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UAV Multispectral Data: A Reliable Approach for Managing Phosphate-Solubilizing Bacteria in Common Bean

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Abstract: Remote sensing can offer stakeholders opportunities to make precise and accurate decisions on agricultural activities. For instance, farmers can exploit aircraft systems to acquire survey-level, high-resolution imagery data for crop and soil management. Therefore, the objective of this study was to analyze whether an unmanned aerial vehicle (UAV) allows for the assessment and monitoring of biofertilization of the common bean upon vegetation indices (VIs). The biological treatment of the legume crop included its inoculation with phosphate-solubilizing bacteria (PSB), namely *Bacillus subtilis* and *B. megaterium*. Indicators of photosynthetic performance, such as chlorophylls (a and b) and carotenoids, were measured from actively growing leaves to determine effectiveness. In addition, images were acquired in the field, both spatially and temporally, to establish functional relationships between biometric and computational features. Microorganisms manifested as growth-promoting agents to the crop as they significantly increased its quantities of light-harvesting pigments. VIs allowed for predicting their impact on photosynthetic performance, making them on-site markers of PSB. Therefore, this research can provide insights into the remote, non-destructive mapping of spectral changes in the common bean upon the application of PSB. Imagery data from UAV would enable producers to generate information on the crop to intervene in the field at the right time and place for improved utilization of biofertilizers.

Keywords: UAV data; photosynthetic pigments; phosphate-solubilizing bacteria; common bean

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) is a significant source of edible grains for humans. It offers people affordable nutrient-dense diets; hence, it can be strategic in developing food security in vulnerable zones worldwide [1]. Smallholder farmers and large-scale producers generally rely on mineral fertilizers to grow such a legume crop. For instance, they introduce phosphorus (P) into intensive systems to enhance the yield and quality of the product. However, synthetic fertilizing inputs can be costly. In addition, an overreliance on their application can negatively impact agroecosystems and surrounding areas by salinization, eutrophication, and volatilization. Therefore, pressing social and environmental issues concerning unsustainable and unsuitable utilization of conventional fertilizers in agriculture drive the need for developing and implementing resource-effective and eco-friendlier solutions, such as inoculants [1].

Microorganisms can offer the global agricultural system disruptive ways to produce food, energy, and raw materials for industrial processing. For instance, *Rhizobium* spp. can fixate N_2 from the atmosphere [2]. Its enzymatic complexes can convert such a compound to NH_3 and NH_4^+ through ammonification [2]. Ammonia can be a precursor for NO_2^- and NO_3^- through nitrification by oxidizing bacteria, such as *Nitrosomonas* spp., *Nitrococcus* spp., and *Nitrobacter* spp. [1]. Nitrate and NH_4^+ are assimilable by crops. Therefore, they contribute to their nutrition while reducing their dependence on fertilizers [1]. As rhizoplane-colonizing fungi, mycorrhizae allow hosts to effectively uptake energy inputs (e.g., water and minerals) from the soil [3]. In exchange for carbon to survive, they mutualistically and synergistically bring nutritive substances to crops, enabling them to resist stresses in harsher environments, such as drylands and those areas whereby fertility is intrinsically poor [3]. In addition, mycorrhizal agents can solubilize P, which generally has low mobility in soil, thereby enhancing its bioavailability [3]. *Azospirillum* spp., *Azobacter* spp., *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Serratia* spp., and *Streptomyces* spp. can act as synthesizers and secretors of phytohormones (e.g., auxin, cytokinin, and gibberellin) for crops [4–6]. Their co-occurrence in the rhizosphere can promote growth and development endophytically, protect against phytopathogens [7], and ultimately increase the cost-effectiveness of the production [4–6]. Therefore, the diversity and multiple functions of beneficial microbial agents can enable stakeholders to address synergies and trade-offs in elaborating sustainable and renewable biotechnologies, such as biofertilizers, to transform agriculture.

Bacillus comprises species capable of fixing N_2 and solubilizing P [8]. Therefore, it can offer significant and effective inoculants for nitrogenous or phosphorous biofertilization [9]. It can also contribute to crop and soil management by making N, Ca, Mn, Zn, and Fe readily available from the system for absorption via roots and hairy fibers [6,7,10]. Relevant studies on rhizospheric N_2-fixing and P-solubilizing research can provide evidence of its ability to enhance the technical performance of cereal and energy crops, such as wheat [11,12], barley [6,12], sugar beet [6], canola [13], maize [14], and the common bean [1]. The common bean is a versatile host for microbial agents. For instance, it can establish a symbiotic relationship with *Rhizobium* spp., which increases yield [1]. However, a co-inoculation of *B. subtilis* and *B. megaterium* can promote further modulation and concentration of chlorophylls, allowing for higher technical performance than what is achievable through conventional biological treatment [1]. Therefore, as bacteria influence pigments, remote sensors onboard orbital (e.g., satellites) or suborbital (e.g., unmanned aerial vehicles; UAVs) platforms could capture their impacts on the crop by spatio-temporal variation in canopy reflectance.

