Supplemental Information

Abbreviations

A-C_i, response of net photosynthesis to leaf intercellular CO_2 concentration
AGB, aboveground biomass
A_{max}, maximum leaf net photosynthesis
A_n, leaf net photosynthesis
BX, “Boax” cultivar
CBD; CBDA, cannabidiol, cannabidiolic acid
CCI, chlorophyll concentration index
C_i, leaf intercellular CO_2 concentration
CO_2, carbon dioxide
CW, “Cherry-Wine” cultivar
EC_b; EC_e; EC_w, bulk soil electrical conductivity, saturated extract EC, and pore H_2O EC
E_L, leaf transpiration
g_s, leaf stomatal conductance
H_2O, water
J_{max}, maximum electron transport rate for the regeneration of RuBP
LRC, light response curve
LSP, light saturation point
N, nitrogen
NPK, Nitrogen-Phosphorus-Potassium ratio
P, phosphorus
PAR; PPFD, photosynthetically active radiation (400-700nm)
P_{atm}, atmospheric pressure
RACiR, rapid A/C_i response method
R_d, dark respiration
RH, relative humidity
SLN, specific leaf nitrogen
SLW, specific leaf weight
T_a; T_{soil}; T_L, air, soil, and leaf temperature
THC; THCA, Δ^9 – tetrahydrocannabinol, tetrahydrocannabinolic acid
TPU, triose phosphate utilization rate
V_{cmax}, maximum carboxylation capacity of the enzyme Rubisco
VPD, vapor pressure deficit
VWC, soil volumetric water content
WUE, water use efficiency
Ψ; $Ψ_{pd}$; $Ψ_{md}$, psi (water potential), predawn foliar Ψ, and midday foliar Ψ

Supplemental: Study Site and Experimental Design

We conducted our research on an upland site with soil types representative of many locations within the Slate Belt geologic region of the North Carolina Piedmont province (Stromquist and Sundelius 1969). Our experimental site (0.16 ha [0.4 acre]) was established on an uncultivated sub-parcel situated within a larger hemp production farm (6.4 ha [15.8 acre]) on well-drained (slope 0–4%) silt loam soils of the Goldston and Nanford NRCS soil series, with a taxonomic classification of Typic Dystrudepts and Typic Kanhapludults respectively (https://websoilsurvey.nrcs.usda.gov/app/). The Köppen climate classification for the site is Cfa - humid subtropical (Beck et al. 2018), with average annual rainfall of 102–114 cm (40–45 in) occurring locally, which is typically well distributed during the growing season, with long-term (1991–2020) rainfall averaging. 76–127+ mm monthly (3 to 5+ in) across the months of April through October (Carthage NC, https://www.ncei.noaa.gov/cdo-web/search). Mean monthly high and low temperatures (Apr–October) for the area averages 21–32 °C (70–89 °F) and 8–20 °C (46–68 °F), respectively.

We established replicated experimental study plots in July 2019, with the study site receiving pre-planting cultivation treatments including plowing, disc harrowing, raising of planting beds, and installation of buried irrigation drip tape. Plants were sown into six different replicate blocks spaced across the 0.16 ha experimental study, with each block containing 4 to 5 planting rows that averaged up to 45.7 m (150 ft) in length, resulting in a total of 28 rows across the 0.16-ha study site (see Table S1 below). Within each row, we planted the plants at either a 1.2 or 1.5 m (4 or 5 ft) spacing between plants, with plant spacing varying by block (n = 3 blocks at 1.2 m spacing, n = 3 blocks at 1.5 m). Individual rows within each block were also laid out at a 1.2–1.5 m spacing between row centers, and this spacing matched the plant spacing intervals by block. We separated each of the six individual replicate blocks by an open 1.5–m spacing gap where nothing was planted. Raised planting beds in each row had dimensions of 15 cm (6 in) height and 61 cm (24 in) width.

