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Effects of Carbon Amendments, Tillage and Cover Cropping on Arbuscular Mycorrhizal Fungi Association and Root Architecture in Corn and Cotton Crop Sequence

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Abstract: A field experiment was conducted to study the effects of carbon amendments, tillage, and cover cropping on arbuscular mycorrhizal fungi (AMF) association and root architecture at Farm Services at Texas A&M University. Three levels of carbon amendments at the rate of 500 kg C ha^{-1} (biochar, composted biosolid, and control (no carbon amendment)), two levels of tillage (conventional disking (CT) and no tillage (NT)), and two levels of cover crop (a mixture of oat, mustard, and pea (CC) and no cover crop (NCC)) were arranged in a split-split plot design with four replications. Over a two-year crop sequence of corn followed by cotton, AMF colonization of roots was 4.43% greater in biochar-treated soil than in the control treatment. Colonization in cotton was 5.17% and 6.09% greater under NT and CC treatments, respectively, compared to CT and NCC. Carbon amendments did not alter corn root length but did alter root angle at 20–30 cm. Carbon amendments did not affect root angle under CC. However, tillage did affect CC root length and angle. Root length and root angle were found to differ among the cover crop species. The results imply that farmers may combine certain practices to optimize and harness the benefits of AMF.

Keywords: carbon amendments; tillage; cover cropping; arbuscular mycorrhizal fungi; root length; root angle

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are known to form mutualistic relationships with host plant roots in almost eighty percent of plant species [1] and have shown substantial potential for increasing the productivity of most globally important agricultural crops [2]. The host plant provides carbohydrate to the AMF. In return, the host plant receives phosphorus (P) and other essential nutrients and water from the AMF [3,4]. Other general benefits of AMF include contributions to soil carbon sequestration and soil aggregate formation [4,5], soil erosion reduction [6], and increased plant tolerance to biotic and abiotic stresses [7,8]. Hence, AMF plays an important role in sustainable agriculture through improvements to soil fertility, soil quality, and crop performance.

The abundance of viable AMF for plant association in the soil is affected by nutrient supply, cropping systems, and soil disturbance [9–12]. Host plant/AMF mutualism is inhibited when certain nutrients are sufficiently supplied [13]. For example, greater P content in the soil limits the exudation of signal molecules that encourage hyphal branching and generally limits host plant initiation of association and transfer of photosynthate [14–16]. In the presence of increased organic matter, or following organic amendment, AMF colonization is often enhanced. In a field receiving animal manure over 14 years in Therwil (CH), AMF colonization in wheat, vetch-rye, and grass-clover was 30–60% greater compared to high fertilizer inputs [4]. In this experiment, the greatest increase in the association was found under no fertilization. In New South Wales (AU), wheat grown organically featured two to three times greater colonization than wheat grown under conventional practice [17]. Colonization was also greatest in this study with no addition of P fertilizer.

Biochar is a carbon (C)-rich material produced by pyrolysis [18] that has been shown to improve soil fertility and enhance the number, diversity, and activity of microbial communities, including AMF [19,20]. It has been argued that biochar serves as a favorable microhabitat for AMF [21–23], which can extend extraradical hyphae into the physical biochar matrix to improve P uptake [24]. Biochar also alters soil physical and chemical characteristics, resulting in improved nutrient acquisition and enhanced host/AMF mutualism [25]. Similarly, the use of biosolids as an organic amendment to fields is widely practiced [26]. Biosolid application increased AMF colonization on western wheatgrass by 33% [27]. Evidence suggests that biosolids alter AMF colonization [28]. Biochar is typically composed of more highly stabilized C compounds than biosolids.

Mechanical disturbances to the soil, such as tillage, damage the AMF propagule, which includes the spores and hyphae of the organism [29,30], leading to reduced AMF association with host plants compared with undisturbed soil. The establishment of AMF was observed to be more rapid in wheat and maize roots in NT soil relative to tilled soil [31,32]. Improved P acquisition has been suggested under NT practices relative to tilled soils [32] due to increased AMF symbiosis. However, it is unclear whether the change in nutrient/soil characteristics or AMF symbiosis improved the phosphorus uptake of the plants [33].