Solano-Alvarez et al. [15] analyzed whether vegetation indices could develop accurate and reliable spectral indicators about the role of *B. cereus* in protecting *Solanum lycopersicum* against *Clavibacter michiganensis*. Therefore, they inoculated the crop with the biological agent and infected it with the pathogen to establish a biodynamic model under biotic stress. Then, they assessed the plant material for ROS-scavenging enzymes while acquiring multispectral (RGB and NIR) imagery data via smartphone for explanatory modeling. The computational features, namely normalized difference vegetation index (NDVI), green normalized different vegetation index (GNDVI), and phenylalanine ammonia lyase (PAL), allowed for distinguishing between symptomatic ad non-symptomatic groups. Their correlations with enzymatic and photosynthetic activities were positive and significant, making them markers for sanitary conditions. By further reviewing the literature, we can identify another relevant study by Kthiri et al. [16]. They tested the accuracy of VIs for detecting changes in Triticum aestivum with inoculation of *Trichoderma harzianum* and *Meyerozyma guilliermondii* for biological control over *Fusarium* spp. As the linear relationship between chlorophylls and NDVI was positive, the authors accurately predicted the effects of seed-coating bioagents on fungal infection spectroradiometric imagery data.

Such studies provide knowledge to progress the field's prominence in remotely analyzing crops with beneficial microorganisms. However, they are still at an early stage of development, driving the need to conduct further in-depth research for scalable and realistic designs. Authors ground their methodologies on portable instrumentation (e.g., spectroradiometer) and perform activities in controlled environments (e.g., greenhouse facilities), which opens an opportunity to investigate reflectance sensors in the field to address full-scale remote sensing. Therefore, the objective of this study was to analyze whether vegetation indices from UAV imagery data allow for the assessment and monitoring of the common bean with phosphate-solubilizing bacteria.

2. Materials and Methods

2.1. Study Area and Experimental Design

This study was conducted in an experimental field at São Paulo State University (Unesp), near 21°14′59′′ S and 48°17′15′′ W, from April to August 2022. The regional climate consists of a dry winter and rainy summer, with annual precipitation and temperatures of approximately 1460 mm and 22.6 °C, respectively. The subject of the investigation was the commercial cultivator IAC 2051, which grows effectively in tropical zones with 300–500 mm, producing approximately 4735 kg of grains per hectare. Twelve seeds were arranged every meter on the field to establish the cultivation. As the average rainfall during the season was 45.6 mm (Figure 1), a supplementary 456.15 mm was automatically applied throughout the plots via a sprinkler irrigation system.

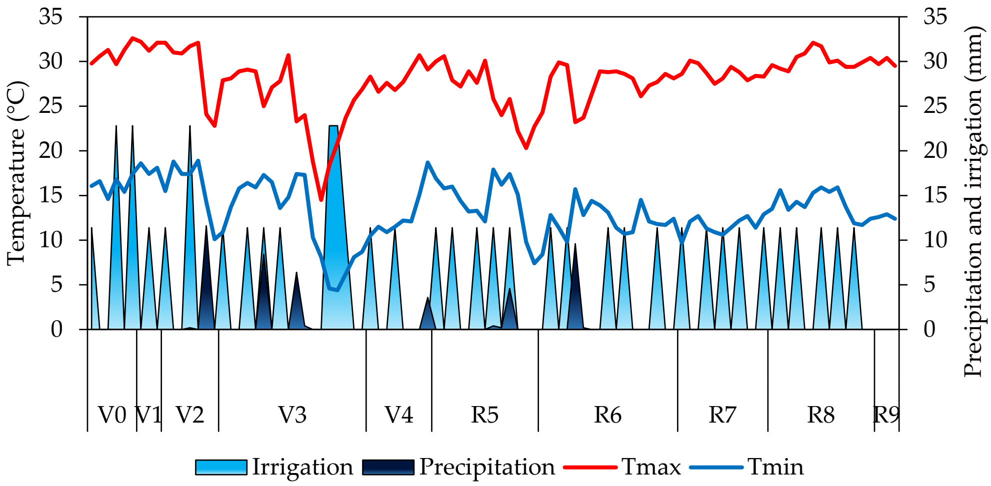

Figure 1. Climatic conditions during the common bean growing stages (from V0 to R9).

In addition, the soil was Oxisol. Chemically, it consisted of 26 g m^{-3} organic matter, 32 mg dm^{-3} P, and 6.3 mmolc dm^{-3} K. Therefore, 20, 20, and 100 kg ha^{-1} of P_2O_5, KCl, and N, respectively, were added to the area to ensure adequate fertilization for the crop. The N was applied once at 20% before seeding, then twice at 30% and 50% at V3 and V4, respectively, to improve the efficiency of its utilization since it is highly volatile and can easily escape from the system via leaching. In addition, P2O5 was at half of the conventional dose to not hinder the potential biofertilization by PSB. An additional management activity included weeding to allow the common bean to grow without competing with weeds over resources.

