Short Communication

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Response of cannabidiol hemp (*Cannabis sativa* L.) varieties grown in the southeastern United States to nitrogen fertilization

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Abstract: With the expanding hemp cannabidiol (CBD) industry in the United States, information on fertilizer recommendations for optimal production under field conditions is deficient in the literature. This study evaluates the effects of nitrogen (N) fertilization on bud biomass and CBD yield of two high-yielding CBD hemp varieties (Spectrum and Therapy) under field conditions. Four application rates of N fertilization (0, 56, 112, and 224 kg N ha^{-1}) were supplied using 2 commercially available N fertilizers. Fresh bud biomass, dry bud biomass, and CBD yield (g $plant^{-1}$) increased quadratically with N fertilization rates, with optimum rates between 140 and 190 kg N ha^{-1}. When pooled across varieties, the tissue N concentration was linearly related to the fresh bud biomass, dry bud biomass, and CBD yield. Our findings show that N fertilization can affect CBD yield under field conditions. The resulting effect of N fertilizer rates on these varieties could serve as a preliminary guide for CBD hemp production under field conditions, although results may differ with variety, location, or fertilizer type.

Keywords: hemp, cannabidiol yield, nitrogen fertilizer, bud yield

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1 Introduction

In recent years, there has been a resurgence in the cultivation of an ancient crop, hemp (*Cannabis sativa* L., Cannabaceae), in the United States following its removal from the lists of schedule 1 controlled substances in the Controlled Substances Act (H.R.5485 – Hemp Farming Act of 2018) after the passage of the 2018 farm bill [1,2]. The increase in consumer demand for secondary metabolites such as cannabinoids (e.g., cannabidiol [CBD]), terpenes, flavonoids, etc., obtainable from hemp (hereinafter referred to as "CBD hemp") for medicinal and recreational purposes has made it an invaluable crop [3–5]. Attempts have also been made in processing hemp biomass residue into biochar to enhance soil quality and reduce nutrient loss [6]. As such, the rapid growth in the hemp industry has led to the emergence of many hemp varieties with unpredictable characteristics and performance [5]. The rapidly increasing cultivation of CBD hemp calls for the development of fertilizer recommendations specific to varieties and locations.

Recently, research studies evaluating the effect of soil nutrients on CBD hemp agronomic performance and cannabinoid production in North America have been published. Most of these studies, however, have been carried out in greenhouses [7–12] with only few under field conditions. Hemp is a dioecious plant and CBD hemp farmers prefer to cultivate the female plant for its high CBD yield. While optimal fertilization requirements for hemp grown for fiber and seed production under field conditions are available [1,3,7,13], few exist for hemp cultivated for cannabinoids even as farmers' interest continue to grow [14,15].

To determine the effect of nitrogen (N) fertilization on CBD hemp, we carried out a 2 year field study evaluating the impact of 4 rates of N fertilizer on plant growth, bud biomass, CBD, and leaf tissue concentrations on 2 high yielding CBD hemp varieties, Spectrum and Therapy, in North Carolina, USA.

2 Materials and methods

2.1 Site description

Field trials were undertaken between June and October in 2019 and 2020 at the North Carolina Agricultural and Technical (NC A&T) State University Research Farm (36°5′N, 79°44′W) located at Greensboro, North Carolina, USA. Soil type is a fine, mixed, active, thermic Ultic Hapludalfs Alfisol (58% sand, 13% silt, and 29% clay) [16]. Soil chemical properties were measured prior to N fertilization in the spring. The soil pH (1:2, soil:water w/v), total organic carbon (TOC), total N (TN), and Mehlich (III) phosphorus (P) values are 7.2, 14.8 g kg^{-1}, 2.1 g kg^{-1}, and 182 mg kg^{-1}, respectively. The TOC and TN were determined via dry combustion using a CHN analyzer 2400 series II, (Perkin/2400 Elmer, Akron, OH, USA). Based on the soil attributes, the pH is circumneutral, the TOC is average, while the TN and Mehlich (III) P values are high, reflecting legacy fertilizer. The mean annual temperature (MAT), mean annual precipitation (MAP), and relative humidity (RH) were obtained from the nearest Weather Station through the North Carolina State Environment and Climate Observing Network (https://econet.climate.ncsu.edu). In 2019, MAT, MAP, and RH were 15.7°C, 110 mm, and 69%, while in 2020, they were 15.6°C, 130 mm, and 68%, respectively.

