Received: 10 October 2024 | Accepted: 4 March 2025

DOI: 10.1002/agg2.70091

Agrosystems, Geosciences & Environment OPEN ACCESS

ORIGINAL ARTICLE

Special Section: Industrial Hemp Production and Management

Performance and mycorrhizal colonization of industrial hemp varieties under regenerative organic systems in Northeastern region

Dinesh Panday[1] | **Wade P. Heller**[2] | **Joseph E. Carrara**[2] | **Nikita Bhusal**[3] | **Nicholas Omoding**[1] | **Tara Caton**[4] | **Ashley Walsh**[5] | **Andrew Smith**[1] | **Arash Ghalehgolabbehbahani**[1]

[1]Rodale Institute, Kutztown, Pennsylvania, USA

[2]USDA-ARS Eastern Regional Research Center, Wyndmoor, Pennsylvania, USA

[3]Department of Food Science and Technology, University of Georgia, Athens, Georgia, USA

[4]Rodale Institute Pocono Organic Center, Blakeslee, Pennsylvania, USA

[5]Pocono Organics, Blakeslee, Pennsylvania, USA

Correspondence
Dinesh Panday, Rodale Institute, Kutztown, PA 19530, USA.
Email: dinesh.panday@rodaleinstitute.org, dinesh.livingsoil@gmail.com

Assigned to Associate Editor Arjun Kafle.

Funding information
Rodale Institute and Pocono Organics

Abstract

Industrial hemp (*Cannabis sativa* L.) is a versatile crop with applications in fiber, seeds, and medicine. Recent legalization has renewed interest in industrial hemp in the United States, particularly in fiber production, which has a critical role in carbon (C) sequestration and various industries, including textiles and construction. A 2-year field experiment (2022–2023) was conducted at Rodale Institute—Pocono Organic Center, Blakeslee, PA, evaluating the performance of four hemp varieties (MS 77, Futura 75, Santhica 27, and Han NE) under regenerative organic systems. Seed rates were considered as 73 kg ha^{-1} for Santhica 27 and Futura 75, 135 kg ha^{-1} for MS 77, and 270 kg ha^{-1} for Han NE, targeting a plant population of 2.47 million plant ha^{-1} across varieties. Data on canopy cover, plant height, yields (biomass, stem, leaf, and flower), and cannabinoids were collected to assess the effects of variety and environmental conditions on growth and yield. Root samples from 2023 were also analyzed for arbuscular mycorrhizal fungi (AMF) colonization. Han NE demonstrated the highest growth, yields, and canopy cover, followed by MS 77, with more favorable growing conditions in 2023. Additionally, AMF colonization was consistent across varieties, reporting a higher colonization in MS 77 (45.37%), suggesting enhanced nutrient uptake and stress tolerance. Based on the results, Han NE and MS 77 are promising hemp varieties for fiber production in this region. Further research is required to explore the impact of mycorrhizal colonization on hemp production under varying nutrient conditions for sustainable production.

Abbreviations: AMF, arbuscular mycorrhizal fungi; CBD, cannabidiol; THC, delta-9-tetrahydrocannabinol.

Plain Language Summary

A field study at Rodale Institute—Pocono Organic Center in Pennsylvania looked at different types of hemp to see which ones grow best for fiber production in the Northeastern region. The goal was to understand how different hemp varieties perform in organic farming conditions and how their roots interact with helpful fungi (called AMF) that aid in nutrient absorption. The results showed that two fiber hemp varieties, Han NE and MS 77, grew the best and produced the highest yields. Han NE also had more leaf cover and stronger growth. The roots of all the hemp plants had good amounts of fungi, with MS 77 having the highest level, which helps the plant absorb nutrients and better handle stress. Based on these findings, Han NE and MS 77 are good choices for growing hemp for fiber in this region. More research is needed to learn how different nutrient levels affect the fungi and hemp growth for more sustainable farming.

1 | INTRODUCTION

Hemp (*Cannabis sativa* L.) is a historically important and highly versatile crop, cultivated worldwide for various applications such as fiber, seeds, and medicinal purposes. Hemp is considered a day-length-sensitive, short-day plant, and floral initiation occurs once the critical photoperiod is reached (Amaducci et al., 2012). Hemp plant contains a rich chemical composition, such as cannabinoids, terpenoids, flavonoids, and fatty acids (Bautista et al., 2021; Kanabus et al., 2021). Of these, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most well-known cannabinoids, with THC being psychoactive and CBD exhibiting potential medicinal benefits (Atakan, 2012). In the United States, hemp is legally defined as containing no more than 0.3% THC by dry weight, facilitating its cultivation and commercialization across various industries (Abernethy, 2019). Beyond its industrial uses, fiber hemp plays an essential role in carbon (C) sequestration, capturing between 7.2 and 13.6 Mg of carbon dioxide (CO_2) ha^{-1}, which is two times higher than the C uptake of traditional forests (Fairs, 2021).

