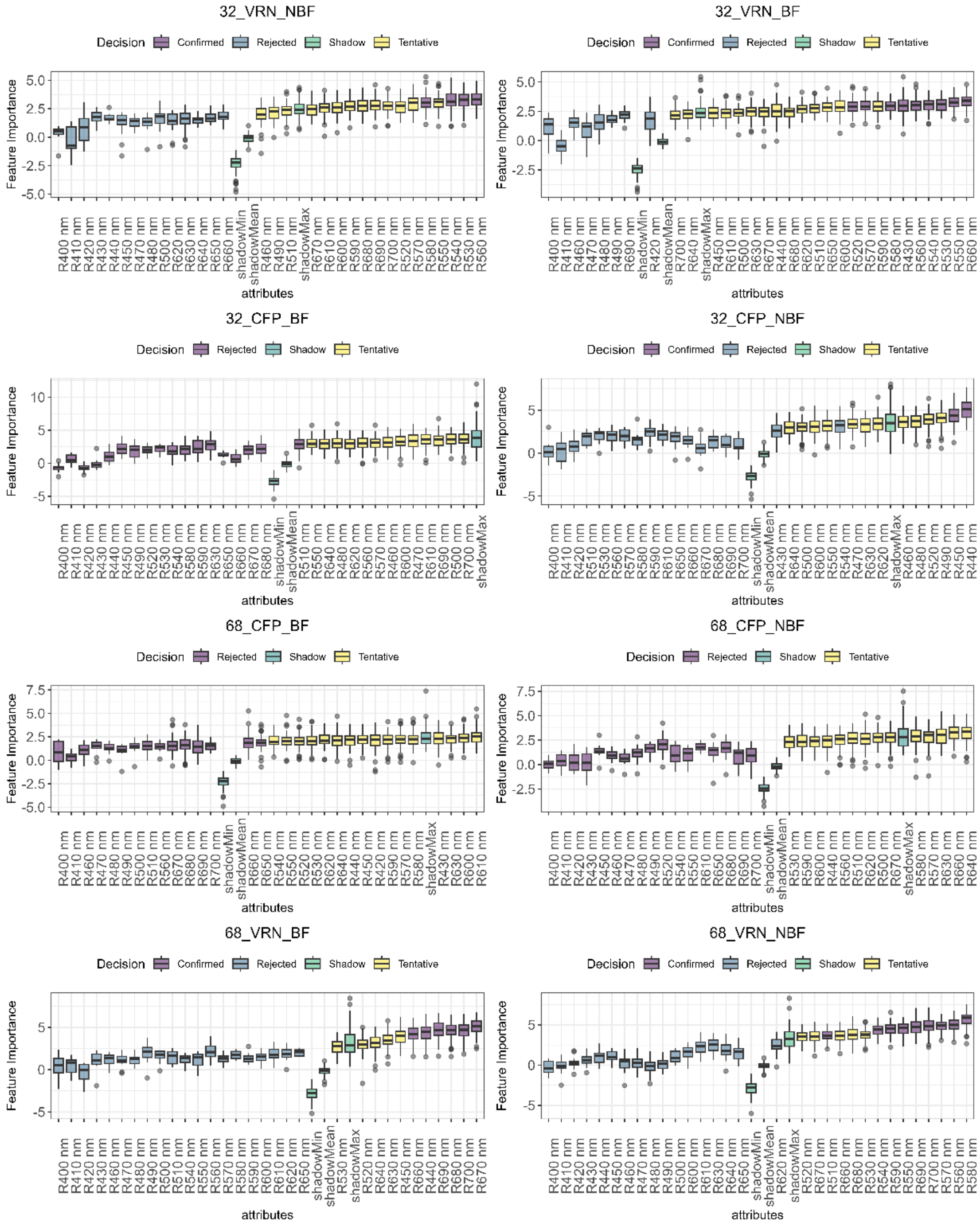

Fig 5. Boruta analysis for chlorophyll *a* at 32 and 68 days after planting. CFP – conventional farm production, VRN – variable rate nitrogen, BF – *Azospirillum brasilense* inoculation and NBF – non-inoculation.