2.2 Experimental design and treatments

Experiments were laid out as a randomized complete block split-plot design with four replicates. Fertilizer N application rate was the main plot effect and variety was the sub-plot effect. The plots were fertilized at the time of planting using two commercially available fertilizers, Nature Safe (NSF) (Cold Spring, KY, USA) and Harmony Ag (HAG) (Roanoke, VA, USA). To achieve equivalent fertilizer N rates of 0, 56, 112, and 224 kg N ha^{-1}, 0, 11.2, 22.4, and 44.8 kg N ha^{-1} NSF fertilizer were combined with 0, 44.8, 89.6, and 179.2 kg N ha^{-1} HAG fertilizer, respectively. The composition of the fertilizers as per manufacturer description are presented in Appendix Table 1. Two high-yielding CBD hemp cultivar cuttings, most readily available in North Carolina, "Spectrum" and "Therapy," were obtained from a mother stock plant and established as clones in the greenhouse in early May. The clones were transplanted to the field in the first week of June 2019 and 2020. Clones were transplanted into holes at 0.3 m depth using a spacing of 1.83 m between rows and between plants with 10 pants in a row, as there is no recommended spacing for CBD hemp [17]. The spacing of CBD hemp, in part, would depend on the canopy width of the variety. At harvest, the canopy width was 0.6 and 1.7 m for Therapy and Spectrum varieties, respectively. The experimental plots were plowed, disked, and beds were prepared using a plastic mulch (76.2 cm wide), to control weed, followed by the installation of drip irrigation lines. The plots were irrigated with approximately 12 cm of water per week, except where there was adequate soil moisture due to precipitation. Pruning was done by cutting the growing apex of the main stem with disinfected clippers at 10 weeks after transplanting. Plants that became infected with southern stem blight were dug up from the field using disinfected materials.

2.3 Plant materials

At the mid-vegetative stage (4 weeks after transplanting, T1) and late vegetative stage (8 weeks after transplanting, T2), 3 most recently matured leaves (MRML) from 3 plants in each plot were sampled to evaluate the critical macronutrient and micronutrient leaf tissue concentrations. The MRML is described as the 3rd to 5th leaf down from the plant apex and could be a good indicator of plant nutrient deficiency [18]. Tissue samples were dried in a forced-air oven at 60°C until a constant weight was achieved and the dry mass was recorded. The oven-dried tissue samples were ground and analyzed at the Analytical Services Laboratory, NC A&T State University for N, P, K, Ca, Mg, Fe, Zn, and Mn after digestion in HNO_3/HF digestion using an Optima 8300 ICP-OES (PerkinElmer, Inc. Shelton, CT, USA). Certified reference materials of highest purity were dissolved in nitric acid and used for standard preparation.

2.4 Cannabinoid analysis

At harvest, three plants were destructively sampled per replicate by collecting the entire plant excluding the roots. The buds were trimmed manually, and fresh weights were taken. The buds were dried at 15–21°C for 1 week in a drying chamber to obtain the dry bud biomass. Composite fresh bud samples from each field replicate were oven-dried to w/w moisture content between 0.5 and 1.25% at 65°C until moisture change was 1 mg min^{-1}. The % w/w on a dry weight basis of two cannabinoids, i.e., CBD and tetrahydrocannabinol (THC) were quantified at the Biological

Engineering Laboratory at NC A&T State University. Certified reference materials, CBD (CAS 13956-29-1), Δ9-THC (CAS 1972-08-3), Δ8-THC (CAS 5957-75-5), from Restek Corporation (Bellefonte, PA, USA) were used in the chemical analysis. Briefly, 0.224 g homogenized dry hemp buds were placed in a 56 mL centrifuge tube. Thereafter, 40 mL of ACS-grade methanol was added, and the tubes were placed on a wrist action shaker for 15 min. A 1 mL syringe was used to pull a clear aliquot and passed it through a 0.22 µm hydrophobic polytetrafluoroethylene filter into a 2 mL amber vial. Samples were analyzed with an Agilent 7890A gas chromatograph, with a flame ionization detector (Agilent Technologies, Inc.). The amount of CBD (g $plant^{-1}$) was quantified.