The commercial inoculant for biofertilization was BiomaPhos (BIOMA, Fazenda Rio Grande, Brazil). It brings *B. subtilis* and *B. megaterium* into its functional composition. The doses tested were 100, 200, and 300 mL. A trial without bacteria was the baseline for comparisons. Each level consisted of four replicates (sample size = 16) to control non-random variability and allow this study to be reproducible by an independent researcher. Therefore, plots with and without biological treatment were monitored and assessed for biometric and spectral characteristics to analyze the effects of PSB on the growth and development of the crop from V4 to R8 to address stages of intensive, rapid, and maximum accumulation of nutrients. Only plants within the working zones that were computationally projected on the field were analyzed to minimize systematic errors (Figure 2).

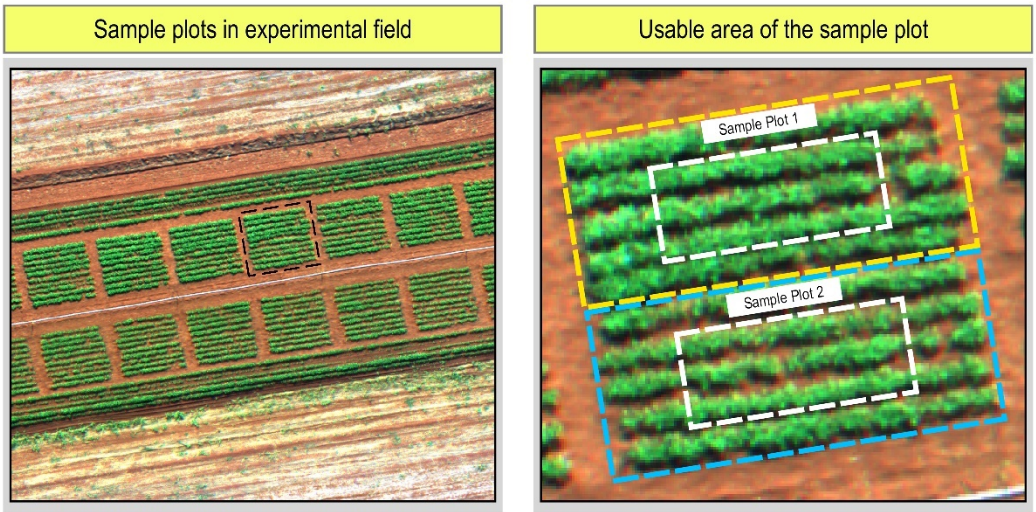

Figure 2. Sample plots in experimental field 5 × 2.25 m (**left**) and usable area of sample plots of 3 × 1.35 m (**right**). Yellow: Plot 1; Blue: Plot 2; and White: usable area of both plot 1 and plot 2.

2.2. Assessment of Photosynthetic Components

A handheld meter (CCM-200 Plus, Opti-Sciences Inc., Hudson, NH, USA) was employed in the field to measure the Chlorophyll Content Index (CCI) by optical absorbance at 653 and 931 nm. In the laboratory, chlorophylls (a and b) and carotenoids were analyzed by spectrophotometry (Beckman Coulter DU 640, Brea, CA, USA) at 470, 645, and 663 nm, respectively [17]. The handheld meter was calibrated by keeping the measuring chamber clear of any material and closed until the screen displayed a message according to the manufacturer's guidelines. As for the spectrophotometer, a series of readings were performed on standard samples in an acetone solution after filling it out in the equipment.

2.3. Acquisition of Spectral Data on the Field by UAV and Processing of Image

The platform for remote sensing was a multispectral UAV (DJI Phantom 4, Shenzhen, China). It provides an onboard sensor established in a 3-axis gimbal with five monochromatic channels: 450 nm ± 16 nm (Blue), 560 nm ± 16 nm (Green), 650 nm ± 16 nm (Red), 730 ± 16 nm (RedEdge), and 840 ± 26 nm (NIR). It also has a natural light sensing device integrated into the upper part, which allows for the compensation of luminance and elimination of environmental noise from the raw imagery data for processing. In addition, it is compatible with a GNSS-RTK receiver, ensuring centimetric positional accuracy and the acquisition of temporal data from the same point. The field was surveyed at noon (±1 h) under the control of a planner platform (DJI GS PRO, Shenzhen, China), from take-off to landing. The flight parameters were: 75/70 (% front: side) overlap and 60 m altitude for high-resolution imagery data and orthomosaic mapping with a ground sample distance (GSD) of approximately 3.5 cm. Images were processed in the Structure from Motion (SfM) software (Agisoft Metashape Professional 1.5.5, Agisoft, St. Petersburg, Russian) to produce orthomosaics. Therefore, VIs (Table 1) were extracted computationally via digital representations by using photogrammetric tools available from the "FIELDimageR" [18] for statistical computing and graphics in RStudio.

Table 1. Vegetation indices for the remote assessment and monitoring of phosphate-solubilizing bacteria in the common bean.

