Impact of Soil Quality on Cannabinoid and Terpenoid Content of *Cannabis sativa* L.

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ABSTRACT

Cannabis sativa L., or 'hemp,' is an expanding commodity that relies heavily on its chemical constituents for its therapeutic effects and legal status. Growing conditions are critical for obtaining an anticipated chemical profile for any given hemp cultivar, a vital component of this process is soil composition. This study evaluates the effect that soil has on cannabinoid and terpenoid content by comparing the extracts of two hemp cultivars grown similarly in two fields differing in soil preparation including a cover crop no tillage field (CC) usage and a conventional tillage field (CF). Hemp inflorescence from two cultivars (Tangerine and CBG Stem Cell), were extracted using supercritical fluid extraction techniques, and extracts were analyzed by a Pennsylvania-approved medical marijuana laboratory. Significant differences in cannabinoid content were observed between field types and cultivars, notably cannabidiol (CBD) levels were1.5x higher in CF for Tangerine cultivars and 2x higher in CC fields for CBG Stem Cell, cannabidiolic acid (CBDA) levels were 6.3x higher in Tangerine CC extracts and 2.2x higher in CF extracts of CBG Stem Cell, cannabigerol (CBG) levels were 3.7x higher in CBG Stem Cell extracts from CC , and Δ^9-tetrahydrocannabinol (THC) were 6x higher for CF Tangerine Extracts. Differences in terpene composition were observed between the cultivars grown in the CF but not between those grown in CC. This is the first study to show differences in extract composition of outdoor cultivated hemp grown in different soil conditions.

INTRODUCTION

Cannabis sativa L., commonly referred to as 'hemp' or 'marijuana' depending upon the Δ^9-tetrahydrocannabinol (THC) content, has become a rising commodity in the United States with values of hemp (low-THC cannabis) production totaling $291 million in 2023 (States and Agriculture, 2024). Hemp is a versatile crop that has many uses depending on the plant part. Parts used include its fibers, leaves, inflorescence, and seeds which have

resulted in a variety of products such as industrial textiles, animal feeds, food supplements, therapeutic oils, tinctures, and topicals (Graybill et al., 2023). *Cannabis sativa* L. is a diecious plant meaning both female and male plants are produced (Small and Cronquist, 1976). Due to the greater abundance of cannabinoid producing trichomes on female flowers, female plants are preferentially grown for bioactive compounds, such as cannabidiol (CBD). CBD has demonstrated potential therapeutic properties including neuroprotective, antiepileptic, anxiolytic, analgesic, and anti-cancer properties (Peng et al., 2022). Aside from CBD, hemp inflorescence also contains a plethora of bioactive compounds including other phytocannabinoids and therapeutic aromatic compounds such as terpenoids (Radwan et al., 2021; Chacon et al., 2022).

Cannabinoids and terpenoids are both classified as plant secondary metabolites that play critical roles in plant defense, communication, and competition (Gülck and Møller, 2020; Ninkuu et al., 2021). When it comes to the biosynthesis of these compounds, studies have described shared precursors between cannabinoids and terpenoids along with evidence of genetic variation for specific enzyme synthases of individual cannabinoids and terpenoids (Flores-Sanchez and Verpoorte, 2008; Booth et al., 2020; Gülck and Møller, 2020; Tahir et al., 2021). Additionally, researchers have explored the effects of environmental factors such as temperature, light quantity and quality, photoperiod, and soil nutrients suggesting these factors may also play a role in the total yield of cannabinoids and terpenoids in hemp extracts (Dang et al., 2021; Saloner and Bernstein, 2021; Wei et al., 2021; De Prato et al., 2022; Park et al., 2022; Trancoso et al., 2022; Massuela et al., 2023). Although the role of soil nutrients in the synthesis of cannabinoids and terpenoids is unclear, changes in macronutrient levels have been observed to alter cannabinoid and terpene yield (Saloner and Bernstein, 2021; Massuela et al., 2023).

Outdoor cultivation has been shown to provide higher biomass at a lower cost when compared to indoor cultivation (Zheng et al., 2021). Agricultural practices can impact soil health and quality and various practices are employed to maintain soil health such as crop rotation, the incorporation of cover crops (CC), and fertilization (Tully and McAskill, 2020). All of these have been found to enhance several aspects of the soil's physical, biological, and chemical characteristics (Tully and McAskill, 2020). To date, there are no studies that have explored the effects of CC soil on cannabinoid and terpenoid content in comparison to conventionally-maintained tilled soil.