2.5 Statistical analysis

Statistical analysis was carried out using the GLIMMIX procedure in SAS [19]. Fertilizer rate was denoted as the main plot fixed effect and variety was specified as the sub-plot fixed effect and year as a repeated measure while block was the random effect. For the leaf tissue nutrient analyses, the plant vegetative stage was included as a second repeated measure. Residuals were checked for normality and homogeneity of variance and where necessary, variables were log-transformed before analysis and back-transformed to obtain the mean values. Differences between the mean values were separated using Tukey-Kramer method ($p < 0.05$). Four response models (linear, quadratic, cubic, and square root) were fitted to the data for each variety for each year using the REG procedure in SAS [19]. The linear model is defined by equation (1), quadratic model by equation (2), cubic model by equation (3), and square root model by equation (4). The correlation between the fresh bud biomass, dry bud biomass, and CBD yield with leaf tissue nutrients was examined using Pearson correlation.

$$Y = a + bX, \quad (1)$$

$$Y = a + bX + cX^2, \quad (2)$$

$$Y = a + bX + cX^2 + dX^3, \quad (3)$$

$$Y = a + bX + eX^{1/2}, \quad (4)$$

where Y is either the fresh bud biomass (kg ha^{-1}), dry bud biomass (g $plant^{-1}$), or CBD yield (g $plant^{-1}$) and X is N fertilizer rate; a (intercept), b (linear coefficient), c (quadratic coefficient), d (cubic coefficient), and e (square root coefficient) are constants obtained by fitting the model to data. To address multicollinearity, the quadratic and cubic models were executed with the centered independent variables.

3 Results

3.1 Fresh and dry bud biomass and cannabidiol yield

For both varieties, fresh bud biomass was between 874 and 3,317 kg ha^{-1} and was influenced by the N rate × variety × year interaction (Table 1 and Figure 1). In the 2 years, the fresh bud biomass for both varieties at 112 and 224 kg N ha^{-1} was significantly higher compared to the 0 and 56 kg N ha^{-1} rates. Furthermore, Spectrum variety had higher fresh bud biomass than Therapy at the 0 and 56 kg N ha^{-1}, but not at the 112 and 224 kg N ha^{-1} rates except in the year 2020.

Evaluating the R^2 values, the quadratic, cubic, and square root models performed better than the linear model (Appendix Table 2). Since the quadratic and cubic

Table 1: Degrees of freedom, *F*-value, and significance level for the effects of N fertilizer rate, variety, and year on fresh bud biomass, dry bud biomass, and CBD yield in 2019 and 2020

Source of variation	Numerator df	Fresh bud biomass (kg ha^{-1})	Dry bud biomass (g $plant^{-1}$)	CBD (g $plant^{-1}$)
N Fertilizer (F)	3	55.7*	43.6*	16.2*
Variety (V)	1	141.5*	74.8*	39.8*
Year (Y)	1	38.6*	20.1*	15.5*
F × V	3	3.20	2.01	0.17
F × Y	3	4.66*	3.33*	1.11
V × Y	1	0.53	0.03	1.26
F × V × Y	3	5.01*	3.40*	4.33*

*Significant at the 0.05 probability level.