Cover crops (CCs) are included in crop rotational systems as a conservation management practice that reduces the fallow period and covers the soil during winter. Grasses/grains, legumes, and brassica species are common CCs planted to increase soil fertility and other benefits for subsequent crops [34]. Maintenance and enhancement of AMF colonization by CCs can improve P uptake in subsequent seasons [5,35]. Winter cover cropping is essential to maintain and enhance AMF inoculum in the soil and roots [36] and to enhance AMF/host plant interaction in subsequent warm season crops [37,38]. Arbuscular mycorrhizae infection has been found to be higher in winter wheat CC relative to fallow soil and resulted in better yields of maize and wheat in subsequent seasons [39]. *Brassicaceae* is one of the few plant families in which AMF colonization is not supported [40,41]. *Brassicaceae* contain anti-fungal chemicals named glucosinolates that inhibit host plant interaction [41].

Soil organic matter (SOM) has been associated with soil aggregation, soil bulk density, soil pore formation, and soil aeration [42,43]. Soil compaction itself has a significant influence on root architecture [44,45]. It has been revealed that greater bulk density decreases the length of seminal, lateral, and nodal roots [46]. When roots are not thick and root angles are not steep enough to penetrate compacted soil, roots are horizontally deflected. Plants such as lupin and triticale developed less steep root angles in compacted soil [47,48].

It is well-documented that AMF and root architecture are important for water and nutrient acquisition. Both AMF association and root architecture are significantly influenced by agricultural practices, such as tillage, fertilization, and crop rotation [49–52]. Very few studies have been conducted to examine the root architecture and AMF association interaction [53–55]. There is a knowledge gap on how combined sustainable agricultural practices affect root architecture and AMF colonization. Therefore, this study aimed to provide an improved understanding of how carbon amendment, no tillage, cover cropping and their interactions affect AMF association and root architecture. This study hypothesized that AMF would increase and root architecture would change with the carbon amendments, no tillage, and cover cropping treatments compared to treatments that did not include the same.

2. Materials and Methods

2.1. Site Description and Experimental Design

The study was conducted at the Texas A&M University Farm Services Facility, located near Snook, TX (30.541670, −96.417575), from March 2019 to March 2021. The soil at the field site is mapped by USDA NRCS soil survey staff [56] as a Weswood series (fine-silty, mixed, superactive, thermic Udifluventic Haplustept). Soil textural analysis found that it contained 22% sand, 37% silt, and 41% clay, consistent with the map designation.

The experiment covered a two-year crop sequence (corn–cover crop–cotton–cover crop). The corn, cover crop, cotton, and cover crop remained in the field during March 2019–August 2019, November 2019–March 2020, June 2020–October 2020, and November 2020–March 2021, respectively. The research was conducted under rainfed conditions for a two-year crop sequence. A combination of urea ammonium nitrate (32-0-0) and ammonium polyphosphate (11-37-0) and an equivalent of 112 kg nitrogen (N) and 50 kg P_2O_5 per hectare for corn and 84 kg N and 50 kg P_2O_5 per hectare for cotton were provided.

The field experimental design was a split-split plot design with four replications. The main effect was carbon amendments: biochar (Bc), composted biosolid (Cb), and control (C). The sub-effect was tillage: conventional disking (CT) and no tillage (NT). The sub-sub-effect was cover cropping: mix of oat, mustard, and winter pea (CC) and no cover crop (NCC). Each plot was 3 m × 5 m in size and totaled 48 plots.

The experimental field was divided into four blocks with two replications each of CT and NT. One block of NT and CT each received the cover crops (mix of oat, mustard, and winter pea), while the other did not. Cover crop mixes were planted at rates of 2.27 kg mustard, 13.61 oat, and 4.54 kg winter pea per acre using a seed drill. Carbon amendments were randomly applied within each block at the rate of 500 kg C ha^{-1}. The carbon was incorporated into the soil in CT blocks by disking 10 cm, while carbon was applied only to the soil surface in NT blocks. The biochar was a pyrolyzed soft wood from pulp waste (Green Texan Farms, Quinlan, TX, USA). The biosolid was composted municipal waste, which included municipal wastewater treatment sludge, food waste, and yard waste from Austin, TX, USA (Synagro, MD, USA). Nutrient content of composted biosolid and biochar are shown in Table 1.

Table 1. Nutrient content of composted biosolid and biochar.

Carbon Source	N g/kg	P g/kg	K g/kg	Ca g/kg	Mg g/kg	Na g/kg	Zn ppm	Fe ppm	Cu ppm	Mn ppm	S ppm	B ppm	%C
Biosolid	28.3	19.5	3.3	115.1	5.6	0.2	751	15,358	237	500	11,765	34	31.7
Biochar	3.1	7.4	53.7	91.5	94.1	1.5	293	6694	135	3061	5207	166	55.7

Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Sulfur (S), Boron (B), Carbon (C).