In Pennsylvania, the hemp industry has grown rapidly since the Industrial Hemp Research Act passed in 2016. By 2023, 121 ha of farmlands were planted, with 69 ha harvested, including 21.5% fiber varieties (PAHIC, 2023). Pennsylvania holds 215 licensed growers and 52 processors, with expanding interest as high-value specialty crop in building a robust bioeconomy and diversifying agriculture to support industrial applications. The Rodale Institute has been instrumental in advancing hemp research since 2017 by developing the best management practices to support growers and associate industries in Pennsylvania and beyond.

Several factors influence hemp yield and fiber quality. These include environmental conditions, soil fertility, and key agronomic practices, such as soil characteristics, fertilizer application rates, cultivars, planting density, planting dates, climate, and so forth (Panday et al., 2025a; Sunoj et al., 2023; Visković et al., 2023). Sustainable agricultural approaches have gained prominence in recent research to optimize cannabis growth and cannabinoid production (Pérez-Bermúdez & Martínez, 2023). However, conventional fertilization methods remain reliant on inorganic inputs, which can degrade soil health and disrupt microbial communities. To mitigate these adverse effects and reduce dependence on inorganic inputs, the utilization of plant growth-promoting microorganisms, including arbuscular mycorrhizal fungi (AMF) and rhizobacteria, has garnered significant interest (Andre et al., 2016; Takishita et al., 2018).

AMF, form symbiotic relationships with the roots of most terrestrial plants, are known for their ability to enhance the host plant's nutrient acquisition, particularly phosphorus (P) and nitrogen (N), in exchange for C compounds derived from the plant's photosynthesis (Bücking et al., 2012). The extraradical mycelium of AMF extends into the soil, absorbing nutrients and transporting them to the intraradical mycelium within the plant roots, where the exchange occurs (Bücking & Kafle, 2015). These fungi have been shown to boost nutrient and water uptake in plants, especially under resource-limited conditions (Sun & Shahrajabian, 2023; Yuan et al., 2024). However, agricultural practices such as tillage can disrupt AMF networks, impairing their functionality and the associated ecosystem services.

In cannabis cultivation, inoculating plants with specific strains of AMF has been shown to improve plant growth and increasing cannabinoids, offering a sustainable alternative to chemical fertilizers (Lyu et al., 2019; Pagnani et al., 2018). For instance, inoculation of hemp KKU05 with the AMF *Rhizophagus aggretatus* demonstrated improved growth, increased leaf, stem, floret, and root biomass, and elevated concentrations of the cannabinoids CBD and THC compared to the controls, including non-mycorrhizal plants that received supplemental synthetic fertilizer (Seemakram et al., 2022). Similarly, Pagnani et al. (2018) demonstrated that using a mixture of plant growth-promoting rhizobacteria increased plant biomass and cannabinoid content, offering benefits comparable to synthetic N fertilizers. Additionally, AMF help to maintain soil structure, preventing issues like soil hardening and acidity (Fall et al., 2022), which are common with synthetic fertilizers. This is especially crucial in regenerative organic systems, where preserving soil health is a priority. However, there is a lack of information, which examines the effects of AMF (inoculation) across different hemp varieties under regenerative organic systems, especially in Northeastern climates. Understanding how AMF influences hemp growth, fiber yield, and root colonization under site-specific conditions is critical for improving sustainable hemp production and maximizing its industrial potential.

A 2-year field experiment was conducted at Rodale Institute—Pocono Organic Center, Blakeslee, PA, to assess the performance of four hemp varieties (MS 77, Futura 75, Santhica 27, and Han NE) under regenerative organic systems. Data collection included hemp canopy cover, plant height, yields (biomass, stem, leaf, and flower), and cannabinoid concentrations. Additionally, mycorrhizal root colonization was analyzed to understand its interaction with variety and environmental conditions. Since hemp yield is linked to the choice of genotype, this study aims to provide valuable insights for producers seeking sustainable and high-performing hemp cultivars.

Core Ideas

- Han NE and MS 77 varieties showed superior growth and yield in fiber hemp production in the Northeastern United States.
- Hemp roots were mostly colonized by *Rhizophagus intraradices* and *Rhizophagus irregularis* among the arbuscular mycorrhizal fungi (AMF) species tested.
- Future research is needed on AMF colonization under varying nutrient conditions for sustainable cultivation.

2 | MATERIALS AND METHODS

2.1 | Site description

A field study was conducted from June to September in 2022 and 2023 at the research site of Rodale Institute—Pocono Organic Center located in Blakeslee, PA. The Pocono Organics farm, established in 2018 on 380 ac of farmland, is Regenerative Organic Certified (ROC). The soil type is a Clymer loam (coarse-loamy, siliceous, active, mesic Typic Hapludults) and the research site had 1%–3% slope. Field varied year to year, where soil pH was slightly alkaline in both study years (Table 1). Total C and N were 1.55% and 0.11%, and 2.50% and 0.15% in 2022 and 2023, respectively. Further details about soil analysis from the plough layer (0–20 cm) is presented in Table 1.

The region experiences a temperate continental climate, characterized by hot and humid summers and cold winters. Weather data were sourced from the Pocono Pines station, Pennsylvania. In 2022 and 2023, the average annual temperature and precipitation were recorded as 8.0˚C and 1472 mm, and 8.8˚C and 1493 mm, respectively. The region has an average annual relative humidity of 68%, a frost-free period of 100–160 days and an annual snowfall of 160 cm.