Abbreviation	Vegetation Index	Equation	Ref.
NDVI	Normalized Different Vegetation Index	$\frac{NIR-Red}{NIR+Red}$	[19]
GNDVI	Green Normalized Different Vegetation Index	$\frac{NIR-Green}{NIR+Green}$	[20]
$CI_{rededge}$	Chlorophyll Indices RedEdge	$\frac{NIR}{RedEdge}-1$	[21]
CI_{green}	Chlorophyll Indices Green	$\frac{NIR}{Green}-1$	[22]
SRPI	Simple Ratio Pigment Index	$\frac{Blue}{Red}$	[23]
RVI	Ratio Vegetation Indices	$\frac{NIR}{Red}$	[24]

2.4. Data Analysis

Box plots were designed from biometric data to summarize information on photosynthetic performance upon biological treatment of PSB. A deeper level of statistical analysis included the calculation of correlations between pigments and VIs to map computational features to spectral changes. Analyses were performed in the environment of RStudio for statistical computation and graphs.

3. Results

3.1. The Impact of Inoculation on Photosynthetic Components

The application of PSB determined the photosynthetic performance, which produces distinctive box plots (Figure 3). Chlorophylls were more sensitive to biological treatment than carotenoids. Therefore, their quantities and the CCI rapidly increased from V4 to R6 while sharply decreasing as the crop matured in R8. In addition, increasing the dosage of PSB allowed the crop to produce more chlorophylls, even at later (reproductive) stages of its cycle. The response of carotenoids to bacteria was inconsistent, making it challenging to attribute its content to the intervention.

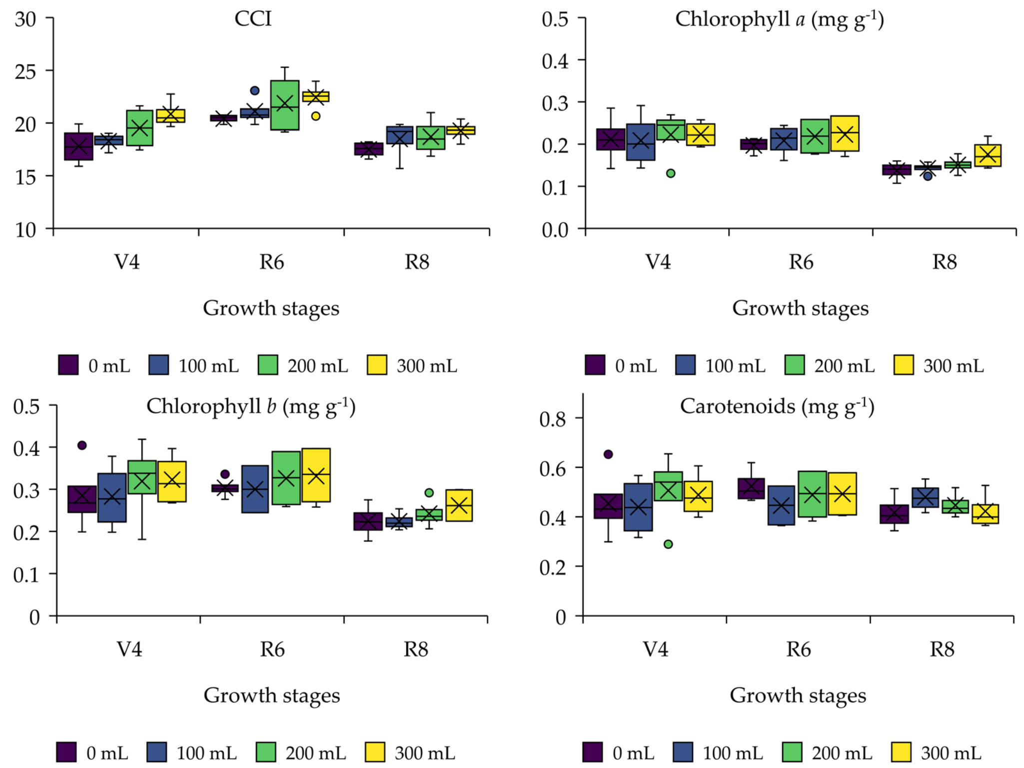

Figure 3. Summary statistics of the effect of phosphate-solubilizing bacteria on pigments. V4, R6, and R8: growth stages; 0, 100, 200, and 300 mL: inoculant levels.

3.2. Mapping Biofertilization by Phosphate-Solubilizing Bacteria upon Vegetation Indices

As PSB positively influenced pigments, VIs that are allowed for mapping its impact on the field do it based on top-of-canopy reflectance (Figure 4). However, it could not be easily recognizable in areas at stages as early as V4 since spectral changes are still not significant. Therefore, reproductive stages would offer better conditions for monitoring biological treatment. For instance, R6 provided the highest values of NDVI, RVI, SRPI, and $CI_{rededge}$, which enhance biophysical modeling. As the crop matured, such VIs sharply decreased by up to 50% at R8, degrading the visual quality of custom diagrams.