2.2. Root Sampling for Root Arbuscular Mycorrhizal Fungi

Corn, cover crop, and cotton root samples were taken before tasseling, before termination, and at the full bloom stage, respectively. Four random soil cores were taken from the middle row of each crop at the depths of 0–15 cm and 15–38 cm for root samples. Roots from each depth were collected, washed, and stored in 70% alcohol until AMF analysis was performed.

2.3. Quantification of Arbuscular Mycorrhizal Fungi

Fine roots were cleared (cell walls made transparent for microscopic examination) by boiling with 10% potassium hydroxide (KOH) solution for 13 min. The cleared roots were rinsed with tap water 4–5 times. The roots were stained with the ink-vinegar method [57]. The staining solution was prepared by diluting 5% ink in vinegar. Pelikan Blue ink (Herlitz PBS AG Company, Germany) was used. The cleaned roots were boiled at 95 °C for 3 min in the ink-vinegar solution. The roots were then rinsed with tap water 4–5 times. The roots were kept in water with a few drops of vinegar for 30 min. This technique is a non-toxic and highly effective method for the staining the AMF, as the fungal structures were clearly visible.

The stained roots were cut into lengths of 0.5 cm each. A random sub-sample of 10 stained root segments of each plot were observed microscopically under 10× magnification (1570022 microscope, Amscope Company, Irvine, CA, USA, also integrated with the 8 megapixels Amscope Microscope Digital Camera). The resulting AMF/root segment images were imported into the Amscope software installed onto a laptop PC. Each

AMF image was divided into ten equal segments. Randomly, 10 subsamples were taken for AMF analysis for each treatment. AMF structures, including hyphae, vesicles, and arbuscules, were counted on 100 grid lines and AMF was quantified using the grid line intersect method [58].

2.4. Measuring Root Architecture

Minirhizotrons with a diameter of 7.6 cm were constructed using polycarbonate tubes (McMaster Carr; Los Angeles, CA) and inserted 45 cm deep from the soil surface. Minirhizotrons were placed near and adjacent enough to plants (corn, cotton) to potentially capture the images of the rhizosphere. Minirhizotron tubes were vertically segmented into 0–10, 10–20, 20–30, and 30–40 cm depths by carefully placing thin strips of water-resistant tape at 10 cm intervals. Root images were captured at each depth using a 360° camera. The images were processed and analyzed with EZ Rhizo software to measure the root length (RL) and root angle (RA). The scale was established using the minirhizotron markings for 10 cm depth segments. Five random quadrants of 2 cm each were created in the software to subsample images from each depth for corn.

Cover crop roots were excavated, and roots were soaked in a 1 L plastic container with water for 30 min to loosen soil particles from the roots. The CC tops were supported by one hand while water-soaked roots were cleaned with a garden wash bottle. One each of the mustard, pea, and oat plants was taken from each plot. A ruler was placed next to the cover crop and images were taken and processed with the free and open-source software Image J (http://rsb.info.nih.gov/ij/, accessed on 28 June 2015) for the analysis of RL length and RA for the top 10 cm depth.

2.5. Statistical Analysis

Data were analyzed using PROC GLM in SAS software (SAS Software, SAS Institute, Cary, NC, USA). Data were sorted by depth and analyzed to determine the effect of carbon amendments, tillage, and their interaction on AMF root colonization, root length and, root angle for corn, cotton, and cover crop. Statistical differences were outlined at $\alpha = 0.05$. Fisher's LSD mean separation was used whenever significant differences ($p \leq 0.05$) were detected in the ANOVA. Root length and angle means were compared with Tukey's post hoc test at $\alpha \leq 0.05$.

3. Results

3.1. Arbuscular Mycorrhizal Fungi Colonization

AMF root colonization was significantly affected by carbon amendments for CCs in the year 2020, as well as cotton plants, at depths of 0–15 cm (Table 2). Arbuscular mycorrhizal fungi root colonization was found to be greater in biochar treatments compared to composted biosolids and control treatments, except for the CC 2021 sample at depths of 0–15 cm (Table 3). The AMF root colonization in biochar-amended treatments increased by 19 and 4% in CCs in the year 2020 and cotton roots, respectively, at depth of 0–15 cm. Similarly, AMF root colonization on biochar-treated soil was 6 and 16% greater for corn and CCs in the year 2020, respectively, at depths of 15–38 cm.