This study aims to assess the effects soil properties have on cannabinoid and terpenoid content in extracts from outdoor-grown hemp inflorescence. Soil health or soil quality are often used interchangeably; however, soil health focuses not just on the inorganic properties of soil but also on the biological properties of the soil and its abilities to promote life (plant, animal, fungal, etc.) (Tully and McAskill, 2020). Soil health can be measured in numerous ways including color, tilth, drainage, crop yield, composition, pH, and nutrient composition, and in fact measures of soil health can be highly controversial (Tully and McAskill, 2020). This study specifically looks at the tilth aspect of soil-health, comparing a CC field to conventional tillage field (CF). Two cultivars of hemp were grown identically in two neighboring fields, a conventional field with tilled soil and a no-till field that has had long-term application of CCs. It is hypothesized that CC application will increase soil health thus, providing elevated levels of these bioactive compounds in hemp extracts. The results of this study demonstrate that soil characteristics and genetics of hemp cultivars play a major role in specific cannabinoid and terpene yield, as well as total terpene content.

MATERIALS AND METHODS

Hemp growth and harvest

Hemp cultivars Tangerine and CBG Stem Cell (Atlas Seed; Sebastopol, CA, USA) were grown and harvested by Cedar Meadow Farms (Holtwood, PA, USA). Both cultivars used are high cannabigerol (CBG) cultivars. Clones of Tangerine (cultivar 1) (10-15 cm in height) were transplanted in June of 2022 and harvested in October of 2022. Clones of Tangerine and CBG Stem Cell (10-15 cm in height) were transplanted in June of 2023 and harvested in October 2023. All plants were planted with a plant-to-plant distance of 3 feet and row-to-row distance of 4.5 feet. Both fields received similar amounts of sunlight and rainfall and no

additional fertilizer or supplementation were applied to either field. Temperature, humidity, and air pressure, during the growth period were retrieved from nearby weather stations (data not shown). Hemp cultivars were grown under similar conditions in two fields that differed in soil preparation: one utilized cover crops (CC), and the other employed conventional tillage field (CF). In the CC field, hairy vetch, triticale, winter oats, and crimson clover were planted prior to transplanting the hemp cultivars. In contrast, no cover crops were used in CF, where the soil was tilled before transplanting.

A comprehensive assessment of soil health was conducted in March of 2021 by the Cornell Soil Health Laboratory (Cornell University; Ithica, NY, USA), at the start of the research project. The initial pilot harvest from 2021 was excluded due to unforeseen events that lead to one field being planted with seedings and the other with clones. This assessment provided information on physical, chemical, and biological soil characteristics with corresponding ratings from 0-100 based on measured values, where high scores indicate higher quality.

All plants were harvested identically, placed within bead trays, and then transferred to designated reefer units (13°C) based on cultivar to eliminate any cross-contamination after drying. A dehumidifier (set to high) was added to each unit where hemp inflorescence was dried over a period of seven days. The plant material was mixed daily to provide for even drying and on day 3 the temperature was increased to 27°C. Dried inflorescence (10-12% moisture) was then vacuum sealed and stored at room temperature with limited light exposure until extraction.

Extraction and Characterization of Hemp Inflorescence

The extraction of dried hemp inflorescence was carried out as previously described (Sepulveda et al., 2022b; Chacon et al., 2024). In short, 250-325g, was ground and extracted using an SFT-SP1100 extraction system manufactured by Supercritical Fluid Technologies, Inc. (Newark, DE, USA). Extractions were performed at 55°C for 35 min at 413 bars, the extract was then collected from the collection vessel. Winterization was performed by dissolving the extract in 90% ethanol (10% extract by weight) for 24-48 h at -20°C and then filtered before ethanol evaporation. The final extract was dissolved in fractionated coconut oil (Pure Body Naturals; West Chester, OH, USA) at 300 mg/ml and decarboxylated at 95°C for 1 h.

Analysis of Extract Composition

Analysis was conducted by Keystone State Testing Laboratories (Lower Paxton Township, PA, USA) as described previously (Raup-Konsavage et al., 2020). Briefly, extract was dissolved in acetonitrile for cannabinoid content and methanol for terpene analysis. Cannabinoid content was determined by reverse phase HPLC using a Restek (State College, PA, USA) Raptor AERC C-18 column (2.7 µm particle size, 150 mm, 4.6 mm ID, and matching guard length 5 mm), mobile phase A: 1% phosphoric acid in LCMS grade water, and mobile phase B: 1% phosphoric acid in LCMS grade acetonitrile (ThermoFisher, Waltham, MA, USA). Data acquisition and integration were achieved with LabSolutions (Ver 5.87 SPI, Shimadzu; Columbia, MD, USA). 11 cannabinoid reference standards (Shimadzu; Columbia, MD, USA) were calibrated with a 7-point curve from 0.5 µg/mL – 100 µg/mL. The detection of cannabis terpenes was carried out using a Shimadzu 8050 GCMS-MS (220-91239-22, Shimadzu; Columbia, MD, USA) and a collection of 42 terpene compounds as calibration standards (CAN-TERP-MIX1H and CAN-TERP-MIX2H,Spex; Metuchen, NJ, USA).