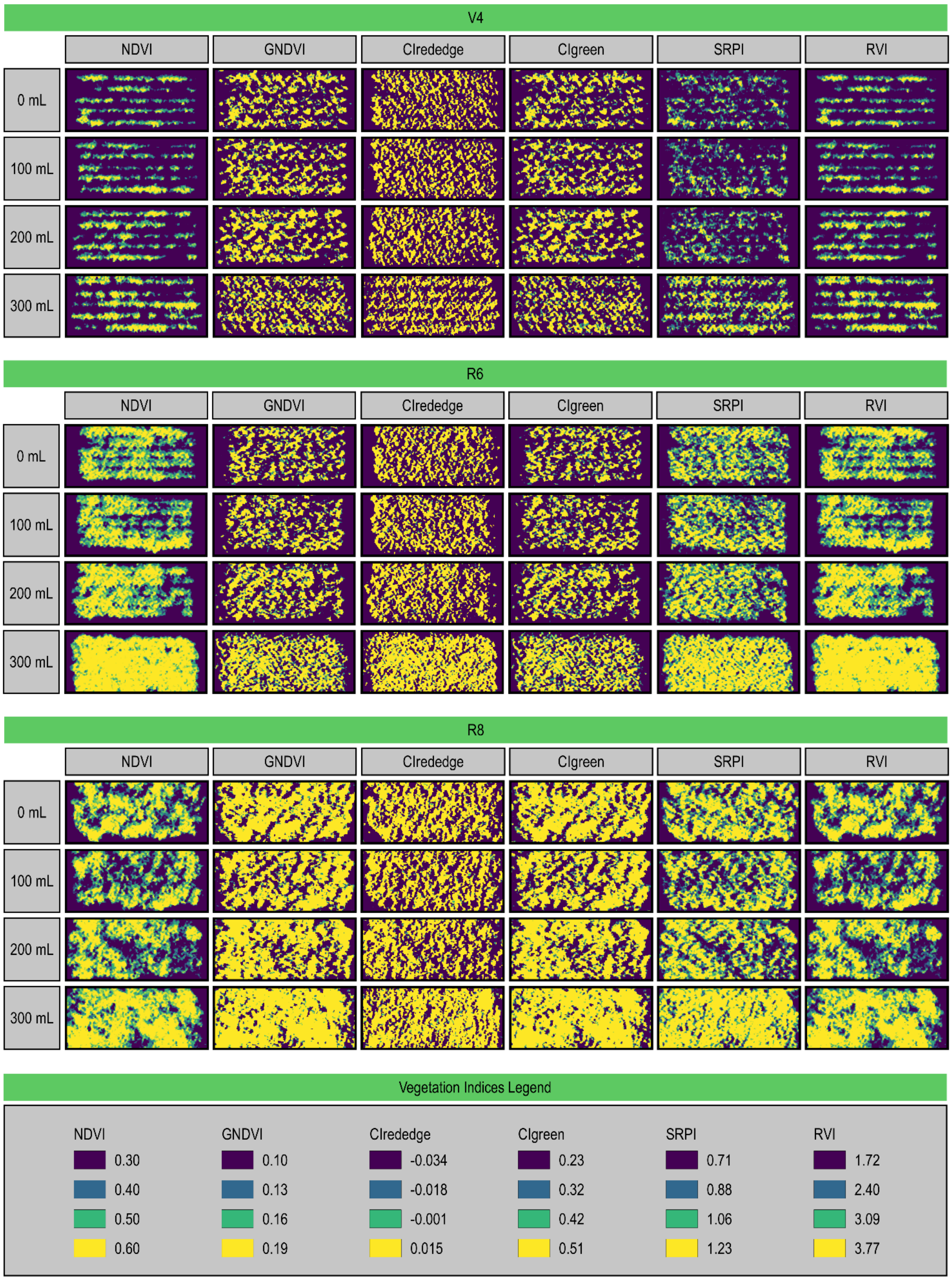

Figure 4. Remote top-down mapping of the application of phosphate-solubilizing bacteria for biofertilization of the common bean.

The higher the dosage, the higher the values of NDVI, $CI_{rededge}$, SRPI, and RVI (Figure 5). However, upsizing the inoculation can decrease GNDVI and CI_{green}. This contrast supported the role of PSB in modulating reflectance and how it can determine positive and negative relationships between physiological, spectral, and computational features. Vegetation indices can allow for the identification of patterns of PSB. However, their utility depends on the phenological stage. For instance, V4 could make it challenging to separate areas with and without inocula upon NDVI, RVI, and SRPI. Therefore, it could not provide VIs for biophysical modeling as effectively as R6 and R8. A variation of approximately 20% in reflectance, irrespective of VI, can occur between the highest and lowest levels of inoculation at R6. However, GNDVI and CI_{green} could offer stakeholders reliable markers of biofertilization by PSB at vegetative stages as early as V4 since their values can range by up to 30% between the highest and lowest levels of biological treatment. Remote sensing at V4 would allow for the development of early assessments and monitoring of the crop.

