

# Nitrogen Fertilizer Effects on Hemp Biomass Production Detected by Drone-based Spectral Imaging

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**Abstract.** The recent introduction of hemp (*Cannabis sativa* L., <0.3%  $\Delta^9$ -tetrahydrocannabinol) to the United States has been met with a limited understanding of cultivation practices and environmental monitoring techniques. Aerial imaging can contribute to site-specific management and improve hemp production by monitoring crop growth and health in response to agricultural inputs. Drone-based spectral imaging was deployed with the objective of identifying a spectral signature for floral hemp variety ‘Wife’; detecting differences in plant size, yield, and spectral reflectance in response to nitrogen (N) fertilizer application rates; and assessing field-level variation in crop health and yield observable by aerial imaging to advance hemp management decisions. We conducted an in-field N experiment to test the effects of six N application rates (0, 56, 112, 168, 224, and 280 kg·ha<sup>-1</sup>) on the high-cannabinoid flower variety ‘Wife’ from 2020 to 2022. A quadcopter with a multispectral sensor collected images from an altitude of 61 m between final fertilization and harvest. Each plant canopy was defined in the image and evaluated for size and reflectance as Normalized Difference Vegetation Index (NDVI) and Green Normalized Difference Vegetation Index (GNDVI). The N application rate of 224 kg·ha<sup>-1</sup> N resulted in significant increase in hemp biomass from 112 and 56 kg·ha<sup>-1</sup>, whereas significant increases in floral yield and canopy area were observed just at 112 and 56 kg·ha<sup>-1</sup> N. The N application rate of 224 kg·ha<sup>-1</sup> N produced significantly greater NDVI values compared with those of the application rate of 112 kg·ha<sup>-1</sup> N, whereas GNDVI was significantly greater at 224 kg·ha<sup>-1</sup> N when compared with N rates less than 168 kg·ha<sup>-1</sup> N. Canopy area and plant indices consistently showed strong positive linear correlation with aboveground biomass. This study revealed that drone-based aerial imaging can be an effective tool for monitoring crop growth and health while informing N management decisions in hemp cropping systems.

The cultivation and commercialization of industrial hemp (*Cannabis sativa* L.) for grain, fiber, and flower production has been revitalized by recent legislation (Tyler et al. 2020). The Agricultural Act of 2014, Section 7606 (7 USC 5940), legalized the cultivation of industrial hemp for universities and state departments of agriculture for research purposes and defined industrial hemp as *C. sativa* containing less than 0.3% total  $\Delta^9$ -tetrahydrocannabinol content (Johnson 2019). Four years later, the Agriculture Improvement Act of 2018, also known as the 2018 Farm Bill, removed industrial hemp from the Schedule I list of controlled substances, which allowed farmers to grow hemp commercially in the United States (Abernethy 2019). In 2022, the National Agriculture Statistics Services found that 500 ha (or 28.3 thousand acres) were dedicated to growing hemp in the open for grain, fiber, and flower production, totaling \$212 million (US Department of Agriculture, National Agricultural Statistics Service 2023). Although hemp has been successfully cultivated, the current lack of hemp N fertilizer recommendations can pose a challenge for profitable hemp production from underfertilization and environmental impacts from overfertilization.

N is an essential nutrient needed in large quantities for plant metabolism and growth (Marschner 2011). When N requirements are unfulfilled, plant growth, productivity, and profitability are adversely affected due to nutrient deficiencies; meanwhile, overfertilization can cause nutrient toxicity, susceptibility to diseases, and environmental pollution (Johnston and Bruulsema 2014; Mylavarapu et al. 2022). The crop nutrient requirement is a specified amount of a particular nutrient (e.g., N application rate expressed in  $\text{kg}\cdot\text{ha}^{-1}$ ) a plant needs to achieve optimal yield (Liu et al. 2015). A crop's requirement for a specific nutrient, like N, is determined by an N fertilizer experiment output, often a yield response curve identifying an ideal N application rate at which the highest percent yield is achieved (Lemaire et al. 2008). Various studies have shown floral hemp may take up  $224 \text{ kg}\cdot\text{ha}^{-1}$  N or more to achieve maximum biomass yield. However, some of this uptake may be in excess of maximum flower production, resulting in greater N losses to the environment (De Prato et al. 2022; Kaur et al. 2023; Wylie et al. 2021). As fertilization recommendations are being established for hemp production systems, remote

sensing technology could be used to manage N fertilizer inputs.

Remote sensing uses unmanned aerial vehicles, such as drones, to capture reflectance data from the field with a multispectral camera sensor to detect plant- and field-level variations in crop health and performance (Chong et al. 2002; Fletcher and Singh 2020). However, the initial development of hemp spectral characteristics and their applications is needed. One study focused on *C. sativa* eradication programs found that *Cannabis* crops with denser plant canopies were more spectrally distinguishable from other vegetation and non-vegetative surfaces (Daughtry and Walthall 1998). This determination is possible because the plant canopy reflects different wavelengths and intensities of electromagnetic radiation (light) in both the visible [red–green–blue (RGB)] and invisible [near-infrared (NIR)] spectra, resulting in a unique spectral signature for improved species identification and differentiation (Walthall et al. 2003). Reflectance data from different spectral bands are combined to calculate vegetation indices (VIs), which are used to assess plant health by linking to features like water status, chlorophyll content, and crop biomass (Agati et al. 2013; Caturegli et al. 2016; Jackson and Huete 1991).

Chlorophyll molecules, green pigments that play an influential role in photosynthesis, require a sufficient supply of N (Marschner 2011; Yang et al. 2021). Healthy vegetation with higher concentrations of chlorophyll or “greenness” absorbs wavelengths of red light and strongly reflects NIR and green light (Bell et al. 2004; Candiago et al. 2015). The Normalized Difference Vegetation Index (NDVI) calculates the ratio between the difference of the NIR band (750 to 830 nm) and the red spectral band (620 to 700 nm) to the sum of the two bands (Jackson and Huete 1991), whereas the Green Normalized Difference Vegetation Index (GNDVI) calculates the ratio between the difference of the NIR band and the green band (510 to 590 nm) to the sum of the two (Gutierrez-Rodriguez et al. 2005). The NDVI and GNDVI values range from  $-1$  to  $1$ , where lower values correlate to nonvegetative objects or unhealthy, stressed, and sparse plants; on the contrary, higher values correlate to healthy vegetation with greater chlorophyll content, canopy coverage, and biomass yield (Candiago et al. 2015; Viña and Gitelson 2005; Xiong et al. 2007). *C. sativa* crops receiving inadequate N supply will display symptoms of deficiency such as chlorosis (i.e., yellowing of foliage) and stunted growth; conversely, *C. sativa* receiving too much N will be unusually dark green and will grow suboptimally (Anderson et al. 2021; Cockson et al. 2019; Edalat et al. 2019; Saloner et al. 2019). Therefore, low NDVI and GNDVI values would correlate to inadequate N supply, whereas the highest VI values would suggest excess N supply from overfertilization.