Statistical Analysis

Total cannabinoid content was calculated by the summation of measured cannabinoids and total terpene content was calculated in a similar manner. The total bioactive compounds is the sum of the total cannabinoid and terpene content measured. Total yield was calculated by dividing the post-extraction weight by the pre-extraction weight of the plant material. Total wax (the non-ethanol soluble portion of the extract) (%) was determined by the weight of the extract subtracted from the winterized extract weight. Welch's t-test, one-way ANOVA with Tukey's multiple comparison or two-way ANOVA statistical test with Sidak's multiple comparison test was employed to determine significant differences in compound concentrations using GraphPad Prism (GraphPad Software, Boston, MA, USA).

RESULTS

Soil Quality Assessment

The soil assessment provided measured values and quality ratings of each field for physical, biological, and chemical soil characteristics. The CC field consisted of 19% sand, 59% silt, and 20% clay and classified as a silt loam soil texture, while CF consisted of 22% sand, 46% silt, and 30% clay and was classified as clay loam (Fig.1A). For physical soil characteristics, each field had similar ratings of predicted available water capacity but differed in aggregate stability with CCs measuring 35.2% and CF at 5.5% (Fig. 1B). Biological characteristics displayed differences in organic matter (%) CCs contained 4.7% resulting in a rating of 97 of the Cornell Soil Health Laboratory (CSHL) and CF with 3.6 % a CSHL rating of 33 (Fig. 1C). CCs reported a high rating in predicted soil protein and soil respiration with a CSHL score of 85 and 84, respectively. CF soil a rating of 59 for predicted soil protein and a CSHL score of 51 for soil respiration. Ratings for active carbon differed between fields as CCs displayed a score of 98 and CF with a score of 35. For chemical characteristics, soil pH for CF was reported at 6.9 while the CCs field displayed a pH of 7.1. Elevated levels of extractable phosphorous were detected in CF with 47.8 ppm in comparison to the CCs field at 19.9 ppm. The amount of extractable potassium also differed between fields with CCs containing 437 ppm and CF with 216.2 ppm. Concentrations of minor elements such as iron, manganese, and zinc were similar across both fields. However, both fields had identical scores for all indicators of chemical characteristic (Fig. 1D).

Extraction Yield

The two hemp cultivars, Tangerine (cultivar 1) and CBG Stem Cell (cultivar 2) had similar extraction yields (of the original biomass), with approximately 6% yield in from CC and 5% from CF (Fig. 2A). The yield from each cultivar was similar with cultivar 1 yielding 6.3% from CC and 5.3% from CF, and cultivar 2 yielding 6.9% from CC and 5.5% from CF (Fig. 2B). The total wax (ethanol insoluble portion of the extract) was not different between growing conditions 43.1% (CC) and 43.7% (CF) (Fig. 3A). Extracts from either cultivar did not differ significantly in wax content based upon growing condition, cultivar 1 43.3% from CC and 39.4% from CF and cultivar 2 44.4% in CC and 47.9% in CF (Fig 3B).

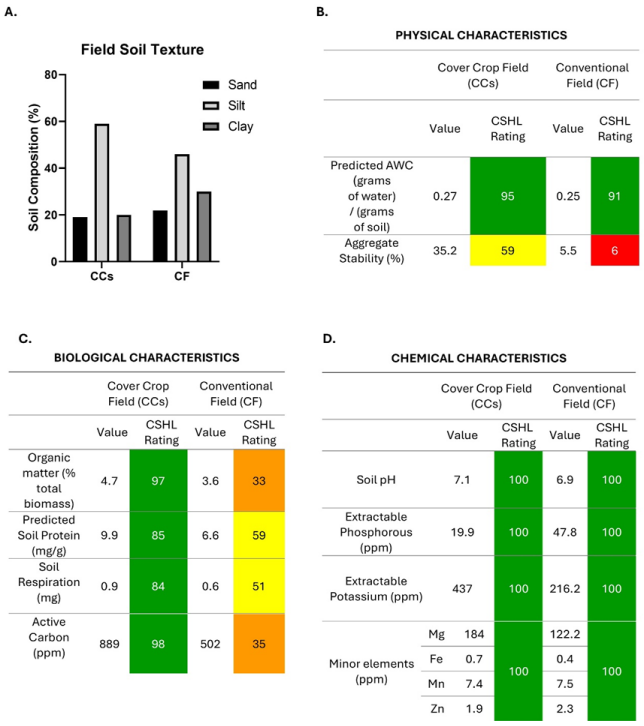

Figure 1. Cover Crop Field (CCs) and Conventional Tillage Field (CF) Soil Assessment. Soil parameter assessment was performed by Cornell Soil Health Laboratory (CSHL) (A) soil composition (%) of no till and conventional soil application (B) physical soil characteristics; AWC-available water capacity (C) biological soil characteristics (D) chemical soil characteristics.