At 68 DAP, a clear contrast emerged between the two nitrogen strategies, independent of inoculation. Neither CFP.BF nor CFP.NBF produced confirmed bands. This is consistent with a homogeneous, saturated canopy under the uniform high nitrogen rate of CFP, with little variation in chlorophyll content among samples, the model lacked the contrast needed to separate informative bands from the shadow variables. Under VRN, by contrast, both VRN.BF and VRN.NBF produced confirmed bands, suggesting that the spatial variation imposed by variable-rate application generated enough variability in chlorophyll *a* for the model to detect informative wavelengths.

The results for chlorophyll *b* followed a similar pattern (Figure 6). At 32 DAP, confirmation was again limited, VRN.NBF and CFP.BF confirmed only bands in the red-edge and green regions (700, 690 and 570 nm), while VRN.BF and CFP.NBF produced no confirmed bands, with most classified as tentative (that is, wavelengths the model could not conclusively assign as informative or not). As observed for chlorophyll a, the blue region (around 410 nm) showed the lowest selection importance, confirming its minor contribution to estimating either pigment.

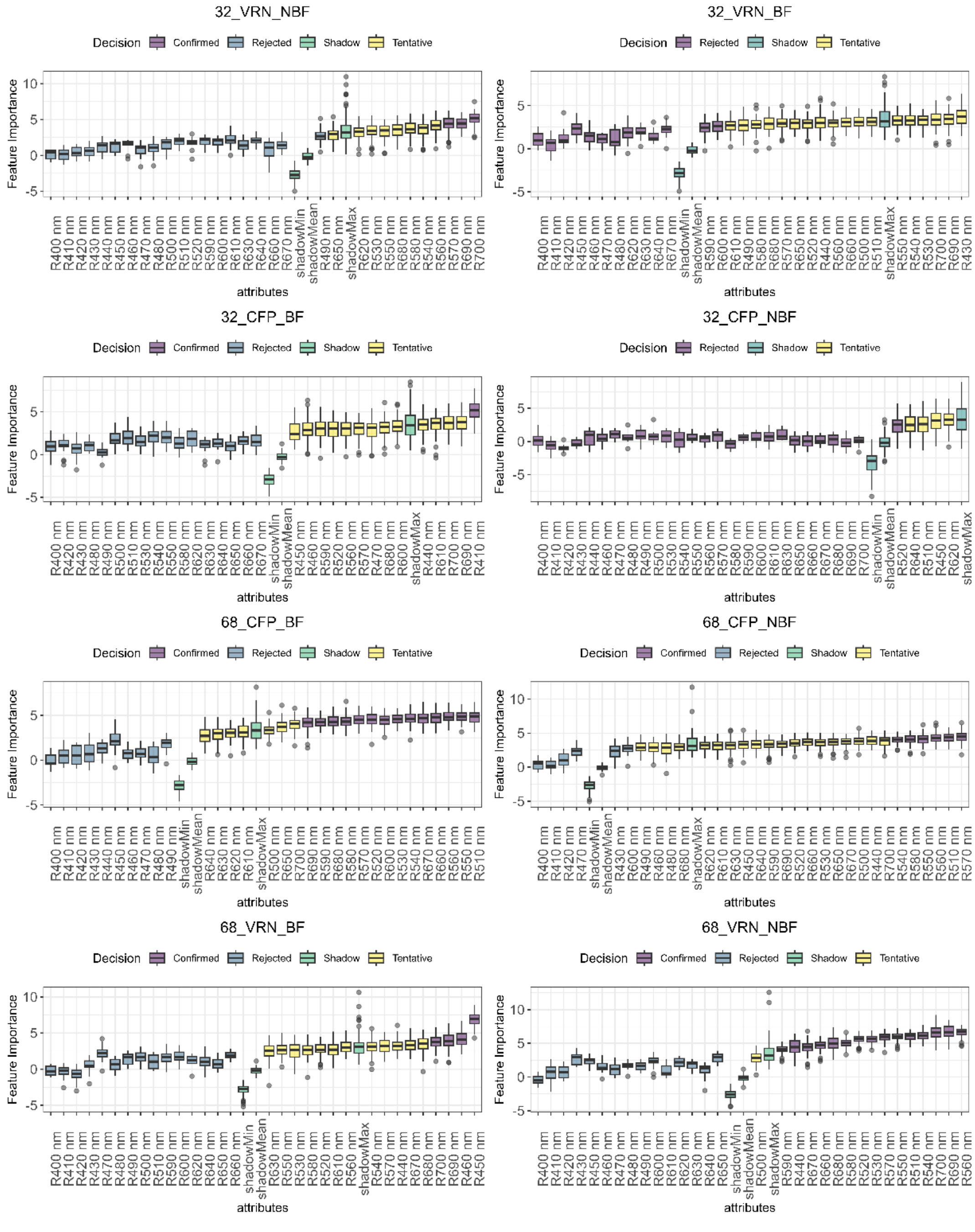

Fig 6. Boruta analysis for chlorophyll *b* at 32 and 68 days after planting. CFP – conventional farm production, VRN – variable rate nitrogen, BF – *Azospirillum brasilense* inoculation and NBF – non-inoculation.