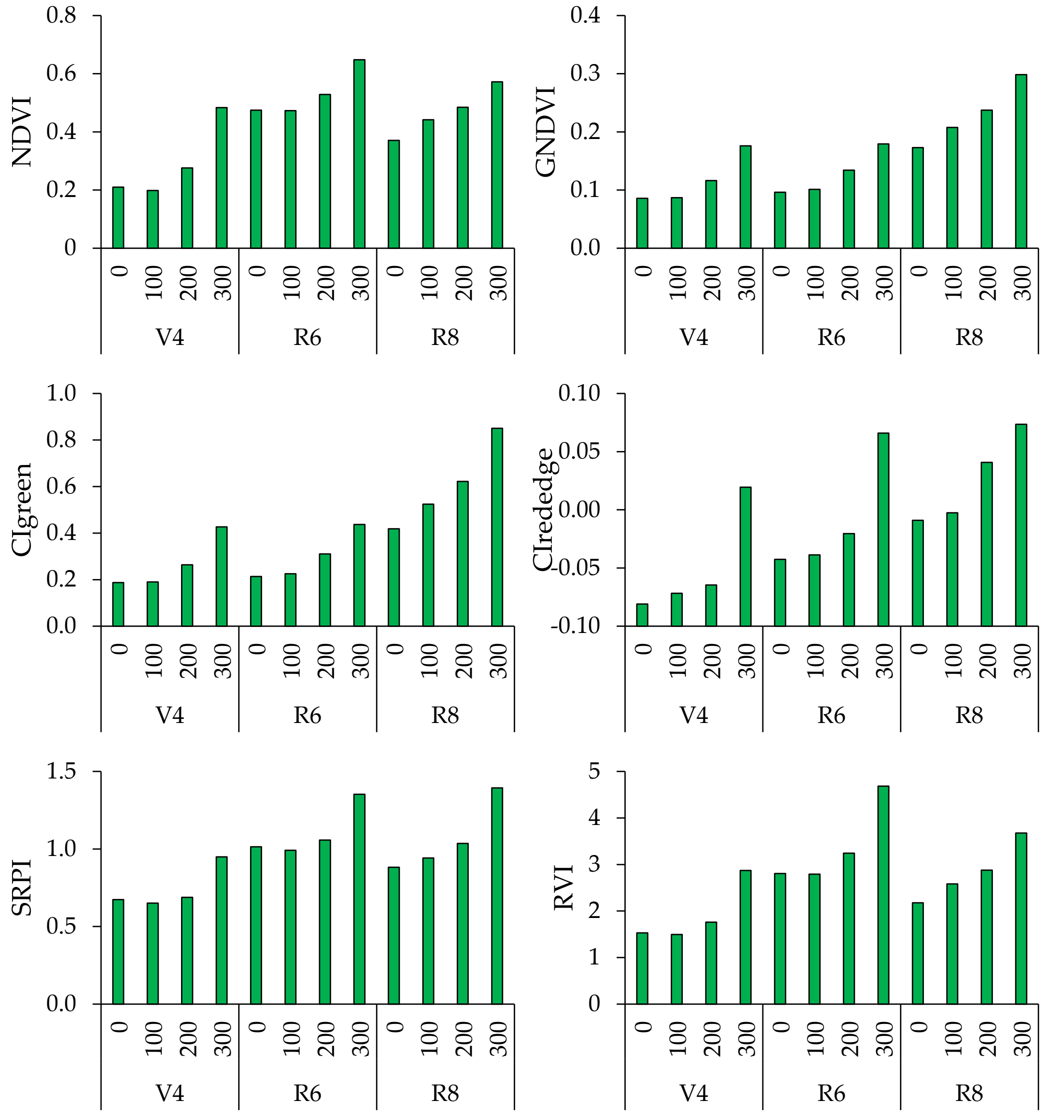

Figure 5. Vegetation indices by inoculant level (0, 100, 200, and 300 mL) for growth stages (V4, R6, and R8).

3.3. Correlations between Vegetation Indices and Indicators of Photosynthetic Performance

The correlation analysis enabled the calculation and validation of relationships between biometric and spectral variables (Figure 6). The values of NDVI, $CI_{rededge}$, SRPI, and RVI increased as the crop matured. Therefore, their correlations with light-harvesting pigments and CCI were positive. As GNDVI and CIgreen decreased at the latter stages, they developed negative relationships with indicators of photosynthetic performance. Therefore, such VIs could not be allowed for describing variability in chlorophylls and carotenoids at R6. They would work better at vegetative stages. Generally, these explanatory relationships would enable stakeholders not to spend resources investigating meaningless and duplicative features; hence, they would instead make precise and accurate decisions on exploiting PSB for biofertilization. As agroecosystems become more data-driven, correlations between physiological and spectral variables would improve analysis of them without obscuring insights.