Cannabinoid and Terpene Content

The total cannabinoid content was determined for both cultivars. There were no significant differences observed based upon field the hemp was grown in CC vs CF (Fig. 4A). There were also no significant differences based upon individual cultivars grown in either location (Figure 4B). The CC fields tend to produce more terpenes than the CF fields, 30 mg/ml vs 21.6 mg/ml respectively but is also highly variable (Fig. 4C). This trend is also apparent with individual cultivars, extract from cultivar 1 grown in CC had 36.1 mg/ml terpene content but only 31.2 mg/ml from CF and for cultivar 2 the terpene content was 20.3 and 6.1 mg/ml respectively (Fig. 4D). Only cultivar 1 from CC had significantly different levels of terpenes compared to cultivar 2 from CF.

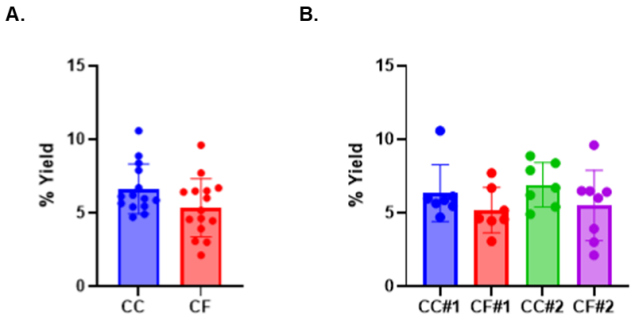

Figure 2. Extract Yield from Cover Crop Field (CC) and Conventional Tillage Field (CF). (A) Combined extraction yield (%) mean ± SD; n=14-15 of two hemp cultivars (Tangerine & CBG Stem Cell) grown in CC, a field with cover crop application with no tillage versus CF a conventional tillage field with no cover crop application (B) Comparison of extract yield of two hemp cultivars, Tangerine (#1) and CBG Stem Cell (#2) grown under CC or CF conditions mean ± SD; n=7-8 per cultivar. No statistical differences observed, Welch's t-test (A) or one-way ANOVA (B).

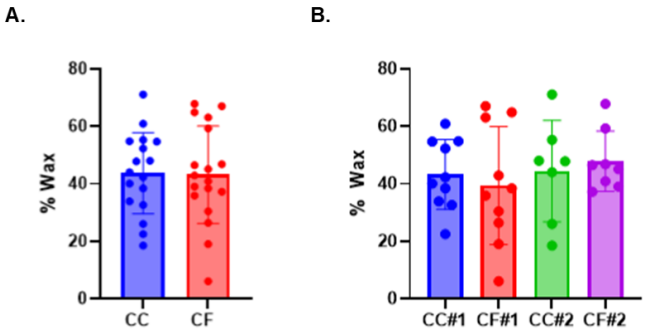

Figure 3. Wax Content from Cover Crop Field (CC) and Conventional Tillage Field (CF) Grown Hemp. (A) Combined wax (%) mean ± SD; n=17-18 of two hemp cultivars (Tangerine & CBG Stem Cell) grown in CC, a field with cover crop application with no tillage versus CF, a conventional tillage field with no cover crop application. (B) Comparison of extract wax from Tangerine (1) or CBG Stem Cell (2) mean ± SD; n=7-10 per cultivar. No statistical differences observed, Welch's t-test (A) or one-way ANOVA (B).

When evaluating the cannabinoid profiles between each field and cultivar, the largest amount of THC (27.5 mg/ml) and CBD (67.9 mg/ml) were observed in the CF extracts for cultivar 1 extracts (Table 1). The greatest amount of the precursor cannabinoid, CBG (53.2 mg/ml), was found in extracts produced from cultivar 2 from the CC fields. For cultivar 1 levels of cannabidiolic acid (CBDA) were the only other significant difference between CC and CF, while for cultivar 2 CBD and CBDA levels were significantly different between CC and CF. Globally CBC and CBG levels tend to be higher in extracts from CC, while no cannabinoids were found to be consistently higher in extracts from CF. Importantly, the levels CBG, in cultivar 1, are consistent with smaller harvest conducted from both fields during the first year (pilot) of this study (data not shown).