We hand-planted 924 hemp clones (*Cannabis sativa* L.) into raised beds on 24 July 2019, using two high CBD yielding (low Δ^9-THC) cannabis cultivars “Boax” and “Cherry-Wine”, hereafter referred to as BX and CW, respectively. The site received 69.9 mm (2.75 in) of rain on 23 July, and subsequently soil moisture was at or near field capacity at time of planting. We tagged all plants with an identification number for tracking purposes at time of planting. During the early vegetative phase of plant development, we hand-watered plants on the 3, 10, and 17 August at a rate of two liters/plant to mitigate water stress and promote root development and initial establishment. We employed mechanical weed control to remove all herbaceous and grass competition within each raised row bed throughout the growing season.

The experimental design used four differing factors, including two levels related to plant variety (i.e., BX vs. CW), two levels of plant spacing comparison (i.e., 1.2 vs 1.5 m.), two levels of row bed mulching cover (i.e., plastic mulch cover versus no plastic), and two levels of nutrient fertilizer amendment (Suppl. Table S1). Cultivars were planted into specific rows within each block as follows; Row-1 (BX), Row-2 (CW), Row-3 (BX), Row-4 (CW), Row-5 (BX & CW). This pattern was repeated across all (n = 6) replicate blocks, resulting in an average of 154 (range 120–180) plants being planted per block depending on spacing and number of rows, split evenly

between the two cultivars. We tested the effects of plastic mulch cover on plant growth and physiological response by assigning a plastic mulch cover treatment to two out of the six blocks, with two other replicate blocks serving as controls which received no plastic mulch cover treatments. Raised planting row beds in blocks assigned a plastic cover treatment were covered with 1.0 mm black plastic ground cover on 10 August and 12 August 2019. We installed the plastic ground cover by hand over raised planting beds approximately 17 to 19 days after planting and achievement of initial establishment. To facilitate installation of the plastic ground cover over plants, an 20x20 cm (8x8 in) opening was cut at the location of each hemp plant.

We measured environmental conditions during the duration of our study (Figs. 1, 2) using a meteorological station installed within our study site. We measured air temperature (T_a), relative humidity (RH), vapor pressure deficit (VPD), barometric pressure (P_{atm}), photosynthetically active radiation (PAR, also referred to as photosynthetic photon flux density (PPFD), 400–700 nm), wind speed and direction, and precipitation utilizing a 2.5m tall station comprising an ATMOS-14 sensor, PAR quantum sensor, Davis cup anemometer, and ECRN-50 rain gauge (METER Group, Inc. USA). Additionally, we measured soil volumetric water content (VWC) ($m^3\ m^{-3}$), soil temperature (T_{soil}), and bulk soil electrical conductivity (EC_b) at two locations and two depths (15 and 30 cm) using four Teros-12 soil sensors (METER Group, Inc. USA). Bulk soil EC_b was converted to saturated extract EC_e and pore H_2O EC_w following methods outlined by METER Group, Inc. USA, #18190-02 (2018). We recorded data at 15-minute intervals using ZL-6 cellular capable dataloggers (METER Group, Inc., USA).

Natural precipitation was supplemented with periodic irrigation via 1.6 cm (0.62 in) diameter drip tape (emitters on 45.7 cm [18 in] spacing) that was installed in each row bed at a depth of 15.2 cm (6 in). We installed drip tape mechanically at the time of bed formation. Irrigation water was sourced from a nearby pond (when available) and supplied to the experimental study field via a mobile 120 GPM pump and filter trailer skid starting in mid to late August as hand watering of plants ceased. Each supplemental irrigation event supplied a depth equivalent of 29.2 mm (1.15 in) of water into planting beds across all six blocks. We applied supplemental irrigation via drip tape to the experimental study plot on the following dates in 2019: 13 August (testing of system), 28 Aug, 4 Sept, 11 Sept, 3 Oct, 10 Oct, 16 Oct, and 22 October (Fig. 2a).

Finally, in four out of six blocks (which matched the n = 4 “plastic versus no plastic cover" replicate blocks), we injected supplemental irrigation with liquid nutrient fertilizers, whereas the two remaining blocks received no nutrient additions (Table S1). Concentrated liquid fertilizers were injected into the irrigation stream at the mobile pump and filter trailer skid at a delivery rate of two gallons per acre (per irrigation event). Nutrient additions (along with a liquid micronutrient amendment) were applied at a cumulative rate of approximately 28 lbs/acre nitrogen, 44.8 lbs/acre phosphorus, and 70 lbs/acre potassium over the course of the growing season, at an NPK ratio of (1):(1.6):(2.5). Details related to nutrient amendments are covered in a separate analysis.