Here, we integrated a remote-sensing platform with an N fertilizer experiment to determine how varying N rates affected

aboveground biomass and yield at harvest, canopy coverage, and VIs, specifically NDVI and GNDVI, for field-grown flower hemp. This study aimed to identify whether drone-based aerial imaging could be used as a monitoring tool to detect differences in plant health and yield. The objectives of this study were to: (1) describe a spectral signature for the floral hemp variety ‘Wife’; (2) quantify significant differences in biomass yield, floral yield, canopy area, and VIs in response to six N rates; and (3) determine measurable differences in biomass yield based on VIs to inform management decisions in hemp cropping systems.

## Materials and Methods

**Study site.** The study site was located at the University of Florida Institute of Food and Agricultural Sciences Tropical Research and Education Center in Homestead, FL (25.5°N, 80.5°W). This location has a subtropical climate with an average annual air temperature of 24°C and an average annual rainfall of 1572 mm (Florida Automated Weather Network 2020, 2021, 2022). The soil is classified as Krome gravelly loam soil (loamy-skeletal, carbonatic, hyperthermic lithic udorthents), which is intensely cultivated and relatively shallow (<23 cm deep) (Noble et al. 1996; US Department of Agriculture, Natural Resources Conservation Service 1991). In all trial years (2020, 2021, and 2022), experiments were performed in different areas of the same field to avoid legacy N effects. Background soil chemical and physical properties were determined annually before fertilization, except in 2020 (Table 1), when the soil was analyzed 4 months after experiment termination, in a nearby uncultivated area, due to pandemic-related setbacks.

**Nitrogen rate experiments.** Nitrogen rate experiments for flower hemp production were conducted to determine whether plant size and color differences resulting from six N fertilizer rates were detectable by drone-based aerial imaging and corresponded to harvested aboveground biomass. Initially, two varieties, ‘Wife’ and ‘Maverick’, were included, but the study focused on ‘Wife’, because it was more reliably detected by drone-based aerial imaging due to its larger plant size and smaller plant density. ‘Maverick’ is a dwarf autoflower variety that did not develop robust canopies for detection. ‘Wife’ is a high-cannabinoid, photoperiod-sensitive variety that was vegetatively propagated and grown in a greenhouse for approximately 5 weeks. Rooted propagules were transplanted into the field on 26 May 2020, 7 May 2021, and 20 Jun 2022 and were grown for 3 to 4 months. The target plant density was approximately 2990 plants/ha. Each experiment consisted of 24 experimental units arranged into a randomized complete block design with four blocks. Each unit ( $28 \text{ m}^2$ ) was planted with six plants and treated with one of six N rates: 0, 56, 112, 168, 224, and  $280 \text{ kg}\cdot\text{ha}^{-1}$  N, applied in three split applications.

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Table 1. Background soil chemical and physical properties across site years (0 to 15 cm depth).

Year	TKN (ppm)	NO <sub>3</sub> -N (ppm)	NH <sub>4</sub> (ppm)	M3-P (ppm)	OM (%)	MWHC (%)
2020	2288.1	9.2	ND	83.3	13.2	74.0
2021	2657.2	27.3	6.0	97.7	9.7	75.5
2022	3343.7	33.4	9.5	88.5	12.6	73.4

M3-P = Mehlich-3 phosphorus; MWHC = maximum water holding capacity; ND = not detected; NH<sub>4</sub> = ammonium nitrogen concentration; NO<sub>3</sub>-N = nitrate nitrogen concentration; OM = organic matter; TKN = total Kjeldahl nitrogen.

In 2020, all units received 56 kg·ha<sup>-1</sup> N monthly until each unit reached its designated N rate. By the third application, the units assigned to treatments of 224 and 280 kg·ha<sup>-1</sup> N received 112 and 168 kg·ha<sup>-1</sup> N, respectively, to reach their total assigned N rate. The first application was applied before transplanting using 44N-0P-0K environmentally smart nitrogen polymer-coated urea (Agrium U.S. Inc., Loveland, CO, USA), and the subsequent two applications were topdressed around the base of each plant (1 m<sup>2</sup>) as 46N-0P-0K prilled urea (TradeMark Nitrogen Corp., Tampa, FL, USA). In 2021 and 2022, N treatments were applied similarly but with one N source (as prilled urea; 46N-0P-0K). In 2021, the split applications were 60% pre-plant, 20% at the vegetative stage, and 20% at flowering and seed formation stage (Mediavilla et al. 1998), whereas in 2022, the split applications of N fertilizer were 30%, 30%, and 40%, respectively, at the same growth stages. In 2020 and 2021, 67 kg·ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> (as triple superphosphate; 0N-46P-0K) and 336 kg·ha<sup>-1</sup> K<sub>2</sub>O (as granular sulfate of potash; 0N-0P-50K) were incorporated into the soil before transplanting. In 2022, the same phosphorus and potassium fertilizer sources were used, although application rates

were reduced by half due to sufficient background soil levels. In the absence of rainfall, drip irrigation supplied 12.7 mm of water column weekly. Weeds were managed mechanically, manually, and with biodegradable paper mulches.

**Harvest procedure.** Flower harvest was determined when at least 50% of floral trichomes' resin transitioned to an amber color. Harvest occurred on 16 Sep 2020, 21 Sep 2021, and 20 Sep 2022. The main stem was cut at the soil surface to harvest aboveground fresh biomass for each plant, which was then weighed and recorded. In 2020, subsamples were taken to estimate dry matter, whereas in 2021 and 2022, each plant was oven-dried and hand-processed into separate flower, leaf, and stem fractions.