Figure 1 shows average monthly temperatures and cumulative rainfall during the growing season, that is, June through September. Temperature trends were similar, starting high in June, though June 2023 was slightly cooler than June 2022, with July temperatures higher in 2023. The rainfall patterns were quite different between the 2 years. In 2022, rainfall decreased from June to September, with the least in July, while in 2023, it followed a bell-shaped pattern, peak rainfall during the mid-season. Optimal temperature ranges vary with hemp growth stages: germination and seedling (8–10˚C), vegetative (21–29˚C), and flowering (18–27˚C). Hence, average temperatures align with hemp's preferred range, supporting healthy development.

2.2 | Experimental layout

The field experiment was arranged in randomized complete block design, with four industrial fiber hemp varieties, MS 77, Futura 75, Santhica 27, and Han NE, each replicated four times (Figure 2). Individual plot dimensions were 2.75 m wide by 60 m long, with a 2 m buffer between plots. MS 77 is a dual-purpose variety from Australia, Han NE is a fiber variety from China, and Futura 75 and Santhica 27 are fiber varieties from Europe. The seeds, all non-genetically modified organism and untreated with fungicides or herbicides, were sourced from different vendors: MS 77 from Ecofibre, Futura 75 and Santhica 27 from KonopiUS, and Han NE from Kanda, United States.

Each year, field was prepared using disk tillage and hemp seeds were sown at different rates (due to difference in seed size), 73 kg ha^{-1} for Santhica 27 and Futura 75, 135 kg ha^{-1} for MS 77, and 270 kg ha^{-1} for Han NE, maintaining a target plant population of 2.47 million plant ha^{-1} for all the varieties. A 2.75-m wide grain drill with 16 nozzles was used for sowing. Seed germination rates were 90%–93% for MS 77, 88%–90% for Futura 75 and Santhica 27, and 75%–79% for Han NE; and seed rates were adjusted accordingly.

TABLE 1 Soil characteristics of 0- to 20-cm depth at research site of Rodale Institute—Pocono Organic Center, Blakeslee, PA, in 2022 and 2023.

Soil analysis	2022	2023
pH	7.4	7.7
OM (%)	2.93	3.60
Total C (%)	1.55	2.50
Total N (%)	0.11	0.15
P (mg kg^{-1})	1.27	13.25
K (mg kg^{-1})	87.64	78.25
Ca (mg kg^{-1})	780.48	1333
Mg (mg kg^{-1})	36.62	10.97
S (mg kg^{-1})	6.46	10.25
Na (mg kg^{-1})	27.44	14.00
Al (mg kg^{-1})	11.86	953
Cu (mg kg^{-1})	0.06	2.10
Fe (mg kg^{-1})	1.14	94.5
Mn (mg kg^{-1})	4.58	36.3
Zn (mg kg^{-1})	0.28	2.78

Abbreviation: OM, organic matter.

Baseline soil samples were collected from plough layer from each block after crop emergence and conducted a routine soil analysis (Table 1). To support early growth, organic fertilizer in the form of blood meal was uniformly broadcasted at rate of 112 kg N ha^{-1} 2 weeks after sowing. Blood meal is an Organic Material Review Institute (OMRI)–listed fertilizer, produced from dried animal blood and provides approximately 12% N for crops in organic farming, but does not supply P and K (Panday et al., 2024). Weeding was conducted periodically by 46-in. wide lawn mower between the plots to maintain plot cleanliness. In both years, seeds were planted in early June and harvested in early September using a sickle bar.

2.3 | Data collection

During the early growing season, data on crop canopy coverage, referred to as fractional green canopy cover (FGCC, %) was collected twice, on June 29 and July 13 in 2022, and on June 20 and July 14 in 2023. This was conducted to quantify chlorophyll intensity and foliar growth in fiber hemp beds and compare these parameters across different varieties. To assess FGCC, foliage photos were taken 1 m above canopy and analyzed using Canopeo smartphone application, which quickly calculates the ratio of green leaves to bare soil. Canopeo, developed with MATLAB (Mathworks, Inc.), differentiates pixel based on red-to-green (R/G) and blue-to-green (B/G) color ratios and an excess green index (2G–R–B) (Patrignani & Ochsner, 2015).

Plant height above the ground was measured prior to the harvest, with an average of 20 samples taken from each plot. Plant biomass data were collected just before harvest, when 70%–80% male plants were in flowering stage, before seeds set. During harvest, biomass samples were collected using two 0.25-m^2 quadrants from each plot. Total biomass (above the ground), as well as leaf, stem, and flower yields of hemp were calculated based on fresh weight of sample plants. These values were later converted to an oven dry weight basis and reported in the manuscript. Additionally, root samples were collected to determine the mycorrhizal colonization in 2023. Due to differences in cultivar traits, Futura 75 and Santhica 27 were harvested earlier than MS 77 and Han NE. Although not required after obtaining a permit to grow fiber hemp in Pennsylvania, we tested THC levels in the commercial lab to ensure they remained below the permissible limit of 0.3% in both years. After data collection, the remaining hemp in the plots were terminated using a 2.75-m wide sickle bar mower attached to the BCS tractor (model 749).