Supplemental: Physiological Measurements

We selected a subset of plants from each replicate block to assess the physiological response of hemp plants to treatment factors and to seasonal change in environmental conditions and plant development over the course of the study (i.e., vegetative phase to early flowering to late flowering and maturation). We randomly selected a total of ten plants from each block for repeated physiological measurements over the course of the study, with two plants being selected from each row, which provided a sample size of n = 5 plants for each cultivar (n = 2) within a specific block. In total, this provided n = 60 plants for a repeated time-series of physiological measurements across the study, with treatment factors having the following number of plants in each treatment factor: a) cultivar (n = 60 total, n = 30 "BX" versus n = 30 "CW"), b) plastic mulching cover (n =4 0 total, n = 20 "plastic cover" versus n = 20 "no plastic"), split evenly between cultivars, c) row spacing (n = 60 total, n = 30 "4 ft" [1.2 m] versus n = 30 "5 ft" [1.5 m]), and nutrient amendment (n = 40 total, n =2 0 "nutrient amendment" versus n = 20 "no amendment"). Hereafter, plants in "plastic" and "no-plastic" treatments will be referenced as "P" and "NP" respectively (i.e., BX-P, BX-NP, CW-P, and CW-NP). For each selected plant, we designated an adjacent plant (of the same cultivar and treatment factor) as a back-up to serve as a replacement in case of mortality, disease, or herbivore damage during the course of the study.

Post-hoc analysis revealed that variation in plant spacing distances at 1.2 to 1.5 m had no detectable effect on plant growth or physiological performance for either cultivar due to a lack of any measurable inter-plant competition for growing space, etc.; therefore, this specific factor was dropped from the analysis in this paper. In this paper, the two experimental factors explicitly examined in our analysis and results are those related to cultivar and the effect of plastic mulching treatment. As stated previously, details related to nutrient amendments are covered in a separate analysis.

To assess changes in leaf level physiology and physiological responses over the course of our study, we made repeated measures of leaf net photosynthesis (A_n), leaf stomatal conductance (g_S), leaf transpiration (E_L), foliar chlorophyll content (CCI), foliar nutrient content (%), leaf temperature (T_L), and plant foliar water potential (Ψ) across a ten week period from 14 August to 17 October 2019 that spanned from the pre-flowering vegetative phase of plant development through flowering to maturation (see Figs. 3, 4). In regard to plant development, flowering initiation was first observed in both cultivars on 24 August (BX) and 25 August (CW), and we harvested mature plants on 29 October 2019.

We performed weekly assessments (n = 8, Fig. 3) of leaf level physiological response across all n = 60 plants via measurements of leaf-g_S (utilizing an SC-1 porometer, METER Group, Inc. USA), foliar CCI (MC-100, Apogee Instruments), and leaf temperature (Fluke, Model 561, infrared thermometer) on fully illuminated leaves in the upper canopy. The SC-1 porometer was calibrated daily before each use, and weekly measurements were made midday between 1100 to 1530+ hrs on dry plant canopies under sunny to mostly sunny conditions. For balanced sampling across the experimental design, we measured two plants from each replicate block (one per cultivar) before proceeding to the next block. This pattern was followed across all n = 6 blocks for an initial total of n = 12 measurements across all blocks, then the pattern was repeated until all n =6 0 plants were eventually measured. On a subset of weekly measurement days, we

assessed plant water status and water stress on n = 16 plants (Fig. 3d) via measurements of predawn water potential (Ψ_{pd}, n = 6 dates) and midday water potential (Ψ_{md}, n = 3 dates) using a Scholander type pressure chamber (PMS Instruments). We collected foliar tissue samples (upper canopy) for nutrient analysis (≈ 2-week intervals) from late vegetative (15 August) through late flower (24 October) growth phases. Samples were dried and shipped to the North Carolina NCDA&CS Agronomy Services Division for laboratory analysis.