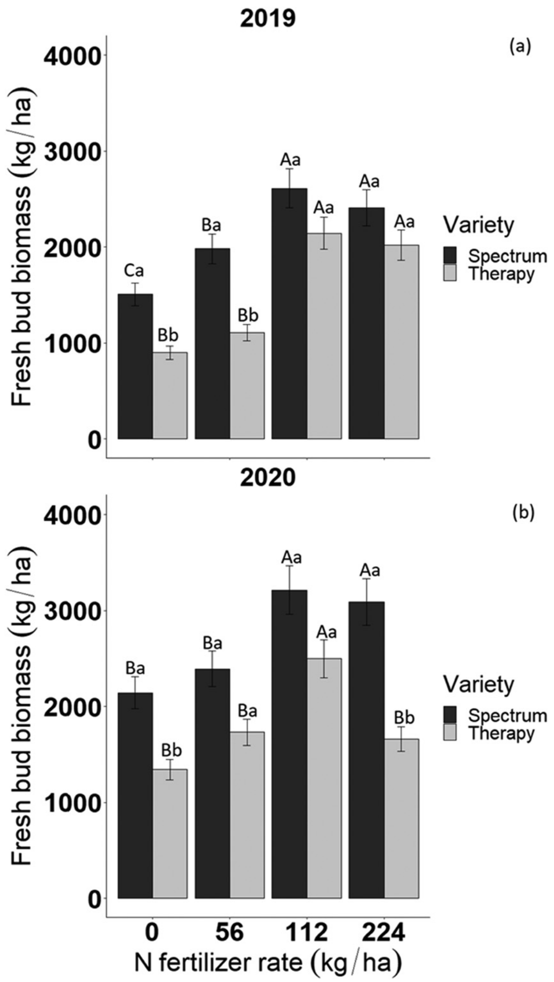

Figure 1: Interaction effects of nitrogen fertilization with year and CBD hemp varieties on fresh bud biomass in (a) year 2019 and (b) year 2020. Mean values designated by different lower-case letters indicate a significant difference between varieties at the same fertilizer level and mean values designated by different upper-case letters indicate a significant difference between N fertilizer rates for the same variety.

models consistently had higher R^2 and lower Bayesian information criterion values than the square root model and the variance inflation factor of the cubic model >10, the quadratic model was prioritized (Appendix Tables 2–3).

The interpolated maximum yields using the quadratic model showed that the relationship between the optimum N fertilizer rate and fresh bud biomass differed between the two varieties. In the year 2019, Spectrum variety fresh bud biomass decreased at rates supplied above 160 kg N ha^{-1} and for Therapy variety, fresh bud biomass decreased above 180 kg N ha^{-1} (Figure 2a and b). However, in 2020, the fresh bud biomass decreased above 180 and 130 kg N ha^{-1} for Spectrum and Therapy varieties, respectively. Similarly, the bud yield responded to N fertilization rates as the plant biomass dry matter (Figure 2c and d). The maximum dry bud biomass for Spectrum variety was obtained between 150 and 160 kg N ha^{-1} and for Therapy variety between 120 and 180 kg N ha^{-1}. The THC concentration, 0.1–0.3% w/w, was within the required limit, while the CBD concentration was between 2.4 and 11% w/w Dry matter (DM) (Appendix Table 4). While there were no significant main effects or interactions on the percent CBD concentrations, the obtainable CBD yield (g plant^{-1}) increased quadratically with N fertilization and reached a maximum at rates between 130 and 200 kg N ha^{-1} (Figure 2e and f). In the year 2019, an optimal N rate of 120 kg ha^{-1} was predicted for Spectrum, while 180 kg ha^{-1} was predicted for Therapy. However, in the year 2020, Spectrum was predicted to require a higher amount of N (250 kg ha^{-1}) for bud production and a lower amount of N (130 kg ha^{-1}) for Therapy. Overall, from the fitted equations, the optimal N application rate using a combination of NSF and HAG fertilizers for maximum CBD yield for Spectrum variety falls between 130 and 250 kg N ha^{-1} and for Therapy variety is approximately 200 kg N ha^{-1}. Furthermore, the linear relationships between the plant dry matter biomass, bud yield, and CBD (g plant^{-1}) were significant ($p < 0.05$).

3.2 Leaf tissue concentration and linear relationship with plant agronomic data

The leaf tissue nutrient concentrations varied greatly by variety (Table 2). The main effect of growth stage, year, and fertilizer and interactions were not significant. Spectrum had higher concentrations of N and P, while Therapy had higher concentrations of Zn. When pooled across varieties, the correlation results showed that leaf tissue N was positively correlated with fresh bud biomass, dry bud biomass, and CBD yield (g plant^{-1}) at T1. However, at T2, only CBD yield (g plant^{-1}) was positively correlated with the leaf tissue N concentration (Table 3).