**Image collection with drone.** We compared the harvested aboveground biomass from the field trials with spectral data collected about 1 month before harvest annually to identify differences in canopy size and reflectance resulting from applied nitrogen rates. Hemp farmers are required to determine harvest date no more than 1 month before harvest so that regulatory testing may be completed to approve the lot for harvest. Furthermore, the

timing of the spectral data collection reflected this timing for harvest decision-making and allowed the hemp to reach harvestable floral maturity. Any plants that died during this period were excluded from the comparison.

Raw drone images were captured by a Sequoia multispectral sensor mounted onto a Parrot Bluegrass Fields drone (Parrot Drone SAS, Paris, France) after an inertial measurement unit and spectral calibration were performed. The drone had an autopilot system that allowed for autonomous navigation of an established flight plan over the field (Fig. 1). The Sequoia multispectral sensor, equipped with a 16-megapixel RGB camera and four multispectral sensors, captured canopy reflectance and drone images in the visible (green, red, and red edge) and NIR spectra with the following band specifications (Table 2). A sunshine sensor (Parrot Drone SAS) located on top of the drone continuously captured incoming light and adjusted for light variability that could alter reflectance data and image processing.

Drone flights and vegetation sensing were conducted between 10:00 AM and 1:00 PM EST to minimize shadows in collected drone imagery. The multispectral camera captured high-resolution images (2.5 cm pixel<sup>-1</sup>) at a

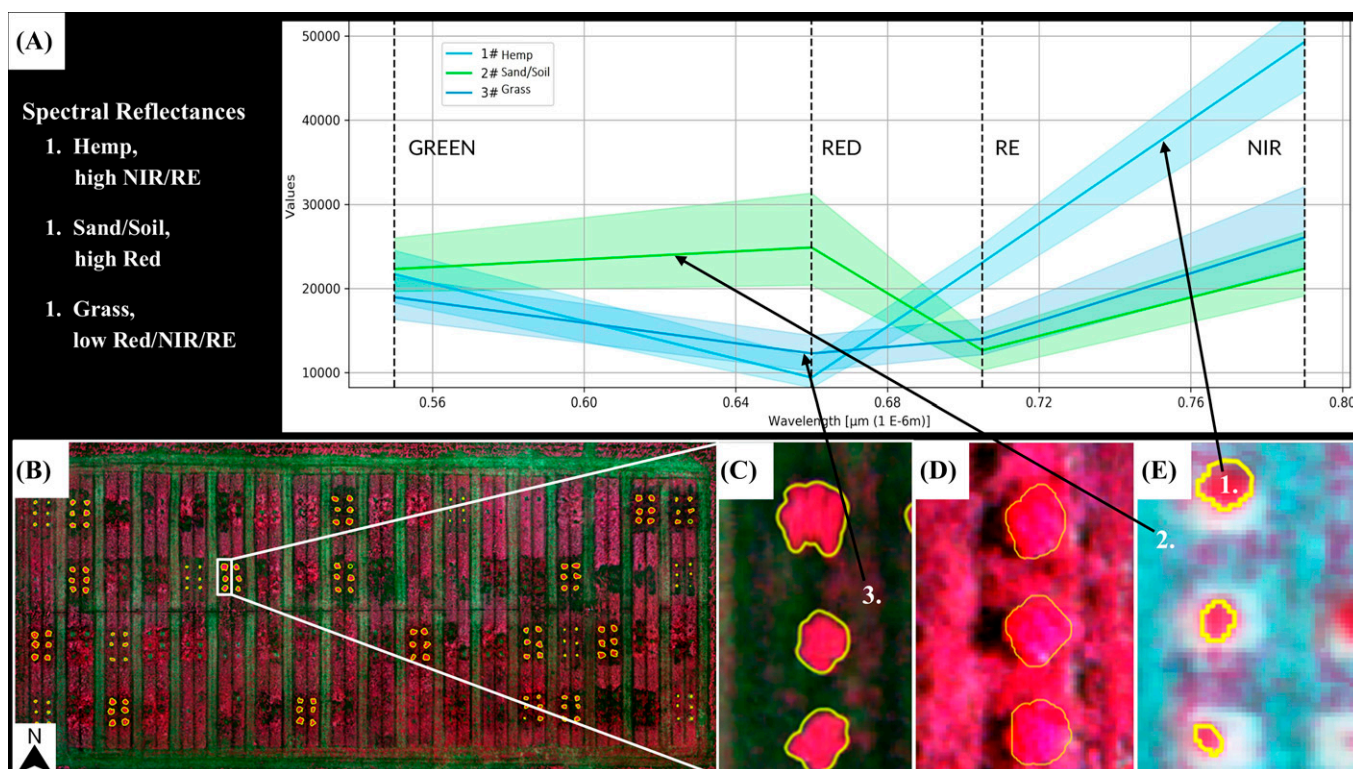


Fig. 1. Spectral reflectance captured from a drone flight conducted in a hemp nutrient management trial at an altitude of 61 m (A) on 12 Aug 2020 (B–C), 5 Aug 2021 (D), and 24 Aug 2022 (E) with the near-infrared (NIR) band shown as pink, and the red band as cyan. Vegetation cover is marked by the intensity of pinkish-red tones, and hemp canopy extractions are outlined in yellow (B–E). RE = Red edge.

Table 2. Sequoia multispectral sensor band specifications.

Sensor band	Minimum (nm)	Maximum (nm)	Width (nm)	Band center (nm)
Green	510	590	40	550
Red	620	700	40	660
Red edge	725	745	10	735
Near-infrared	750	830	40	790

rate of one picture per second with a 70% side and front overlap at an altitude of 61 m. The drone operated at a speed of 5 to 7 m·s<sup>-1</sup> to cover the area of interest (2.2 ha) with a flight time of approximately 10 min.

**Image processing and data products.** Acquired drone imagery was processed with Pix4DCapture software (Pix4D S.A., Prilly, Switzerland). Raw images were aligned and stitched together to create a seamless four-band (green, red, red edge, and NIR) stack orthomosaic with ground control points and a digital elevation model. The GPS locations for the plants were extracted from the flight images, and the final orthomosaic was clipped to the field boundary extent and uploaded to an online server. Plant canopies were outlined and extracted by supervised classification of the digital elevation model after automated spectral selection with artificial intelligence (Stanford et al. 2024). By distinguishing distinct canopy heights from surrounding vegetation and soil, we refined the AI extraction with manual corrections to address any errors related to canopy area overestimation, underestimation, and misidentification.