Taken together, the Boruta analysis converged on a consistent set of informative wavelengths for both pigments. The green (530–580 nm) and red-edge (690–700 nm) regions concentrated the confirmed bands across treatments and dates, whereas the blue region (400–440 nm) and the red absorption peak (620–700 nm) were systematically rejected or unstable, reflecting reflectance saturation in the regions of maximum chlorophyll absorption (Gitelson et al. 2004; Sims and

Gamon, 2002). Band confirmation was markedly stronger under VRN than under CFP, and the selection was generally more robust at 68 DAP than at 32 DAP. Based on this convergence, the bands 530, 560, 570, 690 and 700 nm were retained as the most consistently informative wavelengths.

The results for 32 DAP were presented in figure 7. The spectral bands across the treatments presented significant values Pr(>F) for interaction (Biological and Nitrogen), except for chlorophyll *a* and *b,* that does not present any difference. Besides the significant values, the Tukey's (5%) test did not show any difference for the reflectance values.

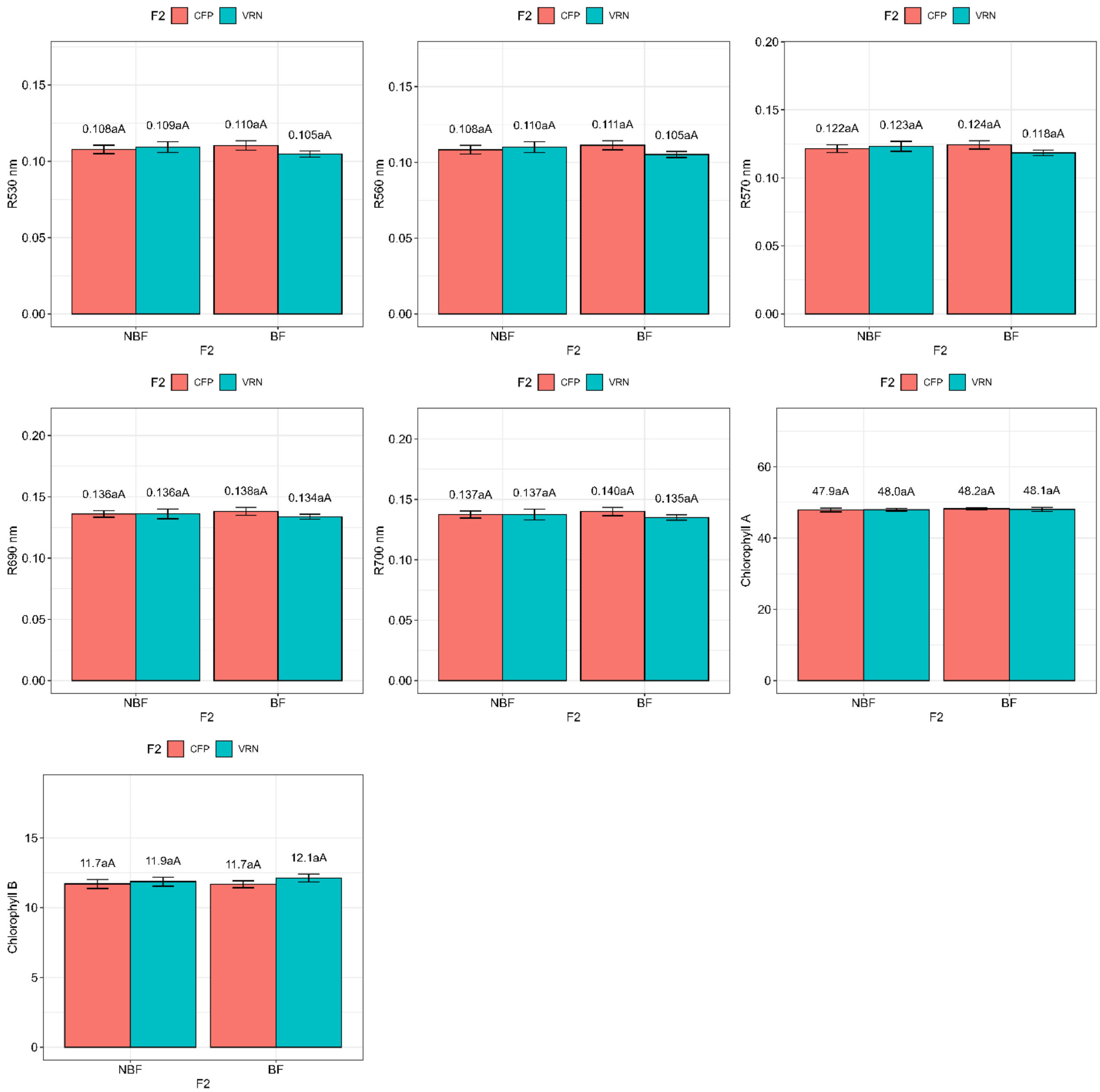

Fig 7. Analysis of variance for 32 days after planting comparing the treatments CFP – conventional farm production, VRN – variable rate nitrogen, BF – *Azospirillum brasilense* inoculation and NBF – non-inoculation. Uppercase compare the same treatment inside each inoculation or not with *Azospirillum brasilense*, while the lowercase compare the nitrogen treatments inside each inoculation or of *Azospirillum brasilense*.

The hypothesis (i) that variable-rate nitrogen guided by remote sensing maintains chlorophyll content comparable to conventional management, was only partially supported. At 32 DAP, chlorophyll a and b differed by less than 3% between VRN and CFP, with no significant differences. By 68 DAP, when the crop reached tasseling (VT), chlorophyll a still showed no significant difference (Tukey, $p < 0.05$), but chlorophyll *b* under VRN was about 11% lower than under CFP (Figure 8). The hypothesis therefore held during early growth but not at tasseling. It is important to distinguish two separate trends, chlorophyll *b* increased over time in all treatments as the canopy reached maximum leaf area and biomass (the temporal pattern seen in the descriptive analysis, Figure 3), while at the single date of 68 DAP it was lower under VRN than under CFP (the treatment contrast). The reflectance data were consistent with this contrast. Except for 530 nm, which was not significant for any treatment or interaction (Pr > F), VRN showed higher reflectance than CFP (Figure 8) at 560, 570, 690 and 700 nm, by 6.4%, 5.5%, 3.8% and 4.4%, respectively. Because lower chlorophyll content reduces absorption and thus raises reflectance, the higher reflectance under VRN at these wavelengths is coherent with its lower chlorophyll *b*, indicating that the spectral and pigment measurements captured the same response.