2.4 | Mycorrhizal colonization analysis

Three root samples from each plot were dug up with spade fork and carefully washed clean with water before snipping off representative samples of fine roots (<2 mm) with scissors, then subsampled for staining and molecular analysis. Deoxyribonucleic acid (DNA) extraction and quantitative polymerase chain reaction analysis of approximately 50 mg root tissue aliquots were performed as in Heller and Carrara (2022), except that the primers and probe for the plant genomic DNA target were substituted for analyzing hemp. The *C. sativa* genomic DNA target consisted of a 100 bp target within the translation elongation factor 1-alpha (EF-1α) gene, which was amplified with primers HempEF1a.F, TGTTTTGCACGGATCAGTTTG and HempEF1a.R, AATGCCGACCGCTACAGTTC from Hu et al. (2023), and quantified using probe HempEF1a.PR, /5Cy5/TCGAGTTGT/TAO/AGAGCTCTTGGAAGGGT/3IAbRQSp/ designed using PrimerQuest Tool (Integrated DNA Technologies). The relative quantification of each AMF DNA target to the hemp DNA target for the different hemp varieties was determined using ΔCt between the means of the AMF and hemp targets, then normalized to Futura 75 as the reference sample (ΔΔCt). The root length colonization was determined by the gridline intersect method (Giovannetti & Mosse, 1980) following staining with trypan blue (Phillips & Hayman, 1970) at 20–50x magnification under a dissecting microscope.

2.5 | Statistical analysis

The statistical analysis was performed using R statistical software (version 4.3.3). An analysis of variance was conducted

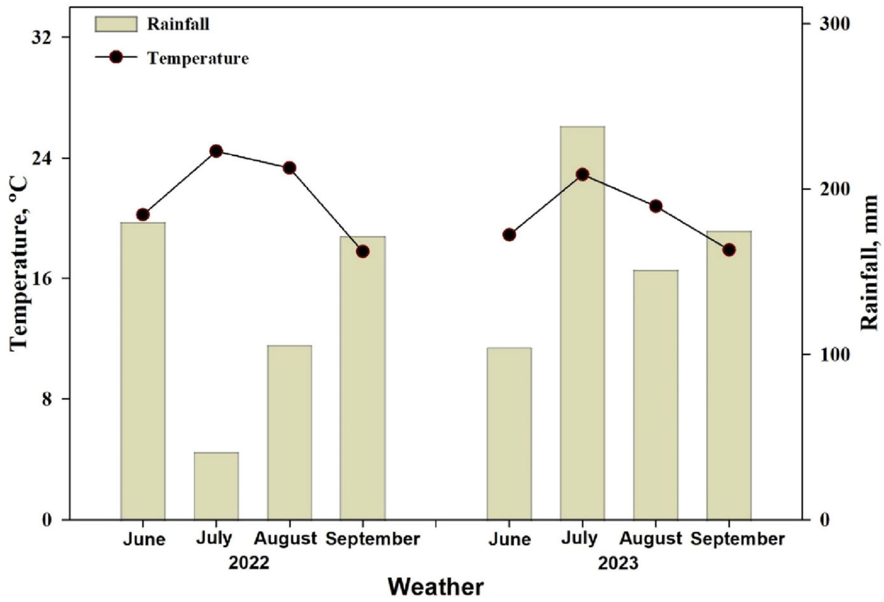

FIGURE 1 Mean temperature and cumulative rainfall during the growing season at research site in 2022 and 2023.

FIGURE 2 Fiber hemp varietal trial field at early (left) and mid-season (right) stages.

to examine the main effects of variety and year, as well as their interactions, on the test variables, utilizing the aov() function. In this analysis, variety and year were treated as fixed independent factors. The post hoc Tukey honest significant difference (Tukey HSD) test was performed using the TukeyHSD() function at a significance level $\alpha = 0.05$. The "ggplot2" package was used for data visualization.

3 | RESULTS AND DISCUSSION

3.1 | Hemp canopy cover, plant height and cannabinoids profile

In each year of the study, Canopeo was able to capture wide variation in canopy cover among varieties, consistent with visual field observations. All varieties showed an increase in green canopy cover from the first to the second measurement (Figure 3). While there were no significant differences among varieties within the same reading date, Han NE consistently showed a higher canopy cover trend in the second reading compared to the first, followed by MS 77 and Santhica 27, while Futura 75 showed variable performance between years. In 2023, Han NE had a higher canopy cover during the first reading compared to other varieties and attained the highest FGCC (85%) during the second reading (Figure 3b). Although Canopeo does not require calibration prior to image processing (Patrignani & Ochsner, 2015), its readings may be over- or underestimated in areas with red or yellow soils (Hale et al., 2023) or in fields with taller crops (Govindasamy et al., 2022). Nonetheless, it remains a valuable tool for monitoring plant