The NDVI and the GNDVI data products were evaluated for each pixel of the image using standard NDVI and GNDVI equations (Eqs. [1] and [2]) with results extracted for each canopy:

$$\text{NDVI} = \frac{\text{NIR} - \text{Red}}{\text{NIR} + \text{Red}} \quad [1]$$

$$\text{GNDVI} = \frac{\text{NIR} - \text{Green}}{\text{NIR} + \text{Green}} \quad [2]$$

where “Red” denotes the red channel, 620 to 700 nm; “NIR” denotes the near-IR channel, 750 to 830 nm; and “Green” denotes the green channel, 510 to 590 nm. The ratio between the difference of the numerator and the sum of the denominator results in a VI value ranging from -1.0 to 1.0. The VI value informs vegetation conditions, such as canopy coverage, biomass, chlorophyll content, and plant health. Lower VI values are associated with nonvegetative surfaces or unhealthy, stressed, and sparse plantings, while higher values are associated with moderate to healthy and dense vegetation (Jackson and Huete 1991; Walthall et al. 2003).

**Analysis.** The average plant aboveground biomass, floral yield, canopy area, NDVI, and GNDVI around harvest were evaluated for each unit with a 95% confidence interval to express variation. Statistical analyses were performed using a two-way analysis of variance [ANOVA, “aov()”] followed by a Duncan post-hoc test [“duncan.test()”; *agricolae* version 1.3-3] for each parameter using R studio software, version 4.0.2 (RStudio, Inc., Boston, MA, USA). The two-way ANOVA

assessed both the main effect of nitrogen rate and the interaction effect of nitrogen rate and year for each variable. Significant ANOVA results ( $P < 0.05$ ) were followed by a Duncan post-hoc test to identify differences between nitrogen rate groups and determine their consistency across years. To further explore the relationship between biomass, canopy area, and VIs, pairwise correlations were conducted using the linear model [“lm()”]. The analysis reported the coefficient of determination ( $r^2$ ) to indicate the strength and direction of their relationship.

## Results and Discussion

**Summary of results.** Hemp spectral characteristics were distinguishable from surrounding weedy vegetation and bare ground, whereby hemp was found to have a higher NIR and red edge reflectance (Fig. 1A). Through field evaluation and aerial imaging, we found that increasing N application rates up to 224 kg·ha<sup>-1</sup> positively increased values for aboveground biomass, floral yield, canopy area, and vegetation indices (Fig. 2). We also observed noticeable interactive effects by years driven by a high level of weed intensity

in 2021 and a delayed planting in suboptimal conditions in 2022 (Figs. 3 and 4). Analysis is presented both in aggregate and by year to thoroughly evaluate trends and deviations. Although the statistical differences showed year-to-year variability, our aggregate results also indicate an overall pattern of plant production and health to N rate. We observed a positive linear correlation between biomass and canopy area across all experiment years (Fig. 5A–C) and also a strong positive correlation between log-transformed biomass and spectral indices (NDVI and GNDVI) over successive study years (Fig. 6A–F).

**Aboveground biomass and floral yield.** Results from the two-way ANOVA for all study years (2020 to 2022) found that both the main effects of N rate and the interaction effects of N rate and year were significant ( $P < 0.001$ ) for aboveground dry biomass (biomass yield) and floral yield (Figs. 2A–B and 3A–F). Overall, maximum mean biomass yield (1252 kg·ha<sup>-1</sup>) was obtained from the nitrogen application rate of 224 kg·ha<sup>-1</sup> N, which was significantly higher than all other treatment rates (Fig. 2A). Hemp definitively benefits from moderate to high rates of N fertilizer indicated by the minimum mean biomass yield (308 kg·ha<sup>-1</sup>) at 0 kg·ha<sup>-1</sup> N without a significant difference at 56 kg·ha<sup>-1</sup> N (Fig. 2A). Maximum floral yield (482 kg·ha<sup>-1</sup>) was obtained from applying 224 kg·ha<sup>-1</sup> N but was not significantly different from 112, 168, and 280 kg·ha<sup>-1</sup> N (Fig. 2B). The minimum floral yield (129 kg·ha<sup>-1</sup>) resulted

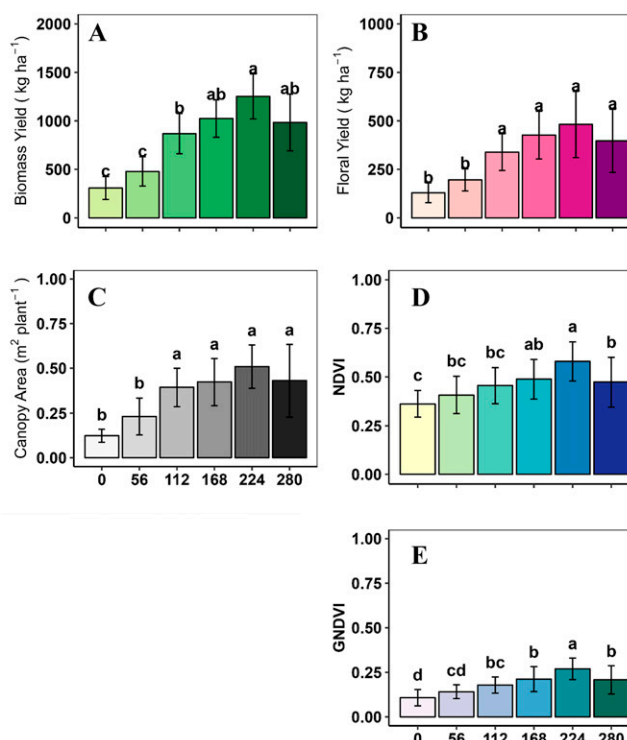


Fig. 2. Biomass yield (A), floral yield (B), canopy area (C), Normalized Difference Vegetation Index (NDVI) (D), and Green NDVI (GNDVI) (E) response to nitrogen fertilizer treatments (0, 56, 112, 168, 224, and 280 kg·ha<sup>-1</sup> N) from combined study years (2020 to 2022). Columns with the same letter are not significantly different at  $P \leq 0.05$  according to Duncan’s new multiple range test. Error bars denote a 95% confidence interval.