4 Discussion

Both CBD hemp varieties responded to N fertilization; however, Spectrum variety produced higher bud biomass than Therapy variety. Our findings indicate that Therapy variety may require greater amounts of fertilizer for maximum growth than Spectrum variety. The difference in the rate of N predicted for the two varieties suggests

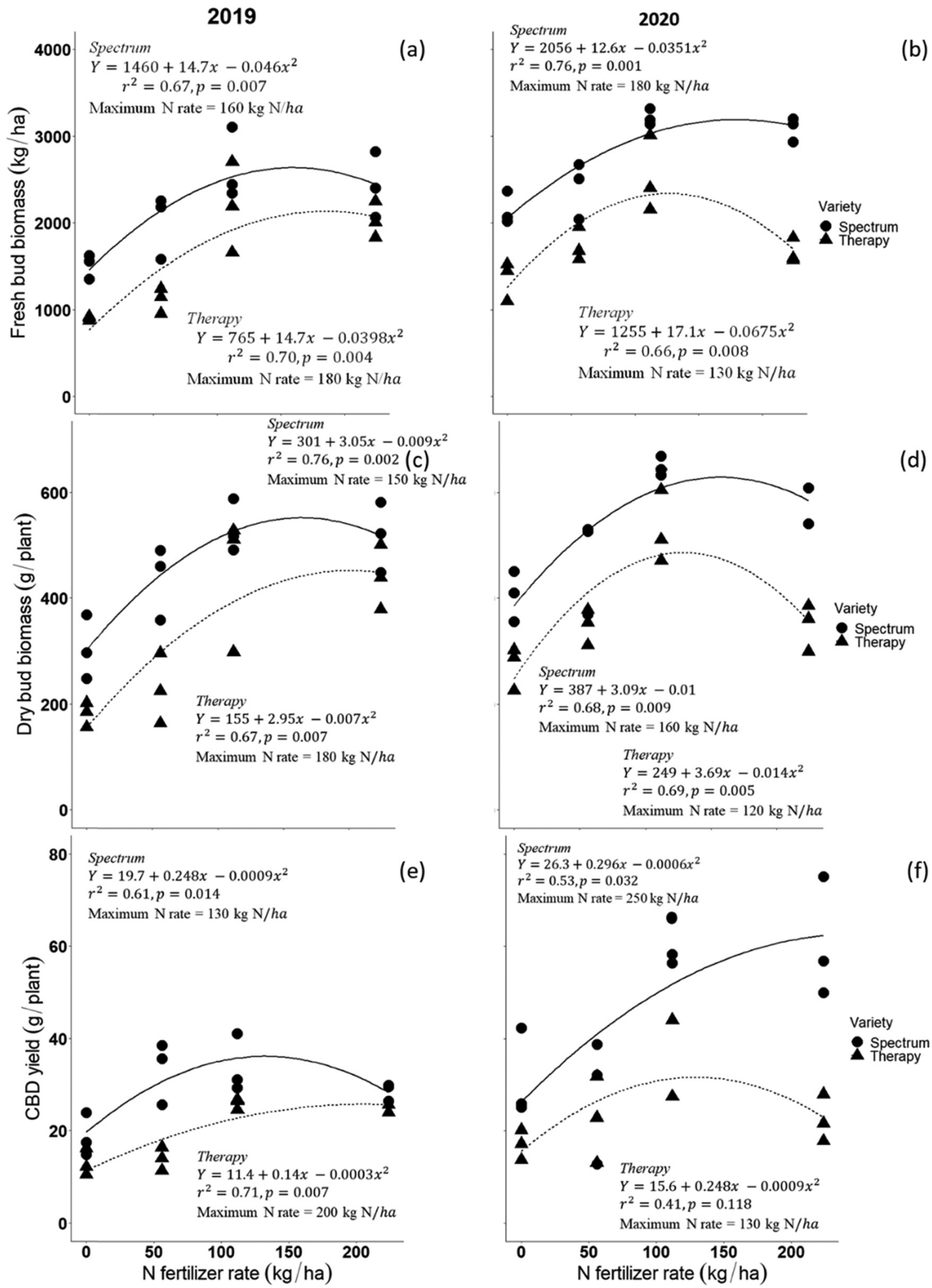

Figure 2: Relationship between fresh and dry bud biomass, and cannabidiol (CBD) yield of two CBD hemp varieties and nitrogen fertilization: (a) Fresh bud biomass in the year 2019; (b) fresh bud biomass in the year 2020; (c) dry bud biomass in the year 2019; (d) dry bud biomass in the year 2020; (e) CBD yield in the year 2019; and (f) CBD yield in the year 2020. The symbols show the data distribution, and the curves are the best-fit regression relationship with $p < 0.05$.