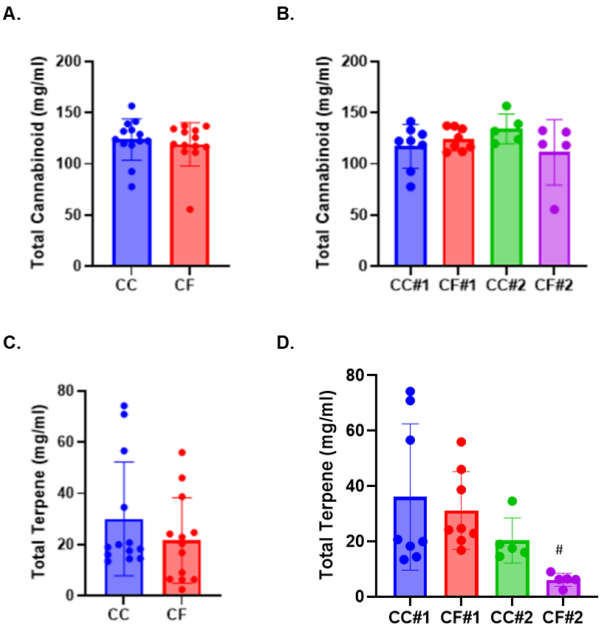

Figure 4. Comparison of Bioactive (Cannabinoid & Terpene) Content from Hemp Cultivars Grown in Cover Crop Field (CC) or Conventional Tillage Field (CF). (A) Combined cannabinoid and (C) terpene content mean ± SD; n=13 per cultivar of both cultivar extracts (mg/mL) grown in cover crop (CC) and conventional tillage field (CF) (B) Comparison of total cannabinoid and (D) terpene content in extracts from two hemp cultivars Tangerine (1) and CBG Stem Cell (2) grown under CC or CF conditions mean ± SD; n=5-8 per cultivar. # $p<0.05$ between cultivars grown in opposite field (CC#1 vs CF#2). Welch's t-test (A & C) or one-way ANOVA (B&D).

Regarding terpenoid profiles the most notable differences in extracts were between the two cultivars grown in CF, cultivar 1 had significantly lower levels of several terpenes, including major terpenes such as α-humulene, β-farnesene, β-myrcene, and trans-caryophyllene (β-caryophyllene) (Table 2). Interestingly, no significant differences in individual terpenes were found in extracts produced from the cultivars grown in CC. One interesting observation for cultivar 1, was that there was a significantly lower level of cannabinoids produced from plants grown in CC during year 2 of this study

(Figure 5A), but extracts from this same cultivar were higher in total terpenes that same year (Figure 5B). This largely accounts for the large variance observed (Figure 4).

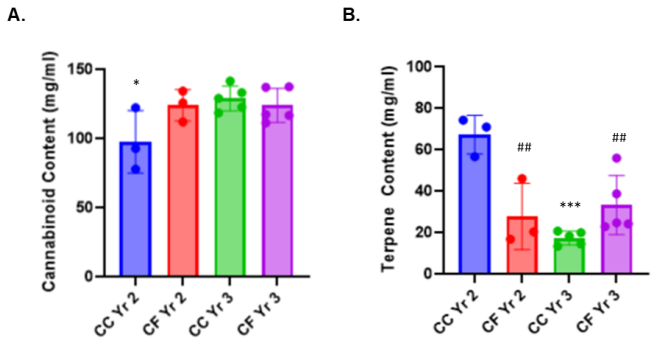

Figure 5: Comparison of Tangerine Extract Composition Between Growing Year 2 and 3 (A)Comparison of year 2 versus year 3 cannabinoid and (B) terpene content from hemp extracts produced from plants grown in cover crop field (CC) and conventional tillage field (CF). Data are mean ± SD, n=3-5. * compare similar fields (CC year 2 vs CC year 3) # compare opposite fields (CC vs CF). * $p<0.05$, , ##$p<0.01$ as assessed by one way ANOVA.

DISCUSSION

This study is the first to evaluate how soil characteristics from CC usage can affect cannabinoid and terpene content of hemp. A comparison of hemp extracts of two different cultivars grown in both CC soil and CF soil revealed significant differences in specific cannabinoid and terpene concentration. Based upon studies done in food crops; it was hypothesized that CC soil would provide elevated levels of cannabinoid and terpene content in hemp extracts (Scavo et al., 2022).

An assessment of soil properties revealed differences in physical and biological soil characteristics at the start of the study. Some of the most notable differences between CC and CF were ratings for aggregate stability, organic matter, predicted soil protein, soil respiration, and active carbon. Each characteristic scored higher in the CC in comparison to the CF. These differences may have contributed to the differences in cannabinoid and terpene content identified in the study. Additionally, these differences may have resulted in part from the farming practices utilized. For example, CC are known to prevent soil erosion and nutrient loss, increase levels of carbon and nitrogen in the soil, and improve water retention (Kim et al., 2020; Tully and McAskill, 2020; Scavo et al., 2022). Higher levels of organic matter, protein, and carbon were all detected in the CC field compared to the conventional field (Fig. 1). These differences were then paired with the measurements of cannabinoid and terpenoid content of extracts from hemp inflorescence grown in the corresponding fields. A comparison of the resulting extracts displayed no significant differences in total cannabinoid content, but did demonstrate contrasting levels of individual cannabinoids, notably THC, CBD, CBDA, and CBG concentration between cultivars with field-type dependency. Total terpene content differed between cultivars regardless of field type, with the most notable changes occurring between the two cultivars grown in the CF. It therefore appears that hemp in CC fields may help to reduce differences in terpene content despite innate differences due to cultivar genetics. This outcome adds to the growing body of evidence suggesting a relationship between the genetics of the hemp cultivar and its effect on soil nutrient uptake (Landi et al., 2019; Veazie et al., 2021; Westmoreland and Bugbee, 2022). However, since several physical and biological soil characteristics differed between each field, the direct effect each characteristic had on the outcome of this study requires further exploration.