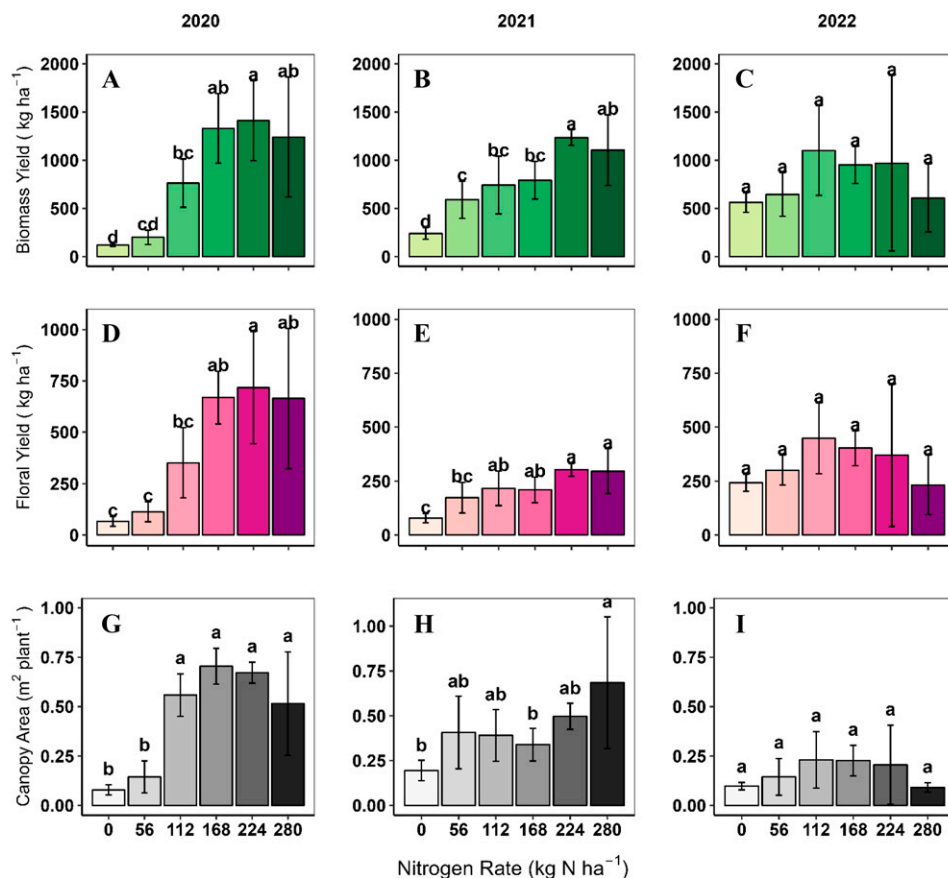


Fig. 3. Biomass yield (A–C), floral yield (D–F), and canopy area (G–I) resulting from each nitrogen treatment (0, 56, 112, 168, 224, and 280 kg·ha<sup>-1</sup> N) during 2020 (left), 2021 (middle), and 2022 (right). Columns with the same letter are not significantly different at  $P \leq 0.05$  according to Duncan's new multiple range test. Error bars denote a 95% confidence interval.

from 0 kg·ha<sup>-1</sup> N and did not significantly differ from 56 kg·ha<sup>-1</sup> N.

Evaluated across years, our results identified an N rate recommendation for floral hemp production between 112 to 224 kg·ha<sup>-1</sup> N. Similar N rate recommendations were established from global studies of hemp crops (Atoloye et al. 2022; Caplan et al. 2017a, 2017b; Papastilianou et al. 2018; Vera et al. 2010; Yang et al. 2021), despite the unique gravelly and calcareous condition of our soil. At nitrogen rates above the recommended range (i.e., 280 kg·ha<sup>-1</sup> N), biomass and floral yield were reduced by 21% and 17%, respectively, compared with the 224 kg·ha<sup>-1</sup> N rate. Similar findings were reported by Anderson et al. (2021) and Saloner et al. (2019), who observed that higher application rates of N fertilizer resulted in reduced plant growth, biomass accumulation, and harvestable yield in *C. sativa* crops. These results could be explained by nitrogen toxicity, which results in cell damage from increased intracellular pH, nitrate accumulation, salinity, and oxidative stress (Saloner and Bernstein 2020). *Cannabis* crops have been shown to express symptoms of N toxicity as unusually dark green foliage with downward curling leaves progressing into leaf necrosis and increased plant mortality (Anderson et al. 2021; Saloner and Bernstein 2020). The expression of phytotoxicity (i.e., dark green foliage) in hemp is an observable

characteristic for understanding crop health from aerial imaging.

For results analyzed separately by year, the N fertilizer rate significantly affected biomass yield in 2020 and 2021 ( $P < 0.001$ ; Fig. 3A and B), similar to the combined-year analysis, whereas no significant effect was found for 2022 ( $P = 0.598$ ). In 2022, the highest mean biomass yield (1101 kg·ha<sup>-1</sup>) was observed at 112 kg·ha<sup>-1</sup> N, and the lowest mean biomass yield (608 kg·ha<sup>-1</sup>) at 280 kg·ha<sup>-1</sup> N (Fig. 3C). Differences between floral yields obtained from applying N at the different rates were significant ( $P < 0.001$ ) for 2020 and 2021, similar to the combined-year analysis, but not significant for 2022 (Fig. 3D–F). Current nutrient recommendations for floral hemp production in Florida suggest application rates of 112 to 168 kg·ha<sup>-1</sup> N (Brym et al. 2024). These rates vary with respect to hemp variety and production target whereby the most vigorous and productive of varieties observed correspond to the higher range of the N rate recommendation. Conversely, poorer performing years that might experience establishment, weed, and disease challenges as were observed in 2021 and 2022 might not respond effectively to higher N rates. A standard nutrient management best practice for Florida is to split N applications to multiple application events during the year, allowing for adaptive timing

and rate of fertilizer to match crop development and performance.