In addition to weekly assessments of hemp leaf physiological responses, we performed comprehensive measurements (Fig. 4) of leaf photosynthesis (A_n), stomatal conductance (g_s), and transpiration (leaf-E_L) on fully illuminated leaves in the upper canopy using an LI-6800 portable photosynthesis system equipped with a 3x3 cm clear top leaf chamber (LI-COR Biosciences, Inc.). From the resulting gas exchange data, we also calculated estimates of both instantaneous and intrinsic water use efficiency (WUE) as follows: a) instantaneous WUE = leaf A_n/E_L, and b) intrinsic WUE_i = leaf A_n/g_s. Measurements were performed on n = 4 dates (22 August through 15 October 2019, both pre and post flower initiation) from late morning through afternoon (1100 to 1700 hrs) on days with sunny to mostly sunny conditions. For each sampling effort utilizing the LI-6800, we measured 24 plants (total of n = 96 measurements across the season), with an equal number of plants measured from each cultivar and plastic mulching treatment factor across blocks. Measurements were taken at ambient T_a (ranging from 24 to 36 °C depending on date and time of day), ambient RH and VPD (ranging from ~50 to 78%, and ~1.0 to 2.2 kPa respectively), and CO_2 concentrations (~400 ppm). Flow rate for the system gas analyzers was set at 500 µmol s^{-1}, and the 3x3 cm clear top leaf chamber light source was set at a saturating PPFD of 1500 to 2000 µmol m^{-2} s^{-1}, depending on the date when measurements were performed. Prior to taking measurements each day, we filled all H_2O scrub, humidifier, and CO_2 scrub columns with fresh Drierite, H_2O (if needed), and soda-lime respectively, and all system warm-up tests were successfully performed/passed. Reference and sample IRGA's H_2O and CO_2 readings were matched prior to measurements each day, and matching of IRGAs was performed every 30 minutes thereafter throughout each measurement session. Post measurement, all LI-6800 leaf gas exchange rates were normalized for chamber leaf area using photographs of foliage inside the chamber, with subsequent analysis and quantification of projected leaf area using Image-J analysis software (U. S. National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/).

Supplemental: Light and CO_2 Response Curves

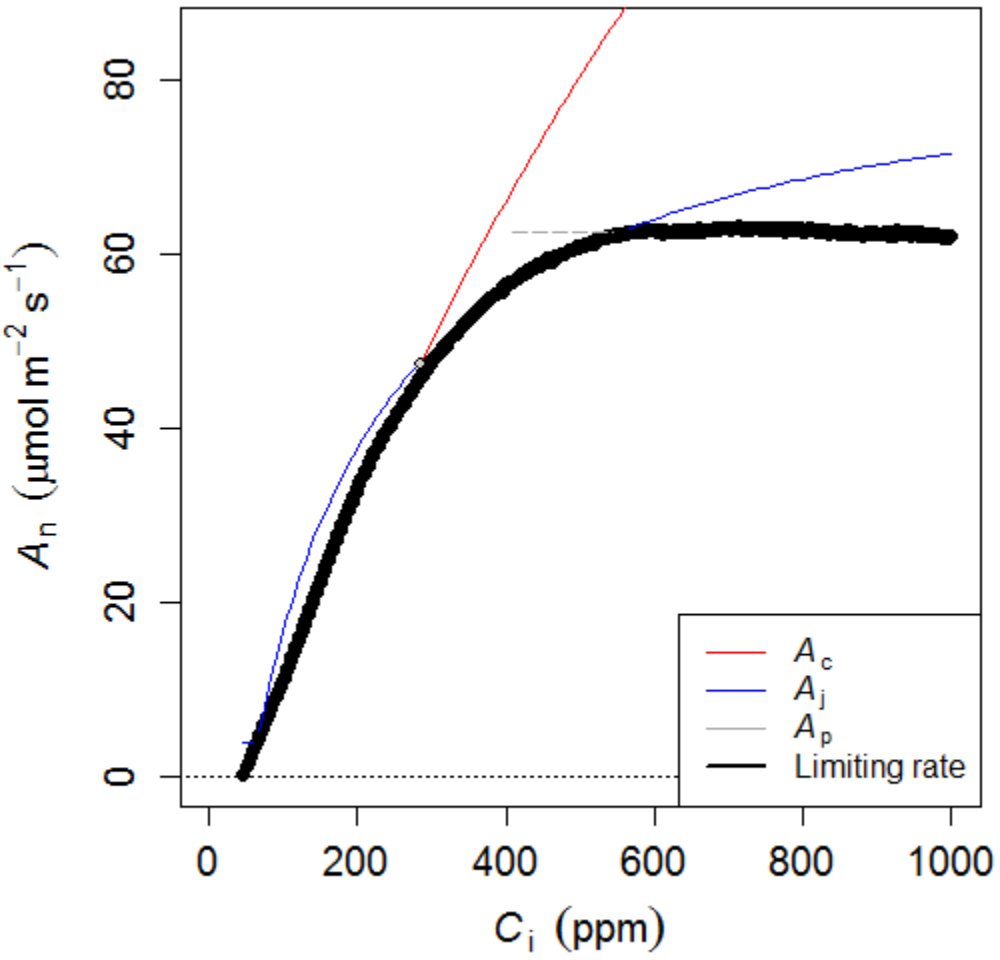
We measure light response curves (LRC) during late vegetative (19 and 20 August) and mid-late flower (9 October) growth periods. Light response curves were measured using the Light Response program (with default settings) provided within the Auto Programs function of the LI-6800. Environmental settings used for the late vegetative period measurements were as follows: PAR range 0 to 2000 µmol m^{-2} s^{-1}, T_L = 33°C, RH= 75% (VPD = 1.26 kPa), and CO_2 sample = 400ppm. Mid-late flower LI-6800 settings were as follows: PAR range 0 to 2000 µmol m^{-2} s^{-1}, T_L = 25°C, VPD = 1.5 kPa, and CO_2 sample = 400 ppm. Sample size was n = 12 plants for each measurement period, which included plants of both cultivars and plastic mulching treatments.