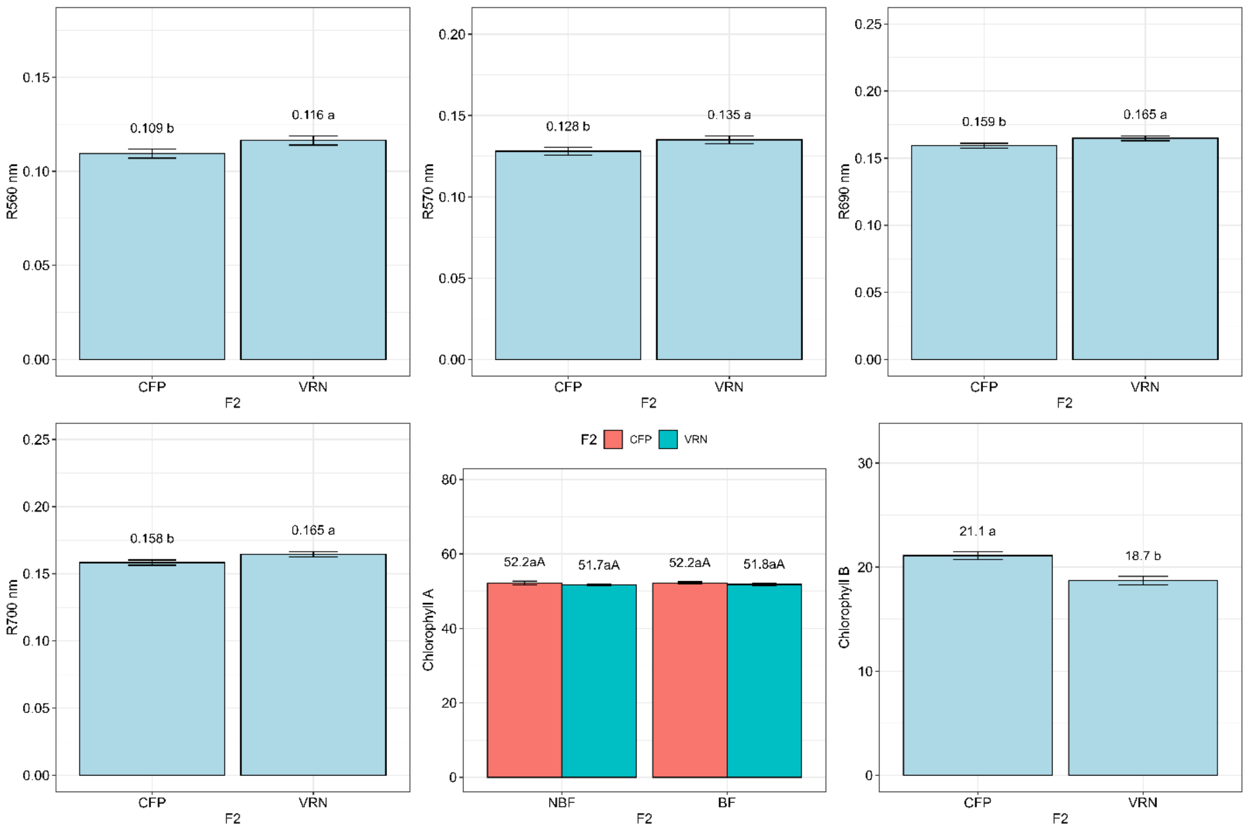

Fig 7. Analysis of variance for 68 days after planting comparing the treatments CFP – conventional farm production, VRN – variable rate nitrogen, BF – *Azospirillum brasilense* inoculation and NBF – non-inoculation. Uppercase compare the same treatment inside each inoculation or not with *Azospirillum brasilense*, while the lowercase compares the nitrogen treatments inside each inoculation or of *Azospirillum brasilense*.

These wavelengths are closely associated with chlorophyll content in literature, although the most informative regions vary across studies. Reddy et al. (2001) found that the 450–520 and 620–680 nm ranges were affected by chlorophyll in corn, groundnut and soybean, whereas Jacquemoud and Baret (1990) identified 548 nm, Zhao et al. (2003) reported 554 and 712 nm under varying nitrogen rates with a hyperspectral sensor, and Strachan et al. (2002) highlighted 556 nm. In the present study, the 560–570 nm range showed the highest importance in both the Boruta and ANOVA analyses, agreeing with the green-region findings of Jacquemoud and Baret (1990), Zhao et al. (2003) and Strachan et al. (2002). The blue region and the 630–680 nm range, by contrast, showed low importance, diverging from Reddy et al. (2001), this is attributable to reflectance saturation in the regions of maximum chlorophyll absorption.

The second hypothesis (ii), that *Azospirillum brasilense* reduces the required nitrogen rate while maintaining leaf chlorophyll content, was not supported by the spectral data. The contribution of inoculation to chlorophyll content and spectral variation was significant only at 32 DAP for the interaction, with a difference for chlorophyll *a* at 68 DAP. This limited effect is explained by the nitrogen rates applied under VRN.BF and VRN.NBF (43.2 and 43.9 kg N ha^{-1}), which were nearly identical and therefore unlikely to produce distinguishable reflectance. A. *brasilense* has been reported to increase shoot and root biomass, produce phytohormones, and raise leaf nitrogen, potassium and boron (Hungria et al., 2010; Batista et al., 2021; Yassue et al., 2022). However, the present study focused on spectral signatures rather than nutrient content, which constrains the interpretation. Consistent with a measurable inoculation effect under other conditions, Souza et al. (2022) reported that Bacillus subtilis and B. megaterium increased chlorophyll and vegetation-index values in bean by almost 12% as inoculation dose and noted the potential of mapping inoculated plants for high-throughput phenotyping using vegetation index.