**Canopy area.** As detected by drone-based aerial imaging, canopy area was observed to positively increase with N fertilization (Figs. 2C and 3G–I). Nitrogen rate, year, and the rate  $\times$  year interaction significantly affected canopy area for combined-year analysis, and 2020 independently showed significant post-hoc groupings ( $P < 0.001$ ). Combining years, the largest mean canopy area (0.51 m<sup>2</sup>) occurred with 224 kg·ha<sup>-1</sup> N but only differed significantly from canopy areas at 56 and 0 kg·ha<sup>-1</sup> N (Fig. 2C). Similarly, in 2020, lower fertilizer rates (0 to 56 kg·ha<sup>-1</sup> N) were statistically different from canopy area values at moderate to high fertilizer rates ( $>112$  kg·ha<sup>-1</sup> N), whereas in 2021, the 280 kg·ha<sup>-1</sup> N rate resulted in an average canopy area that was statistically greater than canopy area at 0 and 168 kg·ha<sup>-1</sup> N yet not statistically different from average canopy area values at 56, 112, and 168 kg·ha<sup>-1</sup> N ( $P < 0.01$ ). In 2022, no significant differences were observed between the fertilizer groupings ( $P = 0.847$ ). In 2021, canopy area was significantly higher at 280 kg·ha<sup>-1</sup> N when compared with 0 and oddly with 168 kg·ha<sup>-1</sup> N (Fig. 3H). In 2022, canopy area was not significantly affected by fertilizer rate, although an increasing trend was observed with intermediate rates (112 to 224 kg·ha<sup>-1</sup> N).

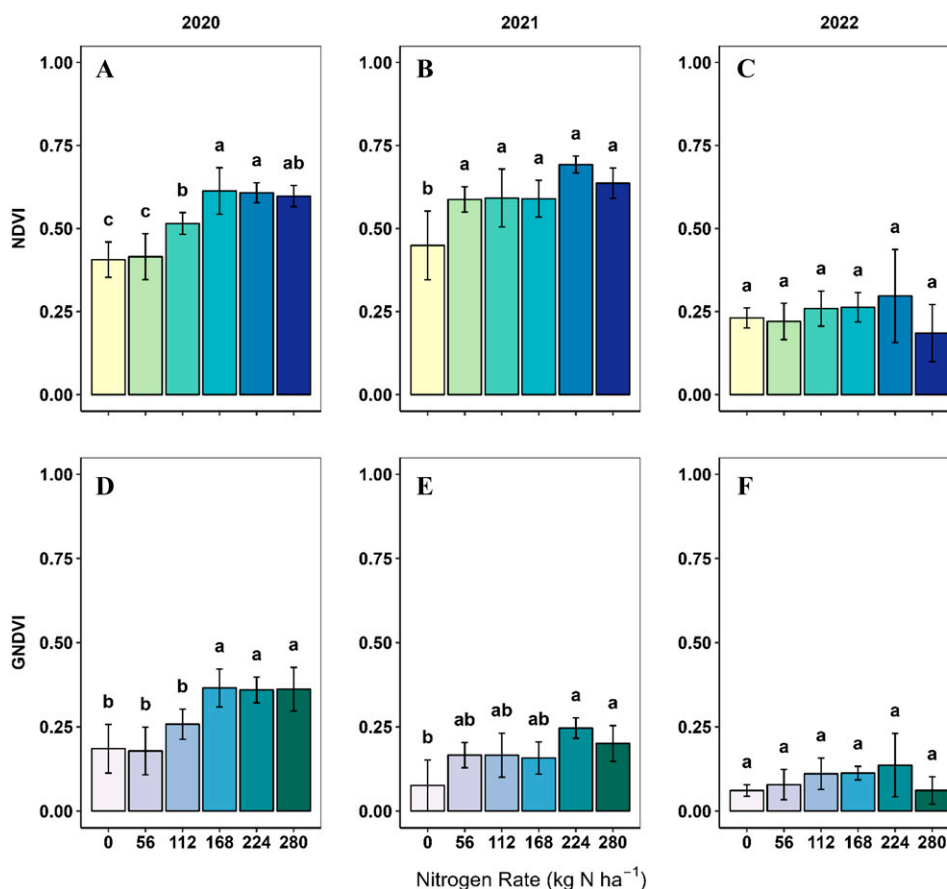


Fig. 4. Normalized Difference Vegetation Index (NDVI) (A–C) and Green NDVI (GNDVI) (D–F) response to varying N fertilizer application rates (0, 56, 112, 168, 224, and 280  $\text{kg}\cdot\text{ha}^{-1}$  N) during 2020 (left), 2021 (middle), and 2022 (right). Columns with the same letter are not significantly different at  $P \leq 0.05$  according to Duncan's new multiple range test. Error bars denote a 95% confidence interval.

Plant canopies appeared to be less sensitive to changes in N application rates than aboveground biomass, with increases at 56 and 112  $\text{kg}\cdot\text{ha}^{-1}$  (Fig. 2A and C). While our study did not report data from the vegetative growth stage, similar N rate studies have shown that leaf and branch counts, as well as canopy coverage, positively responded to increasing N fertilization up to moderate levels (90 to 100  $\text{kg}\cdot\text{ha}^{-1}$ ) during vegetative growth

(Campiglia et al. 2017; Caplan et al. 2017b; Farnisa et al. 2023; Tang et al. 2017), which correlated to increases in growth and yield during flowering (Caplan et al. 2017a; Tang et al. 2018). In addition, canopy development, crop growth, and yield were negatively correlated to N fertilizer rates at or below 60  $\text{kg}\cdot\text{ha}^{-1}$  N. The observed analytical results between plant biomass and canopy area suggest varying sensitivity of plant size and

behavior to limited N rates (Barker and Pilbeam 2006; Saloner and Bernstein 2020; Zhang et al. 2007). For example, we observed a significant decrease in aboveground biomass and some instances of plant mortality at 280  $\text{kg}\cdot\text{ha}^{-1}$  N, but no statistical difference was observed in canopy area. Although canopy area can serve as an indicator of plant size and growth, it may lack the practical resolution to distinguish aspects of nutrition and production status.