Carbon dioxide (CO_2) response curves were measured using the *rapid A-Ci response* (RACiR) procedure and the AutoLog program function of the LI-6800 (as outlined in Stinziano et al. 2017). We measured response curves (RACiR) midday over the course of three seasonal campaigns including: late vegetative (21–23 August), early flower (10–12 September), and late flower (10 and 15 October). For each measurement campaign, we measured n = 24 plants using the RACiR procedure, including plants from each cultivar and plastic mulching treatment (for a total of n = 72 plants measured over the study duration). We used RACiR CO_2 response curves to measure the response of leaf-A_n to increasing leaf internal CO_2 (C_i) concentrations as the LI-6800 reference CO_2 concentration was linearly ramped from 10 to 1500 ppm over a 15-minute period (at a target CO_2 increase of 100 ppm min^{-1}). Due to non-steady state measurement conditions, RACiR measurements require corrections related to offsets and lags between the two infrared gas analyzers in the LI6800 due to constantly changing CO_2 levels in the chamber and system. Data correction was first performed by measuring an empty chamber ramp (i.e., no foliage in chamber) over the same time period and settings used to measure an actual leaf. Measuring the empty chamber serves to capture and quantify the influence of system offsets/lags, etc. on the apparent value of measured leaf A_n. Measured leaf-A_n data (for a foliar sample) was then corrected as follows: $A_{leaf} = A_{meas} - A_{empty}$, with the corrected A_{leaf} data subsequently used to update and recalculate C_i values in the LI6800 output (again, see Stinziano et al. 2017 for details and theory related to this correction procedure). The correction equation to predict A_{empty} for each empty chamber ramp measurement was calculated by fitting a polynomial equation to the relationship between apparent A_n (y-axis) and reference CO_2 (x-axis) for the empty chamber output data, then using that regression to predict A_{empty} across the range of the reference CO_2 ramp values. We performed multiple empty ramp measurements each day RACiR curves were measured, and RACiR data was filtered at the beginning and end of each empty ramp curve as necessary to remove data points when CO_2 reference values were less predictable or out of range (see Stinziano et la. 2017). System and environmental settings (LI-6800) used for the late vegetative and early flower RACiR measurements were as follows: Flow = 500 μmol s^{-1}, P_{atm} = 100 kPa, PAR = 2000 μmol m^{-2} s^{-1}, T_L = 33°C, VPD = 1.5 kPa, and CO_2 reference (prior to ramp initiation) = 400 ppm. Mid-late flower RACiR settings were the same with the exception of PAR (set to saturating light of 1500 μmol m^{-2} s^{-1}) and T_L (set to 25°C).