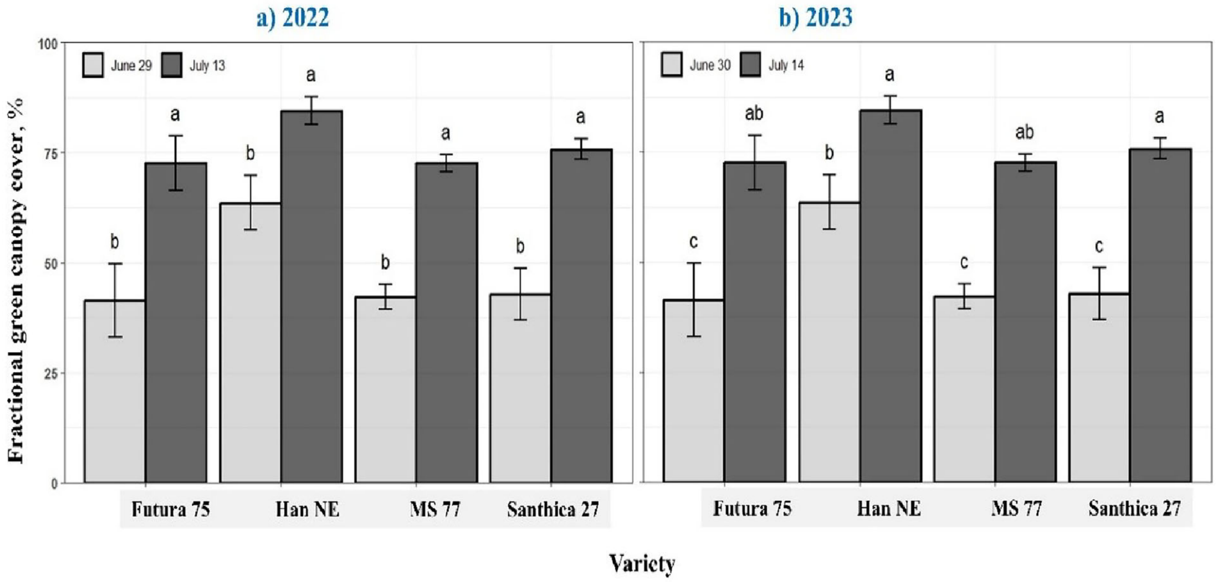

FIGURE 3 Fractional green canopy cover was measured using the Canopeo application for four industrial hemp varieties in (a) 2022 and (b) 2023. Two measurements were taken each year, with the results presented in mean (±SE). Means followed with same letters are not significantly different ($p > 0.05$).

growth and development across diverse backgrounds and have been successfully used in crops such as soybean (Shepherd et al., 2018), bermudagrass (Chhetri & Fontanier, 2021), forage crops (Jauregui et al., 2019), and industrial hemp cultivars (Sandhu, 2022).

The plant height at harvest varied across varieties: 0.47–1.31 m in Han NE, 0.39–0.99 m in MS 77, 0.46–0.74 m in Futura 75, and 0.39–0.88 m in Santhica 27. The plant height was significantly influenced by the main effects of variety and growing year (Table 1). Han NE had the tallest plants (0.91 m), significantly taller than the other varieties. Similarly, greater plant height was observed in 2023 compared to 2022, suggesting that environmental conditions in 2023 were more favorable for fiber hemp production. Elevated temperatures during 2023, except in June, provided optimal conditions in hemp production, suggesting that air temperature could be a limiting factor even with sufficient water supply (Cosentino et al., 2013). It is also important to consider other environmental factors, such as precipitation, photo period, and soil fertility, which play crucial roles in hemp growth and yield (Visković et al., 2023).

Studies highlight that genotype performance can vary widely depending on growing conditions or cropping environments and their interaction, which are crucial for optimizing hemp production and sustainability (Beleggia et al., 2023; Tang et al., 2016; Tsaliki et al., 2021). For instance, a 3-year field study on six monoecious hemp varieties, including Santhica 27 and Futura 75, conducted in a Mediterranean environment with warm, dry summers and cool, humid winters in Northern Greece, consistently showed taller plants (1.16 m for Santhica 27 and 0.81 m for Futura 75) (Tsaliki et al., 2021). However, in the current study, these same varieties underperformed, which underscores the importance of selecting varieties adapted to specific environmental attributes and optimizing management practices accordingly.

At harvest, representative flower samples were collected and analyzed for cannabinoids profile, focusing on THC and CBD. In both years, THC concentrations were notably low in fiber hemp varieties, except for Han NE, which had concentrations of 0.29% in 2022 and 0.20% in 2023—both still lower than the legal threshold of 0.30% (Table 3). In contrast, CBD concentrations were comparatively higher in the European varieties during both years. The industrial hemp is classified under chemotype III, characterized by a THC content of less than 0.3%, and typically has a CBD content ranging from 2% to 3% (Pacifici et al., 2019).

3.2 | Yields of fiber hemp

The hemp plant biomass yield, expressed on a dry weight basis, exhibited significant variation influenced by the main effects of variety and growing year (Table 2). Han NE and MS 77 produced higher biomass and stem yield compared to Futura 75 and Santhica 27. Although biomass and stem yield increased in 2023 compared to 2022, the yield among varieties remained consistent, indicating a strong genetic influence on the biomass production.