**Vegetative indices.** NDVI and GNDVI calculated from canopy reflectance increased with increasing N rate ( $P < 0.001$ ; Fig. 2D–E). The results were significant when aggregated and with an interaction by year (Figs. 2D–E and 4). NDVI values increased from 0.36 to 0.58 as N rates increased from 0 to 224  $\text{kg}\cdot\text{ha}^{-1}$  N, whereas GNDVI values increased from 0.11 to 0.27. The highest mean values for NDVI (0.58) and GNDVI (0.27) were obtained at 224  $\text{kg}\cdot\text{ha}^{-1}$  N, similar to observed biomass and floral yield. NDVI at 224  $\text{kg}\cdot\text{ha}^{-1}$  N was statistically higher than those of all other N rates aside from the 168  $\text{kg}\cdot\text{ha}^{-1}$  N rate. GNDVI at 224  $\text{kg}\cdot\text{ha}^{-1}$  N was also a statistical maximum. At 280  $\text{kg}\cdot\text{ha}^{-1}$  N, both VIs exhibited significant decreases in reflectance values when compared with 224  $\text{kg}\cdot\text{ha}^{-1}$  N, with NDVI and GNDVI reflectance values decreasing by 19% and 22%, respectively

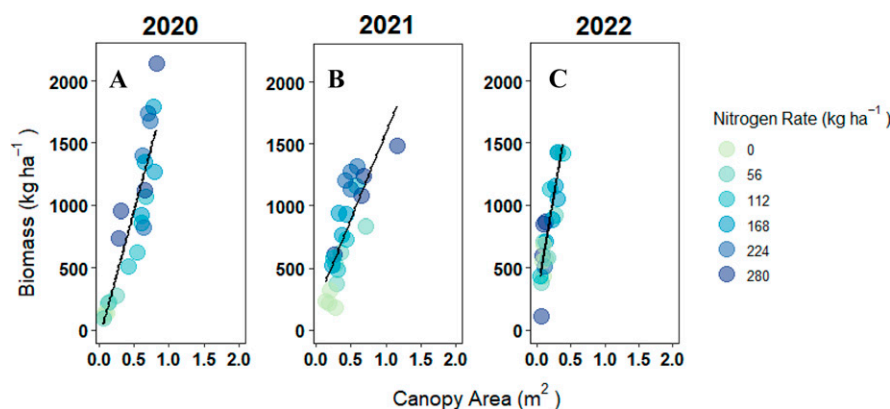


Fig. 5. Linear regression between aboveground biomass ( $\text{kg}\cdot\text{ha}^{-1}$ ) and canopy area ( $\text{m}^2$ ) across 3 years. (A) 2020,  $r^2 = 0.822$ ,  $y = 2051x - 69$ . (B) 2021,  $r^2 = 0.653$ ,  $y = 1391x + 202$ . (C) 2022,  $r^2 = 0.772$ ,  $y = 3190x + 274$ . Data point shade corresponds to the fertilizer application rate (0, 56, 112, 168, 224, and 280  $\text{kg}\cdot\text{ha}^{-1}$  N).

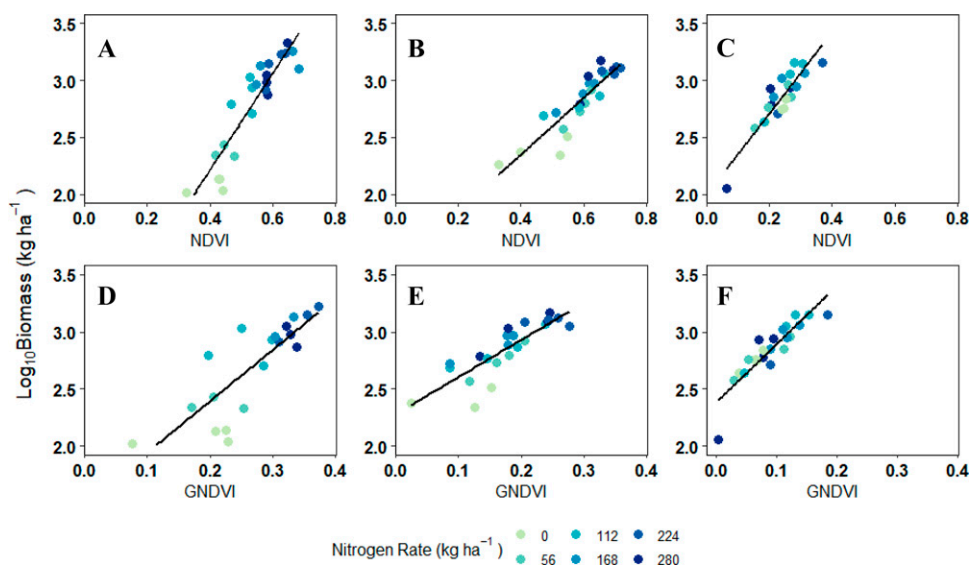


Fig. 6. Linear regression between log-transformed aboveground biomass (kg·ha<sup>-1</sup>) and vegetative indices. (A–C) Normalized Difference Vegetation Index (NDVI). (A) 2020,  $r^2 = 0.849$ ,  $\log_{10}(y) = 4.14x - 0.58$ . (B) 2021,  $r^2 = 0.805$ ,  $\log_{10}(y) = 2.50x - 1.35$ . (C) 2022,  $r^2 = 0.814$ ,  $\log_{10}(y) = 3.56x - 2.00$ . (D–F) Green NDVI (GNDVI). (D) 2020,  $r^2 = 0.768$ ,  $\log_{10}(y) = 4.00x + 1.61$ . (E) 2021,  $r^2 = 0.775$ ,  $\log_{10}(y) = 3.28x + 2.27$ . (F) 2022,  $r^2 = 0.795$ ,  $\log_{10}(y) = 5.09x + 2.39$ . Data point shade corresponds to the fertilizer application rate (0, 56, 112, 168, 224, and 280 kg·ha<sup>-1</sup> N).

(Fig. 2D–E). The lowest NDVI (0.36) and GNDVI (0.11) values were observed at the 0 kg·ha<sup>-1</sup> N rate. These results suggest that NDVI and GNDVI from aerial images are useful indicators for plant N status; however, the practical results were not consistent year to year.