One major limitation of our current study is that soil testing was conducted during our pilot growth year (Supplemental Figure 1) and not collected during the two years for cannabinoid and terpene data presented. The data from year 1 was excluded due to differences in the nature of the plants (CC was planted with tangerine clones, whereas CF was planted with tangerine seeds due to unforeseen events). Although soil health indicators are often stable over several years, and standard agricultural practice is to test soil every two to three years (Vogel, 2021). We cannot rule out potential changes due to farming practices, climate variability, or microbial shifts. Cover crops as well as traditional farming practices have developed ways to preserve and improve the quality of the soil over time and such changes may have occurred during our study (Tully and McAskill, 2020). Because of this the observed differences in our study are best viewed as hypothesis-generating, warranting further investigation.

Moreover, this study evaluates the content of supercritical CO_2 hemp extracts, the industrial standard for cannabis oil production; however, it

has been demonstrated that different extraction techniques produce different profiles (Lazarjani et al., 2021; Chacon et al., 2024). Therefore, different extraction techniques may identify differences in cannabinoid and terpene content that were not detected using supercritical CO_2 extraction. Because supercritical CO2 extraction is the most commonly used commercial procedure, this study provides evidence on how soil quality and farming practices can affect the cannabinoid and terpenoid content of consumer hemp products. Limitations in the complete soil characteristics exist as the tests performed in this study do not provide information on the soil microbiome, which is reported to be stimulated by the use of CCs (Kim et al., 2020).

The exact role that cannabinoids and terpenoids play in the plant remains unclear. The current leading hypothesis is that these compounds play a role in anti-predation and may also be stimulated by other stressors as THC is reported to increase with higher levels of UV light. We consistently found that plants grown in CCs had higher levels of the precursor cannabinoid, CBG. Under normal conditions, CBG is quickly converted into the other three major cannabinoids (CBD, THC, and CBC) (Nachnani et al., 2021). Therefore, future studies examining the levels of the enzymes responsible for converting CBG into CBD, THC, and CBC may provide additional insights into our findings here and provide a better understanding of why CBG levels accumulate in plants grown in cover-crop fields. For the most part the changes we observed were subtle shifts in cannabinoid profile, and without information on how the plant regulates the conversion of CBG into CBD, THC, or CBC, trying to correlate our findings on cannabinoid composition based upon soil characteristics is purely hypothetical at best. Interestingly, with regards to terpene, we observed fewer statistical differences in terpenes between the cultivars when grown in CC compared to CF, suggesting that CC practices may produce more uniform terpene patterns.

Our study adds to a growing literature demonstrating that cannabinoid and terpene profiles are strongly influenced by cultivation conditions, notably cultivation system (indoor vs outdoor), soil quality, and light exposure. Burgel and colleagues reported that growing media (peat moss, peat moss substituted with green fiber, or coco coir fiber) altered hemp growth rate and root density but did not impact CBD/A concentrations (Burgel et al., 2020). This contrasts with our study, we did observe field-dependent differences in both CBD and cannabidiolic acid (CBDA) that appear to be both cultivar and soil-quality dependent. Specifically, in cover crop field the cultivar Tangerine produced relatively higher CBDA and lower CBD levels, whereas CBG Stem Cell exhibited the inverse pattern (higher CBD and lower CBDA). Only minor differences between levels of cannabigerolic acid (CBGA), (CBDA), tetrahydrocannabinolic acid (THCA), and CBCA levels were reported between cannabis grown indoors vs outdoors (Zandkarimi et al., 2023). However, this study did find that indoor production resulted in more oxidized cannabinoids (such as: cannabinol/CBN, cannabicitran/CBT, and cannabielsoin/CBE). This study also found that outdoor cultivation increased the complexity of terpene composition. Our findings are consistent with this in that very low levels of CBN and cannabicylol (CBL) were detected. Another study found that poor soil increased CBDA levels regardless of indoor or outdoor growing conditions (Husain et al., 2019). We did not observe a consistent trend between fields for CBD and CBDA, with the two cultivars studied being impacted differently. It should be noted that none of our conditions were as harsh as that of mine soil used in the study by Husain and colleagues (2019). Light spectrum, a factor that can readily be manipulated in indoor settings, is probably the most studied environmental factor influencing cannabinoid content. Blue light has been found to increase THC and CBG levels in plant biomass but to have limited impact on CBD levels (Danziger and Bernstein, 2021; Morello et al., 2022). Collectively, these results underscore complex cultivar and environmental interactions that influence cannabinoid and terpene composition and highlight the need for cultivar-specific, controlled studies to understand the role environmental factors play in cannabinoid and terpene production.