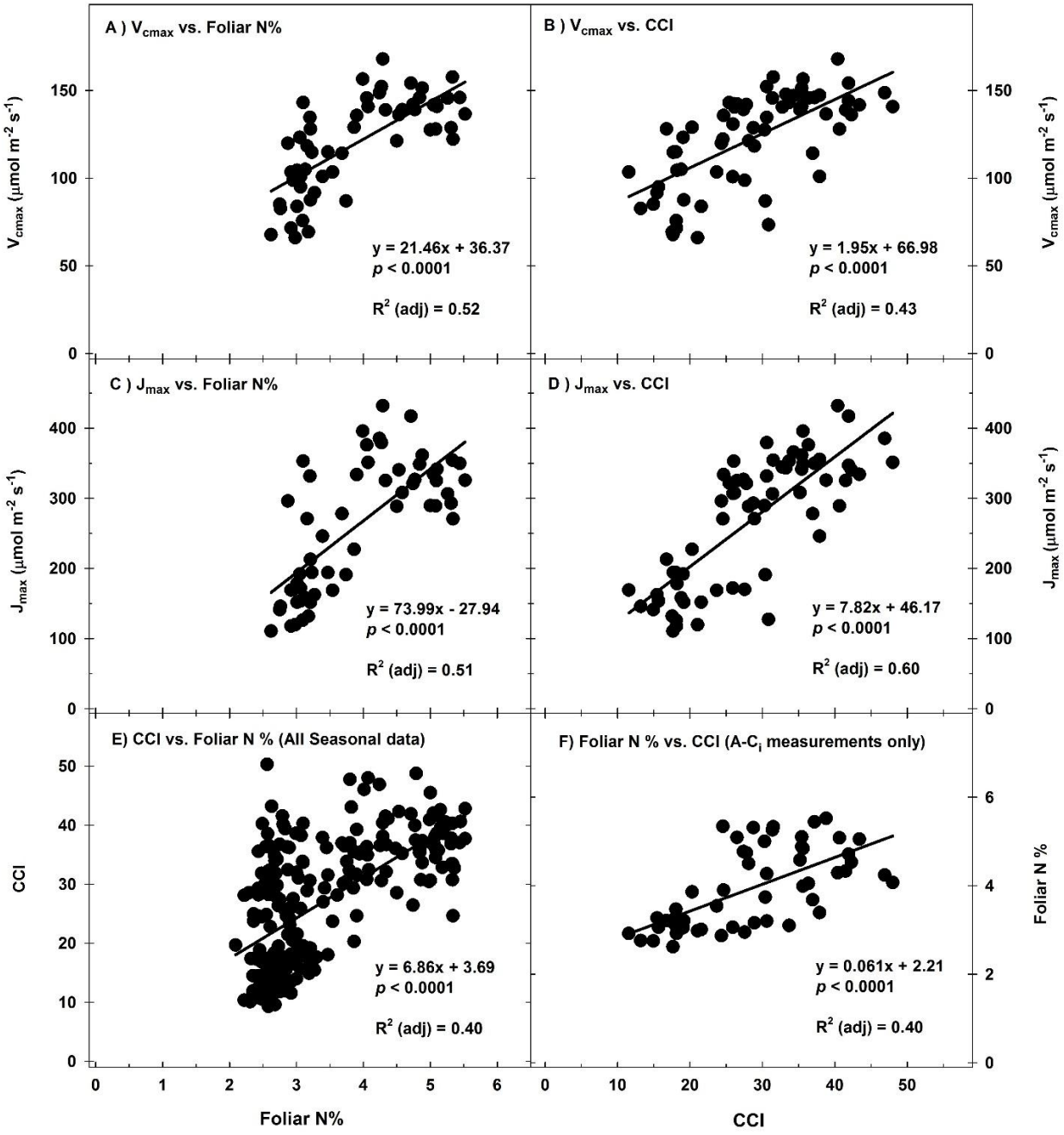
Once processed, we used corrected RACiR data (A_n versus C_i) to obtain V_{cmax} and J_{max} estimates based on the FvCB model (Farquhar et al. 1980) using R open-source software (R version 3.5.3) and the "plantecophys" R package (Ver. 1.4.4, Duursma et al. 2015). The "fitaci" function was used for curve fitting, along with leaf A_n, T_L, PPFD, and C_i (<1000 ppm) as input variables. All parameter outputs were corrected to 25°C during the fitting procedure, and the fit method was set to "bilinear". All fit parameter values used in the fitting procedure, including a temperature adjusted Michaelis-Menten coefficient for the Farquhar model (Km) and *photorespiratory CO_2 compensation point (T*)* are reported in Table 1. Due to the saturation of photosynthesis at high C_i (Long and Bernacchi 2003), the "fit TPU" option in the "fitaci" function was utilized (see Fig. S1 for an example of an A-C_i curve with TPU fitted). Triose phosphate utilization (TPU) was estimated for all A-C_i datasets, and utilizing the TPU fitting option improved overall curve fits at high C_i ($P < 0.0001$ and $R^2 > 0.94$, all fits) and provided for more accurate parameter estimates for J_{max}. See Gregory et al. (2021) for a discussion of potential underestimation of J_{max} when TPU is not accounted for under situations of photosynthesis saturation at high C_i. Finally, no