There was a significant interaction between variety and year on leaf yield of fiber hemp (Table 2). The highest leaf yield was recorded in MS 77 in 2022 (Figure 4a) and Han NE in

TABLE 2 Varietal effect on observed parameters of fiber hemp production in 2022 and 2023.

Source of variation	Plant height (m)	Biomass	Stem	Leaf	Flower	Root length colonization (%)
		Yield (Mg ha^{-1})				
Variety (V)						
Han NE	0.91a	5.20a	4.0a	0.29	0.62	43.96
MS 77	0.73b	4.57a	3.53a	0.27	0.61	45.37
Futura 75	0.64b	2.11b	1.12b	0.10	0.48	45.05
Santhica 27	0.67b	1.37b	0.87b	0.04	0.37	43.66
Significance	***	***	***	***	*	ns
Year (Y)						
2022	0.69b	2.55b	1.89b	0.13	0.37	–
2023	0.72a	4.07a	2.87a	0.21	0.68	–
Significance	**	***	**	**	***	–
Interaction						
V × Y						
Significance	ns	ns	ns	*	**	–

Note: Means in a column followed by same lowercase letter are not significantly different. Yield is presented on a dry weight basis. Root samples data are presented from 2023 only.

Abbreviation: ns, not significant.

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

TABLE 3 Concentrations of cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) in flower samples from four representative industrial varieties in 2022 and 2023.

Variety	2022		2023	
	CBD (%)	THC (%)	CBD (%)	THC (%)
Han NE	0.38	0.29	0.76	0.20
MS 77	0.36	0.16	1.56	0.02
Futura 75	0.26	0.02	2.42	0.04
Santhica 27	0.80	0.07	1.22	0.03

2023 (Figure 4b), while the lowest was in Santhica 27 for both years. A similar significant interaction effect was observed for flower yield of fiber hemp (Table 2), with Futura 75 achieving the highest flower yield in 2023 (Figure 4d). Interestingly, Han NE exhibited consistent flower yields across both years (Figure 4c,d), suggesting a stable plant structure with uniform height and larger stem diameter compared to other varieties.

Although fiber yield was not directly measured from the harvested stalks in the current study, it is estimated that approximately 25% of stem yield in fiber-type varieties would contribute to bast fiber yield (McLennon et al., 2024). This translates to an estimated 1.0 Mg ha^{-1} for Han NE, 0.3 Mg ha^{-1} for Futura 75, and 0.2 Mg ha^{-1} for Santhica 27 varieties. For the dual-purpose variety, around 23% of the stem yield is expected as bast fiber (McLennon et al., 2024; Tsaliki et al., 2021), resulting in an estimated 0.8 Mg ha^{-1} for MS 77. In a recent 2-year research trial at the Rodale Institute, Kutztown, PA, the dual-purpose variety, Canda, yielded 23.9% bast fiber from harvested stalk (Panday et al., 2025b).

Many studies have shown a strong positive correlation between hemp fiber yield and both total biomass and stem biomass yield (Baldini et al., 2020; Tsaliki et al., 2021; Yazici, 2023), indicating that higher biomass production typically leads in greater fiber yields. There was no significant interaction between variety and year, except for leaf and flower yields (Table 2). Fiber yield, which is dependent on total biomass or stalk production, remains consistent across varieties under similar environmental conditions. This further highlights the importance of selecting appropriate varieties for optimal fiber production.

3.3 | Root mycorrhizal colonization

The percentage of root length colonization measurements in fiber hemp samples ranged from 37% to 54%. Colonization rates were relatively consistent across all hemp varieties tested (Table 2). The highest average root length colonization was recorded in MS 77 (45.37%), while Santhica 27 had the lowest (43.66%), though the differences were not statistically significant. Higher root length colonization in hemp varieties, such as MS 77, may contribute to improved nutrient uptake and stress tolerance, as suggested by available research on the role of AMF in plant stress responses (Kakabouki et al., 2021; Sun & Shahrajabian, 2023; Yuan et al., 2024; Zhang et al.,