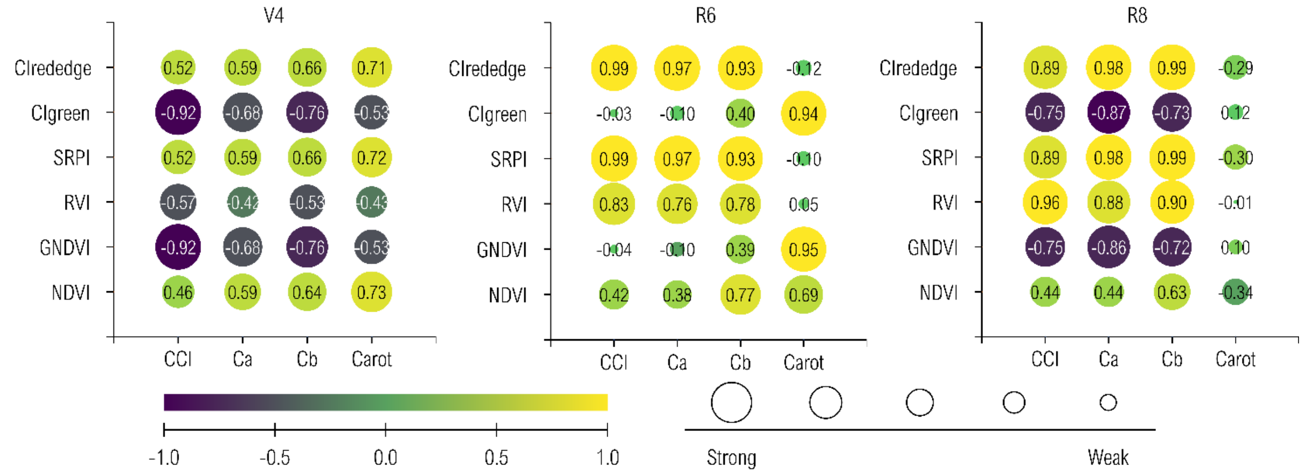

Figure 6. Explanatory relationships between vegetation indices; Chlorophyll content index, CCI; chlorophyll *a*, Ca; chlorophyll *b*, Cb; and Carotenoids, Carot.

4. Discussion

This study hypothesized that imagery data would allow for mapping the role of PSB in producing the common bean. Therefore, the crop was biologically treated via seed and then assessed for indicators of photosynthetic performance. Spatio-temporal changes in canopy reflectance were monitored and captured via a multispectral UAV. Vegetation indices were calculated from imagery data and then analyzed for accuracy in discriminating areas with and without biofertilization. The inoculation positively impacted the growth and development of the crop by increasing quantities of light-harvesting pigments, especially chlorophylls, making it an alternative for biofertilization. A series of VIs could predict such a positive effect. Therefore, they could offer stakeholders the possibility to develop airborne markers for remotely assessing and monitoring the common bean with PSB.

4.1. The Impact of PSB on Photosynthetic Components

This study provides insights into the biofertilization of the common bean with PSB. The inoculant positively impacts the accumulation of pigments. The higher the dose, the higher the quantities of chlorophylls and carotenoids. Increasing P in the system intensifies the synthesis and deposition of chlorophyll in photosynthetic active tissues [25], supporting the upward tendencies for the biological treatment from this study. Phosphate-solubilizing bacteria delivered a synergistic effect on primary light-harvesting pigments, enhancing the growth and development of the crop from V4 to R6. The common bean accumulated the most chlorophylls at R6. As it matured, it transported more nutrients from vegetative organs to pods; hence, the concentrations of chlorophylls and carotenoids (auxiliary photosynthetic pigments) in leaves decreased. However, introducing PSB into the system at 200–300 mL can decelerate the downward tendencies in photosynthetic activity.

Phosphate-solubilizing bacteria can intensify the photosynthetic performance of the common bean. This biotechnological solution relies on microorganisms capable of solubilizing phosphate, enhancing its availability from the soil to the crop. Its action generally involves the release of low-molecular-density organic compounds through which their hydroxyl and carboxyl groups chelate the cations bound to insoluble phosphate, thereby promoting its solubilization [26]. The scientific community places adequate emphasis on its application to crops such as soybean [27,28], corn [29], and millet [30]. The common bean also has importance to agriculture and the bioeconomy. This study demonstrates its benefits to the common bean, which offers a staple food in vulnerable zones. A legume crop and PSB can develop synergies, increasing canopy spectral reflectance. Vegetation indices can predict and map this relationship in the field with pinpoint (leaf-level) accuracy, driving the need to address them in precision agriculture for crop nutrition and soil management [31].

4.2. The Assessment and Monitoring of PSB upon Imagery Data

Vegetation indices are enablers of remote sensing for agriculture. They assist stakeholders in making precise decisions for their production systems. For instance, their introduction into the management of fertilizers can support applying nitrogen [32–34], phosphorus [35–37], and potassium [36–38] to strategic points of the area to maximize yield and quality while minimizing losses and costs. Those studies can offer insights into mapping conventional crop nutrition using UAV imagery data. However, they do not provide knowledge about the feasibility of this platform for assessing and monitoring biofertilization. Therefore, this study brings innovation into aerial remote sensing.