estimates for dark respiration (R_d) are reported here, as the RACiR method can produce poor estimates for R_d (Taylor and Long 2019), with Saathoff and Welles (2021) documenting the occurrence of unreliable and negative R_d estimates resulting from A-C_i curve fitting using the RACiR method.

Supplemental Figures



Supplemental Figure S1. Representative example of hemp A-C_i curves measured during the late vegetative growth phase at the Robbins hemp field study during 2019 using the RACiR technique. Note A_c, A_j, and A_p limitations, especially A_p (TPU) limitations at high C_i. Fit statistics = $R^2 > 0.99$, $P < 0.0001$.



Supplemental Figure S2. Relationship between a) V_{cmax} and foliar N%, b) V_{cmax} and CCI, c) J_{max} and foliar N%, and d) J_{max} and CCI for all late vegetative, early flower, and late flower A-C_i measurement campaigns at the Robbins hemp study in 2019. Seasonal relationship between CCI and foliar N% for all (n = 6) tissue sampling periods across the study (panel e), and CCI versus N% relationship (panel f) for measurements and leaf samples measured during A-Ci campaigns.

Supplemental Table

Supplemental Table S1. Experimental design for 0.16 ha hemp experimental study at Robbins, NC. Cultivars were planted at 1.2 or 1.5 m spacing between plants and row centers (by block). Raised planting beds had dimensions of 15 cm height and 60 cm width. The experimental design utilized four factors, including: n=2 levels of cultivar (Boax, i.e., BX vs. Cherry-Wine, i.e., CW), n=2 levels of plant spacing (i.e., 1.2 vs. 1.5 m), n = 2 levels of bed mulching cover (i.e., plastic mulch vs. no plastic), and n = 2 levels of nutrient fertilizer amendment. Cultivars were planted into specific rows ***within each block*** as follows: Row-1 (BX), Row-2 (CW), Row-3 (BX), Row-4 (CW), Row-5 (BX & CW, when applicable). Individual plants selected for physiological measurements were repeatedly measured throughout the duration of the experiment (repeated measures on the same subject).

Block #	Rows/ block	Plant & Row spacing (m)	Row Length (m)	# Plants/ Row	Total # plants per block (by variety)	# Cultivars selected for repeated physiological measurements/block	Plastic mulch	Fertigation
1	5	1.2	44	36	180 (BX=90, CW=90)	BX=5, CW=5	NO	NO
2	5	1.5	46	30	150 (BX=75, CW=75)	BX=5, CW=5	YES	YES
3	5	1.2	44	36	180 (BX=90, CW=90)	BX=5, CW=5	NO	YES
4	5	1.5	46	30	150 (BX=75, CW=75)	BX=5, CW=5	YES	YES
5	4	1.2	44	36	144 (BX=72, CW= 72)	BX=5, CW=5	NO	YES
6	4	1.5	46	30	120 (BX=60, CW=60)	BX=5, CW=5	NO	NO