When the results were analyzed for each year, N fertilization positively influenced VIs in 2020 and 2021, but not in 2022 ( $P < 0.01$ ; Fig. 4). In 2021, fewer groupings limited the interpretation of VI. Canopies of hemp plants were extracted from aerial images to evaluate the VI, which were challenged by weed competition in 2021 and late season planting in 2022, likely affecting the outcome. Overall patterns in the results remain, such as the maximum mean value for NDVI observed at 168 and 224 kg·ha<sup>-1</sup> N in 2020 (0.61) and 2021 (0.69) and the minimum mean reflectance values under no fertilization in 2020 (0.40) and 2021 (0.25). The highest GNDVI values occurred between 168 and 224 kg·ha<sup>-1</sup> N in 2020 (0.37) and 2021 (0.25). In 2022, both VIs obtained their greatest values at 224 kg·ha<sup>-1</sup> N followed by a reduction in reflectance values at 280 kg·ha<sup>-1</sup> N, although these results were not statistically significant.

NDVI is used to monitor and assess crop health and nutrition, where an NDVI value of 0.58, as observed in our study, is associated with moderately healthy plants with dense canopies and good fertility (Nichiporovich and Radevich 2012). Conversely, NDVI values below 0.40 are associated with unhealthy, stressed, and sparse vegetation with nutrient deficiency. GNDVI is sensitive to chlorophyll content in vegetation associated with an increase in photosynthetic activity and crop biomass (Candiago et al. 2015; Cherlinka 2019; Li et al. 2008; Viña et al. 2011). An increase in N rate has been shown to affect chlorophyll content in *Cannabis* plants, where those treated

with inadequate N resulted in N stress and lower chlorophyll content. Both indices are viable candidates for crop status determination and likely both obtainable by a multispectral camera. From our study, NDVI was more effective than GNDVI in detecting differences in N rate treatments, and the year 2020 was more discernable than the other years. Dense canopies of *Cannabis*, which were well-fertilized and watered, were found to be more spectrally discriminable than sparse canopies (Daughtry and Walthall 1998). Conversely, high weed pressure and smaller-sized *Cannabis* plants were a challenge to separate by drone imagery (Walthall et al. 2006). Well-maintained hemp crops make strong candidates for evaluation by aerial imaging with detailed in-season information possible to assist management decisions such as split N rate applications.

**Plant biomass correlation with aerial imaging.** Plant biomass correlates well ( $r^2 > 0.65$ ) with features generated by aerial imaging (i.e., canopy area, NDVI, GNDVI) evaluated independently for each year studied (Figs. 5 and 6). When evaluated across years, canopy area maintained a moderate relationship with plant biomass ( $r^2 = 0.557$ ,  $y = 1412x + 317$ ), whereas NDVI and GNDVI resulted in weak relationships with plant biomass ( $r^2 = 0.214$ ,  $\log_{10}(y) = 2.45x + 5.26$ ;  $r^2 = 0.397$ ,  $y = 2704x + 311$ ). NDVI was the strongest correlation with aboveground biomass at harvest evaluated independently for each year ( $r^2 > 0.80$ ), whereas GNDVI also showed strong correlation ( $r^2 > 0.76$ ). This is consistent with studies reporting that NDVI was best suited for estimating aboveground biomass of wheat under different N fertilizer rates (Erdle et al. 2011; Geipel et al. 2016; Viña and Gitelson 2005), although an obvious operational preference would be a model that performs effectively

across years instead of a model adapted for each year. These results indicate that plant identification, selection, and quantification from aerial imaging is a viable tool for determining hemp growth and production with the important consideration of specific site and year (Daughtry and Walthall 1998; Hall et al. 2014) (Fig. 1B). The observations from our study are well associated with changes in plant performance due to fertilizer application that can be further inferred from an assessment with aerial imaging. This study indicates that a strong correlation between aboveground biomass and NDVI can be adapted to different plants, environmental conditions, levels of weed intensity, and plant-level response to fertility.

The relationship of aboveground biomass and canopy area were best fit across years with a linear regression (Fig. 5). Some variability in estimated regression slope was observed between years with closer slope estimates for 2020 and 2022 than for 2021. In 2021, the plants were exposed to higher weed pressure and overall smaller at harvest than in 2020. In 2022, plants were also smaller due to later planting. The relationship between aboveground biomass and canopy area in 2021 was characterized with a shallower slope and showed slight improvements in model performance when aboveground biomass was log-transformed (linear,  $r^2 = 0.822$ ; log,  $r^2 = 0.890$ ). Features of plant size may be expected to follow scaling laws (Deng et al. 2012; Nilas 2004); for example, offering the opportunity to link remotely sensed canopy area to aboveground biomass. Growth in plant size occurs in multiple dimensions leading to nonlinear (i.e., exponential or allometric) patterns. Canopy area is a two-dimensional feature, whereas aboveground biomass is three-dimensional. Plants in the study were

effectively estimated with a linear regression indicating that plants were relatively similar in size, although some signs of the nonlinear relationship were detected in 2021.

The linear regression models with NDVI were best fit when aboveground biomass was log-transformed, illustrative of the expected nonlinear scaling of aboveground biomass (Niklas 2004). Consistent with the relationship between aboveground biomass and canopy area, the highest correlation with NDVI was observed in 2020. However, for GNDVI, 2021 and 2022 were slightly higher correlations than 2020. Correlation of aboveground biomass with GNDVI was improved in 2020 and 2022 when aboveground biomass was modeled as linear and not log-transformed. These correlation estimates generated by the study appear strong, although additional calibration of observed features and predicted values must account for variability in hemp variety, field characteristics, and weed pressure.

## Conclusions

Drone-based aerial imaging can effectively detect and monitor differences in crop health and biomass yield for field-grown floral hemp. Differences in canopy size and spectral reflectance were detectable in drone-based aerial images and positively correlated with harvested aboveground biomass across years with varying abiotic conditions and high weed pressure. When comparing aboveground biomass, flower yield, canopy area, NDVI, and GNDVI, suggested floral hemp production may require 112 to 224 kg-ha<sup>-1</sup> N for production in south Florida on Krome gravelly loam soil. Our study supports the use of drone-based aerial imaging to detect differences in crop health and yield for field-grown floral hemp and inform N management decisions for growers and researchers. In-season monitoring of canopy area and NDVI may be used for crop health assessments and determination for later season split applications of fertilizer. Future studies are needed to expand aerial imaging detection for additional hemp varieties, growth stages, and field conditions and explore practical aspects for site-specific adaptive management using information from aerial imaging.

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