The differences in cannabinoid content, particularly with regards to CBG and THC levels may have direct impact and relevance for farmers/producers/industry. For example, hemp with >0.3% THC content must be destroyed as it is considered marijuana and not hemp under the 2018 Farm Bill in the USA, and therefore conventional fields may pose an increased risk of producing hemp that

exceeds this limit. Conversely, CBG levels were consistently higher in cultivars grown in CC. Prior work, in our lab and others, has demonstrated increased pain reducing activity in extracts high in CBG, and if our findings hold for other cultivars it could lead to extracts higher in CBD than typically produced/marketed (Sepulveda et al., 2022a; Khajuria et al., 2023; Nachnani et al., 2023; Weerts et al., 2024). Increased levels of THC and CBC may also contribute to increased anti-nociceptive activity (King et al., 2017; Rock et al., 2018; Henderson-Redmond et al., 2021; Sepulveda et al., 2022b; Raup-Konsavage et al., 2023). Notably, while CBC levels were not significantly higher in extracts from the CC field, they did trend higher. Additional studies into the biomedical properties of the extracts from this study may help to elucidate further differences, resulting from the various combinations of cannabinoids and terpenoids, that soil quality may have on hemp extracts.

Overall, this study provides evidence of alterations in cannabinoid and terpene profile between extracts of hemp cultivars grown in CC soil and tilled soil. Two cultivars were grown identically in both tilled soil and CC soil, changes in cannabinoid content appear to be dependent upon both differences in cultivar (genetics) and soil characteristics. Interestingly, the terpene content of the extracts appeared to be more dependent upon cultivar for the CF, and less so for extracts from CC, as noted by fewer differences between terpene levels from CC extracts.

The outcome of this study provides outdoor growers with information on the effects soil health can have on cannabinoid and terpene content in hemp. Poor soil quality (CF) appears to result in higher levels of THC production, whereas higher soil quality (CC) may result in higher levels of the precursor cannabinoid, CBG. Further studies that incorporate synchronized soil and plant analyses across multiple time points, as well as studies with isolated treatments of the characteristics that contribute to the health of the soil are required to delineate the main driver between soil health and hemp cannabinoid and terpene content. Additionally, the impact of soil quality on cannabinoid synthesizing enzyme levels and activity may shed light on why CBG builds up in CC but not in CF.

Table 1: Comparison of cannabinoid concentrations (mg/mL) from hemp inflorescence grown in a cover crop field (CC) and a conventional tillage field (CF) for two cultivars Tangerine (1) and CBG Stem Cell (2).

Cannabinoid	CC #1	CF #1	CC #2	CF #2	Summary
	(mg/mL)				
CBC[1]	3.4±1.5	3.0±1.7	7.7±1.9	4.2±1.2	n.s.[2]
CBD	44.7±14.2	67.9±17.9	39.8±26.8	19.1±12.6	*aaa, ccc, ddd, eee, ff*
CBDV	0	0.3±0.1	1.7±0.2	0.6±0.4	n.s.
CBDA	40.9±24.8	6.5±10.3	26.2±24.9	58±16.2	*aaa, b, cc, dd, eee, fff*
CBG	15.7±7.6	13.2±16.3	53.2±25	14.3±8	*bbb, ddd, fff*
CBGA	9.1±6.7	0.1±.0.1	0.7±0.2	10.5±5.4	n.s.
CBL	0.1±0.02	0.03±0.04	0.2±0.1	0.2±0.1	n.s.
CBN	0.2±0.1	1.2±1.1	0.4±0.3	0.1±0.1	n.s.
THCV	0.3±0.2	0.3±0.1	0.3±0.1	0.2±0.1	n.s.
THCA	0.5±0.1	0.3±0.2	0.5±0.06	0.7±0.3	n.s.
Δ8-THC	0.1±0.1	3.6±4.0	0.1±0.1	0	n.s.
Δ9-THC	4.6±2.2	27.5±9.4	3.5±1.1	3.4±1.1	*aaa, ddd, eee*

[1]CBC (Cannabichromene), CBD (Cannabidiol), CBDV (Cannabidivarin), CBDA (Cannbidolic Acid), CBG (Cannabigerol), CBGA (Cannabigerolic Acid), CBL (Cannabicyclol), CBN (Cannabinol), THCV (Tetrahydrocannabivarin), THCA (Tetrahydrocannabinolic acid), Δ8-THC (Delta-8-Tetrahydrocannabinol), Δ9-THC (Delta-9-Tetrahydrocannabinol)

[2]Data means ± SD of n=5-8. Statistical significance was determined by a two-way ANOVA (n.s. not significant, *a*(CC#1 vs CF#1), *b*(CC#1 vs CC#2), *c*(CC#1 vs CF#2), *d*(CF#1 vs CC#2), *e*(CF#1 vs CF#2), *f*(CC#2 vs CF#2) one symbol $p<0.5$, two symbols $p<0.01$, three symbols $p<0.005$) Two-way ANOVA.