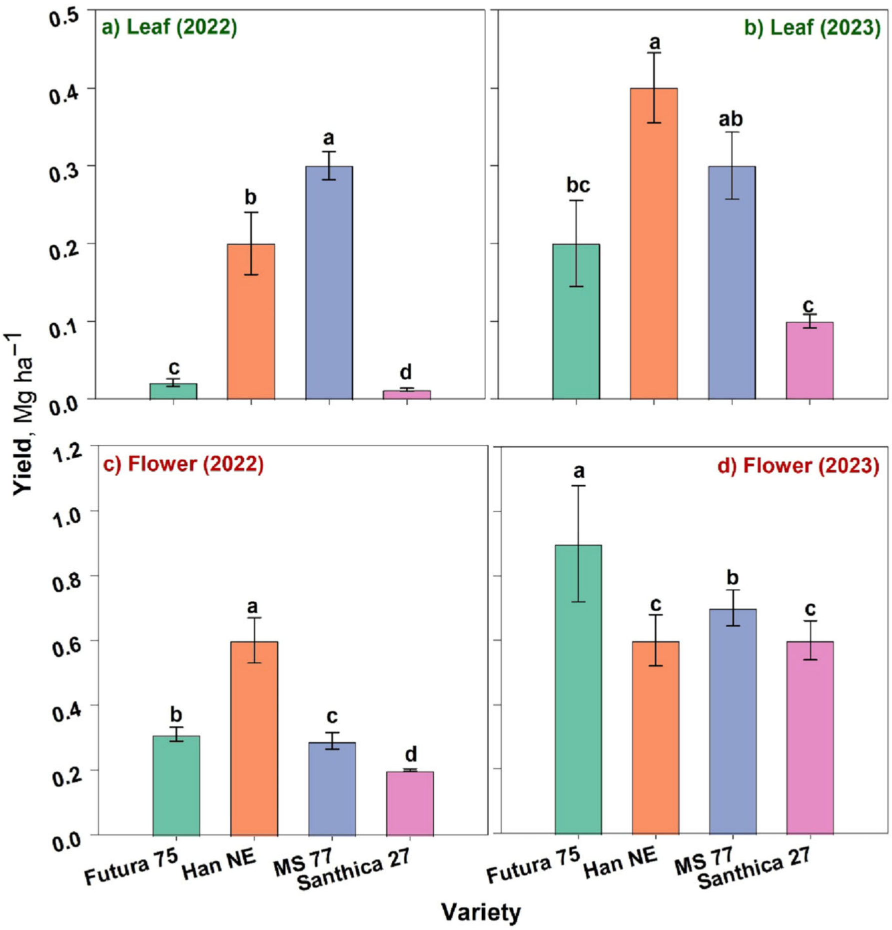

FIGURE 4 Effects of variety and year on fiber hemp leaf yields in (a) 2022 and (b) 2023 and on flower yields in (c) 2022 and (d) 2023, expressed in Mg ha^{-1} unit on dry weight basis. Means (±SE) with different lowercase letters are significantly different at $p < 0.05$.

2019). Studies have shown that AMF colonization boosts the production of key secondary metabolites, such as terpenoids, phenolic compounds, and nitrogenous compounds, which play important roles in plant growth and defense mechanisms (Bahador et al., 2023; Khaliq et al., 2022; Yan et al., 2007). Nonetheless, the current study did not find a significant relationship between root colonization and biomass yield (data not shown).

Emerging research suggests that AMF colonization may reduce the need for application of inorganic N fertilizers by enhancing nutrient uptake (Qian et al., 2024; Tajini et al., 2011). AMF can transfer 20%–75% of the N they acquire from the soil to their host plants (Hashem et al., 2018). This symbiosis improves the availability of N and P in the soil, and as a result, the root system was further developed through the formulation of more branches and root tips, as observed in crops like industrial hemp (Bahador et al., 2023). Root samples from the Rodale Institute's long-term vegetable systems trial (VST) showed an approximately eight times higher abundance of one of the AMF species under organic best management practices for sweet corn production compared to conventional methods (Heller & Carrara, 2022). This further highlights the importance of AMF in regenerative organic systems, in which the absence of inorganic fertilizers supports greater colonization efficiency. However, despite the benefits, further research is required to fully understand the underlying mechanisms of AMF in response to environmental stressors and nutrient dynamics. Interestingly, certain hemp varieties with lower AMF colonization rates, like Han NE, still exhibited higher biomass yields, indicating that other factors beyond root colonization significantly influence overall growth and yield.

Further analysis of root samples from four fiber hemp varieties revealed that two AMF species, *Rhizophagus intraradices* (detected in 42 out of 48 samples, i.e., 87.5%) and *Rhizophagus irregularis* (31 out of 48, i.e., 64.5%), were predominant. There were also lower frequency detections of a few other AMF species (data not shown). Notably, Santhica 27 and MS 77 exhibited higher relative abundances of *R. intraradices* (Figure 5a), suggesting a favorable interaction that may enhance nutrient acquisition, particularly P, essential for plant growth (Seemakram et al., 2022). For *R. irregularis*, Santhica 27 exhibited the highest relative abundance, while MS 77 had the lowest (Figure 5b). The more