Overall, these findings indicate that variable-rate nitrogen can sustain crop chlorophyll status comparable to conventional management for most of the cycle, while substantially reducing the applied rate (almost 43 kg N ha^{-1}), with the green and red-edge regions emerging as reliable, non-destructive indicators of chlorophyll content. Three directions follow from this work. First, the consistently informative 560–570 and 690–700 nm bands should be combined into spectral indices, which can reduce the confounding effects of leaf thickness, viewing angle and illumination that limit single-band measurements. Second, validating these handheld-sensor relationships against UAV- and satellite-borne sensors would enable scaling from leaf to field and support operational variable-rate prescriptions. Finally, future studies should pair spectral signatures with direct nutrient and biomass measurements to clarify the agronomic contribution of *A. brasilense* under nitrogen rates wide enough to reveal a treatment effect, which the near-identical VRN rates here could not.

Conclusion

The handheld reflectance sensor was able to track chlorophyll *a* and *b* across contrasting nitrogen strategies, with the green (560–570 nm) and red-edge (690–700 nm) regions identified as the most informative wavelengths by both the Boruta selection and the analysis of variance, while the blue region carried little information. Variable rate nitrogen maintained chlorophyll content comparable to conventional management during early growth and for chlorophyll *a* throughout the cycle, differing only in chlorophyll b at tasseling, while reducing the applied rate to approximately 43 kg N ha^{-1}. The contribution of *Azospirillum brasilense* could not be resolved spectrally, as the nitrogen rates under the inoculated and non-inoculated variable-rate treatments were nearly identical. Together, these results support variable-rate nitrogen as a strategy to lower nitrogen inputs without compromising crop chlorophyll status and point to non-destructive spectral sensing in the green and red-edge regions as a practical tool for in-field diagnosis.

Acknowledgments

We would like to highlight the acknowledgements for Matheus Araujo from Fortaleza farmers and his family for his effort in support the development of the project. The Digital Agriculture Extension and Research Group (GEPAD) members for the estimated support for data collection. We extend the acknowledgments for Brazilians agencies: Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES), National Council for Scientific and Technological Development (CNPq) and The Research Support Foundation of the State of Minas Gerais (FAPEMIG) for the financial support to researchers, professor and students. Additionally, we would to thank you for Federal University of Lavras (UFLA) in especial for Department of Agriculture (DAG) for the support with materials and structure to developed the project.

References

United States Department of Agriculture (USDA). (2026). Global Production. USDA. https://www.fas.usda.gov/data/production?commodity=almonds . Acessed on 01 June 2026.

Adalibieke, W., Cui, X., Cai, H. You, L., & Zhou, F. (2023). Global crop-specific nitrogen fertilization dataset in 1961–2020. *Scientific Data*, doi: https://doi.org/10.1038/s41597-023-02526-z

Scharf, P. C., Wiebold, W. J., & Lory, J. A. (2002). corn yield response to nitrogen fertilizer timing and deficiency level. *Agronomy Journal*, doi: https://doi.org/10.2134/agronj2002.4350

Miflin, B. J., & Lea, P. J. (1976). The pathway of nitrogen assimilation in plants. *Phytochemistry*, doi: https://doi.org/10.1016/S0031-9422(00)84362-9

Xu, G., Fan, G., & Miller, A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, doi: https://doi.org/10.1146/annurev-arplant-042811-105532

Ustin, S. L., Gitelson, A. A., Jacquemoud, S., Schaepman, M., Asner, G. P., Gamon, J. A. & Tejada, P. Z. (2009). Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. *Remote Sensing of Environment*, doi: https://doi.org/10.1016/j.rse.2008.10.019

Kursa, M. B., Jankowski, A., & Rudnicki, W. R. (2010). Boruta – a system for feature selection. *Fundamenta Informaticae*, doi: https://doi.org/10.3233/FI-2010-288

Liakos, K. G., Busato, P., Moshou, D., Pearson, S., & Bochtis, D. (2018). Machine Learning in Agriculture: A Review. *Sensors*, doi: https://doi.org/10.3390/s18082674

Thompson, L. J., Ferguson, R. B., Kitchen, N., Frazen, D. W., Mamo, M., Yang, H., & Schepers, J. S. (2015). Model and sensor-based recommendation approaches for in-season nitrogen management in corn. *Soil Fertility & Crop Nutrition*, doi: https://doi.org/10.2134/agronj15.0116

Holland, K. H., & Schepers, J. S. (2013). Use of a virtual-reference concept to interpret active crop canopy sensor data. *Precision Agriculture*, doi: https://doi.org/10.1007/s11119-012-9301-6

Sims, D. A., & Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, doi: https://doi.org/10.1016/S0034-4257(02)00010-X

Olivoto, T. (2022). Lights, camera, pliman! An R package for plant image analysis. *Methods in Ecology and Evolution*, doi:10.1111/2041-210X.13803.