Table 2: Leaf tissue concentration of macronutrients and micronutrients in the two varieties

Variety	N	P	K (%)	Ca	Mg	Fe	Zn (ppm)	Mn
Spectrum	4.38[a]	0.29[a]	1.73	4.30	0.30	135	27.7[b]	61.4
Therapy	3.86[b]	0.27[b]	1.63	4.23	0.30	133	29.2[a]	60.2

Mean values with the same lower-case letter within the same column are not significantly different at the 0.05 probability level.

Table 3: Correlation between the leaf tissue macronutrient and micronutrient concentrations and fresh bud biomass (kg ha^{-1}), dry bud biomass (g $plant^{-1}$), and CBD yield (g $plant^{-1}$) at the mid-vegetative (T1) and late-vegetative (T2) stages

	T1			T2		
	Fresh bud biomass	Dry bud biomass	CBD	Fresh bud biomass	Dry bud biomass	CBD
N	0.508*	0.520*	0.466*	0.402	0.343	0.477*
P	0.050	0.022	−0.061	−0.141	−0.227	0.067
K	−0.139	0.000	0.138	0.121	0.153	0.052
Ca	0.158	0.161	0.290	−0.058	−0.118	0.067
Mg	0.165	0.142	0.237	−0.165	−0.232	−0.010
Fe	−0.186	−0.188	−0.112	−0.184	−0.202	0.024
Zn	0.133	0.138	−0.001	−0.258	−0.211	−0.163
Mn	0.038	0.100	0.022	0.212	0.158	0.324

*Significant at $p < 0.05$.

that the N uptake and use efficiency of the two varieties are different [20]. Also, the variance in leaf tissue N suggests different photosynthetic rates and physiological mechanisms between the two varieties [7]. Other studies have found positive correlation between the leaf chlorophyll content and tissue N concentration of hemp grown for seeds [21]. In addition, N is usually remobilized from lower leaves to upper leaves and floral parts as plants age and the amount of leaf N can affect bud production [21].

Differences in the dry bud biomass may have also been accounted for by the variation in plant growth. For example, higher bud biomass was measured for Spectrum variety that grew more vigorously than Therapy variety. Dry bud weight (g $plant^{-1}$) of CBD hemp has been shown to increase quadratically with N fertilization [22].

Our results indicate that while CBD% w/w was not different for the different N fertilizer rates, CBD yield was different. Caplan et al. [22] also demonstrated that while the CBD% w/w did not respond to varying N fertilizer rates, the CBD yield (g $plant^{-1}$) increased linearly with increasing N fertilization. The CBD yield was different for both the varieties; Spectrum with higher dry matter biomass had higher CBD yield than Therapy variety. Elsewhere, increasing N fertilization (above 150 ppm N) has been shown to decrease cannabinoid concentrations of hemp grown for CBD [23]. The CBD hemp varieties, Cherry Blossom, Cherry Blossom Tuan, Berry Blossom, Cherry Wine, and Cherry Blossom × Trump were different from the varieties, Spectrum and Therapy, we grew in our study. Crop varieties are known to vary in their physiology and morphology and as such respond differently to fertilizer applications [24]. Furthermore, the amino acid profiles of the two CBD hemp varieties are likely to be different given that the N concentration in the leaves of Spectrum variety is approximately 1.2-fold greater than that in the Therapy variety.

Currently, there is little research on leaf tissue nutrient concentrations specific for the field production of CBD hemp. The leaf macronutrient concentrations were within the range reported by Landis et al. [8] but lower than the concentrations reported in Kalinowski et al. [10] for CBD hemp produced under greenhouse conditions. On the other hand, the leaf tissue micronutrient concentrations were within the range reported by Kalinowski et al. [10] except for Mn. The differences in the leaf tissue concentration between the varieties suggest that nutrient uptake is different between the two CBD hemp varieties used in this study. Although N fertilization did not impact the foliar nutrient concentration, other studies have found that magnesium and phosphorus fertilizers increased their foliar concentrations [9,12]. The significant positive relationship between the leaf N concentration and fresh bud biomass, dry bud biomass, and CBD yield can be important for understanding the effect of management decisions on hemp grown for CBD. Our findings suggest that leaf samples collected at the mid-vegetative stage could help determine the critical nutrient concentration required for optimal CBD hemp production.