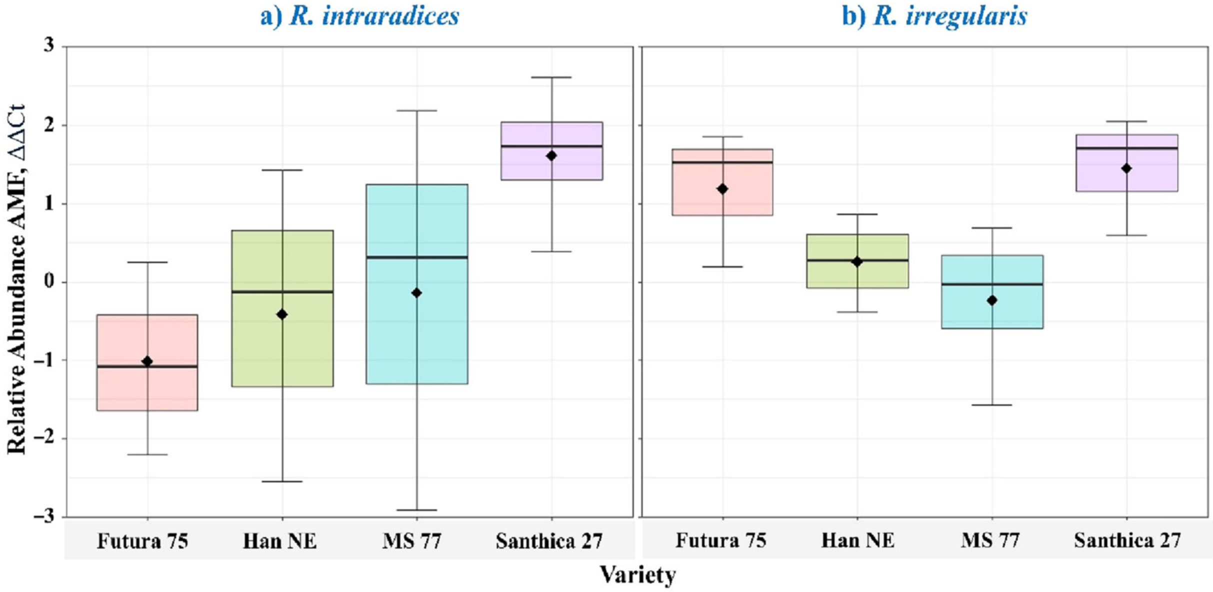

FIGURE 5 Boxplot showing relative abundance of arbuscular mycorrhizal fungi (AMF) species (a) *Rhizophagus intraradices* and (b) *Rhizophagus irregularis*, expressed in ΔΔCt unit.

prevalent association by *R. irregularis* across varieties implies its role in stabilizing nutrient uptake and thereby supporting overall plant development (Ahmed et al., 2021), leading to improved yield. A similar trend of higher (30%) and consistent fractional colonization of *R. irregularis* in hemp roots (variety USO 31) was reported from a greenhouse experiment, resulting in improved seedling quality (Kakabouki et al., 2021).

4 | CONCLUSIONS

Our research demonstrated the wide variability in canopy cover, plant height, biomass yield, and fiber yield among the four industrial hemp varieties evaluated for over 2 years in the Northeastern United States. Han NE consistently exhibited the best growth, reaching the highest canopy cover and plant height across both years, with MS 77 following closely behind. The observed improvements in hemp production parameters in 2023 suggest that the weather conditions that year were more favorable compared to 2022. Although there were variations in plant height and yield, root colonization by AMF was consistent across all varieties, with a higher colonization in MS 77, and may be an important factor to improve nutrient absorption and stress tolerance. However, factors such as genetic traits and environmental conditions play critical roles in determining overall growth and yield. Based on our findings, we suggest Han NE and MS 77 varieties for fiber hemp cultivation in this region. Further research is necessary to understand the full impact of AMF colonization on hemp production to enhance biomass and fiber yield under varying nutrient conditions and how additional management practices can optimize yield in regenerative organic systems.

AUTHOR CONTRIBUTIONS

Dinesh Panday: Conceptualization; data curation; formal analysis; methodology; resources; software; validation; visualization; writing—original draft; writing—review and editing. **Wade P. Heller**: Methodology; resources; software; validation; writing—review and editing. **Joseph E. Carrara**: Data curation; validation; writing—review and editing. **Nikita Bhusal**: Data curation; resources; visualization; writing—review and editing. **Nicholas Omoding**: Formal analysis; writing—review and editing. **Tara Caton**: Writing—review and editing. **Ashley Walsh**: Project administration; resources; writing—review and editing. **Andrew Smith**: Project administration; writing—review and editing. **Arash Ghalehgolabbehbahani**: Conceptualization; data curation; funding acquisition; methodology; project administration; resources; supervision; validation; visualization; writing—review and editing.

ACKNOWLEDGMENTS

The authors extend their gratitude to the research technicians and interns at the Rodale Institute in Pennsylvania for their invaluable support in both field and laboratory activities. Their special thanks to Amiya Kalra, Tim Wilson, Dennis Altemose, and Lindsay McKeever for their exceptional contributions.

DISCLAIMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture (USDA). USDA is an equal opportunity employer.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this research are available on request from the corresponding author.

ORCID

Dinesh Panday https://orcid.org/0000-0001-8452-3797
Wade P. Heller https://orcid.org/0000-0002-5964-9715
Nikita Bhusal https://orcid.org/0000-0002-9840-8244
Nicholas Omoding https://orcid.org/0009-0004-2108-4132
Andrew Smith https://orcid.org/0000-0001-6812-1106
Arash Ghalehgolabbehbahani https://orcid.org/0000-0001-9410-7842

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How to cite this article: Panday, D., Heller, W. P., Carrara, J. E., Bhusal, N., Omoding, N., Caton, T., Walsh, A., Smith, A., & Ghalehgolabbehbahani, A. (2025). Performance and mycorrhizal colonization of industrial hemp varieties under regenerative organic systems in Northeastern region. *Agrosystems, Geosciences & Environment*, *8*, e70091. https://doi.org/10.1002/agg2.70091