Shimizu, G. D., Marubayashi, R. Y. P., & Gonçalves, L. S. A. (2024). AgroR: experimental statistics and graphics for agricultural sciences. *R package version 1.3.6.* https://cran.r-project.org/web/packages/AgroR/index.html . Acessed 01 June 2026.

Pareek, S., Sagar, N. A., Sharma, S., Kumar, V., Agarwal, T., Aguilar, G. A. G., & Yahia, E. M. (2018). Chlorophylls: Chemistry and Biological Functions. In Fruit and Vegetable Phytochemicals, E.M. Yahia (Ed.). https://doi.org/10.1002/9781119158042.ch14

Santos, C., Pinto, S. I. d. C., Guelfi, D., Rosa, S. D., da Fonseca, A. B., Fernandes, T. J., Ferreira, R. A., Satil, L. B., Nunes, A. P. P., & Silva, K. P. (2023). Corn cropping system and nitrogen fertilizers technologies affect ammonia volatilization in brazilian tropical soils. *Soil Systems*, doi: https://doi.org/10.3390/soilsystems7020054

Ma, B. L., Wu, T. Y., Tremblay, N., Deen, W., McLaughlin, N. B., Morrison, M. J., & Stewart, G. (2010). On-farm assessment of the amount and timing of nitrogen fertilizer on ammonia volatilization. *Agronomy Journal*, doi: https://doi.org/10.2134/agronj2009.0021

Gitelson, A. A. (2004). Wide dynamic range vegetation index for remote quantification of biophysical characteristics of vegetation. *Journal of Plant Physiology*, doi: https://doi.org/10.1078/0176-1617-01176

Reddy, G. S., Rao, C. L. N., Venkataratnam, L., & Rao, P. V. K. (2001). Influence of plant pigments on spectral reflectance of maize, groundnut and soybean grown in semi-arid environments. *International Journal of Remote Sensing*, doi: https://doi.org/10.1080/01431160152609218

Jacquemoud, S., & Baret, F. (1990). PROSPECT: a model of leaf optical properties spectra. *Remote Sensing of Environment*, doi: https://doi.org/10.1016/0034-4257(90)90100-Z

Zhao, D., Reddy, K. J., Kakani, V. G., Read, J. J., Carter, & G. A. (2003). Corn (Zea mays L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. *Plant and Soil*, doi: https://doi.org/10.1023/A:1026233732507

Strachan, I. B., Pattey, E., Boisvert, J. B. (2002). Impact of nitrogen and environmental conditions on corn as detected by hyperspectral reflectance. *Remote Sensing of Environment*, doi: https://doi.org/10.1016/S0034-4257(01)00299-1

Hungria, M., Campo, R. J., Souza, E. M., & Pedrosa, F. O. (2010).Inoculation with selected strains of *Azospirillum brasilense* and *A.lipoferum* improves yields of maize and wheat in Brazil. *Plant and Soil*, doi: https://doi.org/10.1007/s11104-009-0262-0

Batista, B. D., Dourado, M. N., Figueredo, E. F., Hortencio, R. O.,Marques, J. O. P. R., Piotto, F. A., Bonatelli, M. L. C., Settles, M.L., Azevedo, J. O. L., & Quecine, M. C. (2021). The auxin-producing *Bacillus thuringiensis* RZ2ms9 promotes the growth and modifies the root architecture of tomato (*Solanum lycopersicum* cv. micro-tom). *Archives of Microbiology*, doi: https://doi.org/10.1007/s00203-021-02361-z

Yassue, R. M., Galli, G., Neto, R. F., & Morota, G. (2022). Classification of plant growth-promoting bacteria inoculationstatus and prediction of growth-related traits in tropical maize using hyperspectral image and genomic data. *Crop Science*, doi: https://doi.org/10.1002/csc2.20836

Souza, A. E. S., Barbosa Júnior, M. R., de Almeida Moreira, B. R., da Silva, R. P., & Lemos, L. B. (2022). UAV multispectral data: a reliable approach for managing phosphate-solubilizing bacteria in common bean. *Agronomy*, doi: https://doi.org/10.3390/agronomy12102284