As VIs offer multispectral markers for mapping the common bean with PSB, they drive the need to narrow gaps in the knowledge of their functions for this purpose. The concept of remote top-down mapping from this study can push the frontiers of this field of research toward implementing a blueprint for high-throughput phenotyping (HTC) since it delves deeper into applying imagery data to analyze beneficial microorganisms than those approaches available in Solano-Alvarez et al. [15] and Kthiri et al. [16]. These authors contributed to the fundamental research in addressing imagery data that are used to predict the effects of bioagents (i.e., *B. cereus* and *C. michiganensis*) on crops (i.e., *S. lycopersicum* and *T. aestivum*) under stressful environments. However, they performed their experiments and analyses in controlled facilities, which was not an assumption of this study to bring it closer to realistic conditions and make it easier to obtain findings and novelties off the academic ground.

Vegetation indices brought discriminant computer vision features into the remote sensing of biological treatment. Therefore, they allowed for realistically and accurately separating regions with PSB from those without the inoculant in map-quality images (orthomosaics). As crops are sensitive to the environment, their spectral signatures depend on many factors, such as radiation, temperature [39], nutrients [40], and PSB in this study. For instance, higher VIs could be indicators of greener regions in the field, thereby discriminating between photosynthetically active and visually less vigorous individuals, especially among extreme zones with the lowest and highest dose of the inoculant.

The inoculant impacted the photosynthetic components of the crop. However, it would not provide better insights into the remote sensing data than phenological stages. For instance, at the earliest stage, the vegetation was not sufficiently dense to fully cover the soil; hence, the background negatively contributed to the reflectance. Its exposure to the incident radiation yielded lower values of VIs, making it harder to establish significant correlations between NDVI, $CI_{rededge}$, SRPI, or RVI and indicators of photosynthetic performance. Although RedEdge and NIR are highly sensitive to greener crops [41,42], they could not accurately describe changes in the content of chlorophylls within earlier stages. The portion of soil in the images from the field at V4 degraded their quality for computational processing, making it challenging to calculate VIs and map them to features of green regions in digital representations of the experimental area. The environmental noise contributed to negative correlations between CI_{green} or GNDVI and chlorophylls for early-stage individuals, driving the need to remove it from imagery data before biophysical modeling.

As the crop approached the reproductive stage, its branches completely covered the rows, eliminating soil exposure. In addition, its leaves became greener, yielding higher values of VIs at R6. At later stages, they changed their color from green to yellow while their effective area decreased. Maximum values of NDVI, RVI, SRPI, and CIR_{ededge} occurred at R6. Therefore, as the crop matures, it reflects more photosynthetically active radiation (400–700 nm) and less NIR; hence, the expressiveness of VIs involving Red or NIR in their mathematical combinatory expressions decreases [39,43]. However, CIrededge, SRPI, and RVI would work better than CI_{green}, GNDVI, and NDVI in mapping biofertilization in the field between R6 and R8 since they developed stronger correlations with chlorophylls. Vegetation indices that are sensitive to high canopy covers (e.g., NDVI) limit the detection of sudden changes in reflectance. Hyperspectral sensors would be alternatives to acquiring information in narrow bandwidths; however, they can be costly.

5. Conclusions

Remote sensing for crops with beneficial microorganisms is still at an early stage of development. Therefore, this study analyzed the possibility of developing VIs from UAV imagery data into spectral markers of PSB in the production of the common bean. As the biological treatment positively impacted photosynthetic performance, VIs allowed for accurately predicting its impact on the field. Therefore, they can offer multispectral markers to establish relationships between biometrical and computational features based on spatio-temporal variations in canopy reflectance. For instance, correlations between chlorophylls (a and b) and $CI_{rededge}$ or SRPI are significant, making it possible to visualize the effects of PSB in the area at reproductive stages. In addition, imagery data from the crop at vegetative stages can bring functional relationships between CCI and CI_{green} or GNDVI into the biophysical modeling. It can assist in implementing early-stage assessment and monitoring. Therefore, this study can provide knowledge about the applicability of UAV imagery data in mapping the production of the common bean with PSB. Its conceptual and technical ramifications can strengthen and broaden the range of functions of aerial remote sensing toward developing analytical and prescriptive agriculture and high-throughput phenotyping for crop nutrition.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CCI	chlorophyll content index
CI_{green}	chlorophyll indices green
$CI_{rededge}$	chlorophyll indices red edge
GNDVI	green normalized different vegetation index
GNSS	global navigation satellite system
GSD	ground sample distance
HTP	high-throughput phenotyping
NDVI	normalized difference vegetation index
NGBVI	normalized green–blue vegetation index
NIR	near infrared
PSB	phosphate-solubilizing bacteria
RGB	visible wavelengths
RTK	real time kinematic
RVI	ration vegetation indices
SfM	structure from motion
SRPI	simple ratio pigment index
UAVs	unmanned aerial vehicles
VIs	vegetation indices

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