5 Conclusion

To our knowledge, this is one of the first studies to report the effects of N fertilizer application rates on CBD hemp varieties grown under plastic mulch in field conditions. Our results indicated that N fertilization increased CBD hemp fresh and dry bud biomass, and CBD yield. The leaf tissue N concentration at the mid-vegetative stage could be used in determining N fertilization requirements for field cultivation of CBD hemp. Our results suggest that Spectrum and Therapy CBD hemp varieties require different N fertilization rates for attaining maximum yield. From the fitted equations, the optimal N application rate required for maximum CBD yield ranged between 130 and 250 kg N ha^{-1} for Spectrum variety and 200 kg N ha^{-1} for Therapy. These findings could be applied for field cultivation of CBD hemp for similar fertilizer and varieties, although results may vary with location.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix

Table 1: Chemical composition of commercial NSF and HAG fertilizers used

Property	Nature safe	Harmony
Ammoniacal N	0.19	–[†]
Water soluble N (%)	0.77	1
Water insoluble N (%)	12.0	4
P_2O_5 (%)	—	4
K_2O (%)	—	3
Ca (%)	—	9
Mg (%)	—	0.08
S (%)	—	0.65
Fe (%)	—	0.2
Zn (ppm)	—	370
Cu (ppm)	—	40
B (ppm)	—	35

–[†] Data not available.

Table 2: The *F*-values, coefficients of determination (R^2), and BIC for models describing relationships between N fertilizer rate and fresh bud yield, dry bud yield, and CBD yield using centered independent variables

Variable	Year	Variety	Linear			Quadratic			Cubic			Square root		
			F-value	R^2	BIC	*F*-value	R^2	BIC	*F*-value	R^2	BIC	*F*-value	R^2	BIC
Fresh bud biomass	2019	Spectrum	7.35*	0.42	149	9.24*	0.67	146	6.27*	0.7	149	6.7*	0.6	149
		Therapy	13.1*	0.46	151	10.5*	0.7	150	14.7*	0.84	149	7.25*	0.61	152
	2020	Spectrum	14.8*	0.59	143	14.5*	0.76	141	18.7*	0.87	137	9.34*	0.68	144
		Therapy	0.63	0.06	151	8.66*	0.66	143	9.7*	0.78	139	3.58*	0.44	148
Dry bud biomass	2019	Spectrum	7.35*	0.51	110	14.3*	0.76	104	8.56*	0.76	107	11.7*	0.72	105
		Therapy	13.1*	0.58	115	8.95*	0.67	113	9.23*	0.78	114	6.96*	0.61	114
	2020	Spectrum	11.1*	0.53	112	9.8*	0.69	110	8.76*	0.76	109	6.98*	0.61	112
		Therapy	0.96	0.08	114	9.99*	0.69	107	15.0*	0.85	106	3.78	0.46	112
CBD yield	2019	Spectrum	1.36*	0.11	53	7.11*	0.61	46	5.27*	0.66	48	8.98*	0.66	45
		Therapy	14.5*	0.62	37	9.77*	0.7	37	27.1*	0.92	14	6.97*	0.63	38
	2020	Spectrum	9.98*	0.5	68	5.14*	0.53	70	8.04*	0.75	66	4.5*	0.5	71
		Therapy	0.55	0.06	51	2.83	0.41	49	2.6	0.53	46	1.51	0.27	51

*$p < 0.05$; BIC, Bayesian information criterion.

Table 3: Variance inflation factor (VIF) of the *X*, *X*2, *X*3, and $\sqrt{X}$ in the quadratic, cubic, and square models centered independent variables. *X* = N fertilizer rate

Variable	Quadratic	Cubic	Square root
X	1.29	24.5	10.4
*X*2	1.29	1.99	—
*X*3	—	28.8	—
$\sqrt{X}$	—	—	10.4

Table 4: Mean concentrations of tetrahydrocannabinol (THC) and cannabidiol (CBD) following different rates of nitrogen (N) fertilization for two CBD hemp varieties

N fertilizer rate (kg ha^{-1})	Variety	THC (%)		CBD (%)	
		2019	2020	2019	2020
0	Spectrum	0.14	0.19	6.12	7.60
50		0.29	0.22	7.58	6.33
100		0.26	0.25	6.32	9.51
200		0.26	0.24	5.57	9.70
0	Therapy	0.28	0.13	7.18	6.26
50		0.28	0.15	6.33	6.48
100		0.26	0.30	6.28	6.55
200		0.23	0.19	6.09	6.40