Table 2. Comparison of terpene concentrations (mg/mL) from hemp inflorescence grown in a cover crop field (CC) and a conventional tillage field (CF) for two cultivars Tangerine (#1) and CBG Stem Cell (#2).

Terpenoid	CC #1	CF #1	CC #2	CF #2	Summary
	mg/mL				
(+)-Cedrol	0.266±0.144	0.15±0.052	0.124±0.019	0.172±0.097	n.s.*
(-)-Caryophyllene Oxide	0.156±0.083	0.028±0.013	0.112±0.2	0.07±0.048	n.s.
3-Carene	0	0	0.004±0.008	0.002±0.004	n.s.
Borneol	0.004±0.005	0	0.002±0.004	0.004±0.005	n.s.
Camphene	0.006±0.005	0	0.008±0.004	0.004±0.005	n.s.
Endo-Fenchyl Alcohol	0.14±0.136	0.016±0.005	0.084±0.035	0.156±0.089	n.s.
Eucalyptol	0.02±0.02	0	0.006±0.005	0.006±0.005	n.s.
Fenchone	0.016±0.011	0	0.01±0.0	0.022±0.015	*c*
Geranyl Acetate	0.018±0.013	0.01±0.0	0.008±0.004	0.024±0.009	n.s.
Guaiol	1.558±1.027	0.934±0.355	0.758±0.128	0.758±0.366	n.s.
Linalool	0.046±0.021	0.012±0.004	0.084±0.028	0.166±0.107	n.s.
R(+)-Limonene	0.214±0.286	0.016±0.005	0.102±0.023	0.284±0.125	*cc,e*
Terpinolene	0.008±0.004	0.002±0.004	0.164±0.075	0.29±0.236	n.s.
Valencene	0.85±0.559	0.114±0.057	0.684±0.157	1.288±0.521	*ee*
α-Bisabolol	3.424±1.269	1.87±0.697	2.082±0.476	2.778±1.373	n.s.
α-Cedrene	0.226±0.189	0.026±0.011	0.066±0.015	0.166±0.063	*ee*
α-Farnesene	1.108±1.017	0.14±0.119	1.324±0.433	4.118±2.298	*e*
α-Humulene	1.19±0.711	0.208±0.092	1.132±0.196	2.064±0.826	*c,ee*
α-Phellandrene	0.02±0.044	0.024±0.009	0.014±0.019	0.012±0.004	*e*
α-Pinene	0.01±0.017	0	0.01±0.007	0.018±0.013	n.s.
α-Terpinene	0	0	0.006±0.005	0.006±0.009	n.s.
α-Terpineol	0.244±0.173	0.064±0.044	0.134±0.046	0.18±0.083	n.s.
β-Farnesene	5.436±1.692	1.67±0.957	5.438±1.762	11.09±5.276	*c,e,f*
β-Myrcene	0.306±0.379	0.018±0.008	0.228±0.069	0.6±0.351	*ccc,e*
β-Pinene	0.08±0.115	0	0.032±0.024	0.082±0.055	n.s.
Cis-Nerolidol	0.04±0.007	0.03±0.007	0.018±0.011	0.006±0.008	n.s.
Cis-Ocimene	0.012±0.008	0	0.026±0.011	0.056±0.047	n.s.
γ-Terpinene	0.002±0.004	0	0.008±0.004	0.008±0.008	n.s.
Trans-Caryophyllene	4.776±3.581	0.734±0.328	4.526±0.818	8.666±3.105	*c,eee*
Trans-Nerolidol	0.156±0.077	0.049±0.008	0.118±0.033	0.19±0.095	n.s.
Trans-Ocimene	0	0	0.044±0.064	0.002±0.004	n.s.

*Data means ± SD of n=5-8. Statistical significance was determined by a two-way ANOVA (n.s. not significant, *a*(CC#1 vs CF#1), *b*(CC#1 vs CC#2), *c*(CC#1 vs CF#2), *d*(CF#1 vs CC#2), *e*(CF#1 vs CF#2), *f*(CC#2 vs CF#2)one symbol p<0.5, two symbols p<0.01, three symbols p<0.005) Two-way ANOVA.

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