Table 2. Analysis of variance (ANOVA) results for arbuscular mycorrhizal fungi colonization percentage at depths of 0–15 and 15–38 cm as influenced by carbon amendments, tillage, and cover cropping at $\alpha = 0.05$.

| Source | Arbuscular Mycorrhizal Fungi Root Colonization Percentage | | | | | | | |
| | Corn | | CC 2020 | | Cotton | | CC 2021 | |
	0–15 cm	15–38 cm	0–15 cm	15–38 cm	0–15 cm	15–38 cm	0–15 cm	15–38 cm
C amendment	ns	0.0180	<0.0001	<0.0001	0.0214	ns	ns	ns
Tillage	ns	ns	0.0341	0.0003	0.0002	0.0085	0.0002	0.0085
Cover Crop	-	-	-	-	<0.0001	0.0036	-	-

Table 3. Treatment effects on arbuscular mycorrhizal fungi colonization percentage at depths of 0–15 and 15–38 cm for corn, cotton, and cover crops.

| Crop | Carbon Amendment | | | | | | Tillage | | | | Cover Crop | | | |
| | Biochar | | Biosolid | | Control | | CT | | NT | | CC | | NCC | |
	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
							0–15 cm							
Corn	47.25 [a]	5.31	44.63 [a]	5.39	40.88 [a]	4.73	42.25 [a]	4.69	46.25 [a]	5.91				
CC	66.75 [a]	4.2	56.13 [b]	4.70	48.00 [c]	2.93	55.25 [b]	9.29	58.67 [a]	8.16				
Cotton	44.06 [a]	6.61	41.19 [b]	5.66	39.63 [b]	5.25	39.04 [b]	4.54	44.21 [a]	6.31	44.67 [a]	5.78	38.58 [b]	4.65
CC 2021	36.5 [a]	13.67	35.5 [a]	10.59	39.63 [a]	6.69	45.83 [a]	6.16	28.58 [b]	5.04				
							15–38 cm							
Corn	50.88 [a]	5.06	44.88 [b]	3.09	44.75 [b]	4.89	45.75 [a]	5.07	47.92 [a]	5.21				
CC	51.25 [a]	3.99	44.00 [b]	3.46	35.13 [c]	6.38	40.25 [b]	7.68	46.67 [a]	7.56				
Cotton	32.19 [a]	4.56	31.94 [a]	4.71	32.75 [a]	5.29	30.54 [b]	4.15	34.04 [a]	4.79	34.25 [a]	4.61	30.33 [b]	4.16
CC 2021	30.5 [a]	10.35	27.50 [a]	9.69	27.38 [a]	8.39	36.83 [a]	4.69	20.08 [b]	1.56				

CC: cover crop, NCC: no cover crop, CT: conventional tillage, NT: no tillage; same letter indicates no significant difference among means by LSD comparison of means at α = 0.05. Letters are within carbon amendments and tillage. avg = mean and sd = standard deviation for the mean of treatment replicates.

Arbuscular mycorrhizal fungi root associations in CCs for both years and depths were affected by tillage (Table 3). No tillage resulted in greater AMF root colonization compared to CT in the year 2020. The NT treatments increased AMF root colonization in CCs in the year 2020 by about 3 and 6% at 0–15 and 15–38 cm, respectively. Tillage also had a significant effect on cotton AMF root association at depths of 0–15 and 15–38 cm. Table 4 represents AMF root colonization in the two-year crop sequence with carbon amendments and tillage treatments.

Table 4. Mean separation of arbuscular mycorrhizal fungi colonization percentage after the harvest of corn, cotton, and cover crops influenced by carbon amendments and tillage at depths of 0–15 and 15–38 cm.

| Crop | Biochar | | | | Biosolid | | | | Control | | | |
| | CT | | NT | | CT | | NT | | CT | | NT | |
	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
						0–15 cm						
Corn	45.00 [a]	3.83	49.50 [a]	6.14	42.75 [ab]	5.74	46.50 [a]	5.07	39.00 [b]	2.94	42.75 [a]	5.85
CC 2020	66.50 [a]	4.95	68.00 [a]	4.59	53.25 [b]	0.95	59.00 [b]	5.35	46.00 [c]	2.16	50.00 [c]	2.61
Cotton	40.88 [a]	4.09	47.25 [a]	7.32	39.25 [a]	4.59	43.13 [a]	6.24	37.00 [a]	4.62	42.25 [a]	4.68
CC 2021	48.75 [a]	4.99	24.25 [a]	3.30	43.75 [a]	8.46	27.25 [b]	2.98	45.00 [a]	4.97	35.00 [a]	3.30
						15–38 cm						
Corn	49.25 [a]	6.02	52.50 [a]	4.04	45.50 [ab]	4.04	44.25 [b]	2.21	42.50 [b]	3.32	47.00 [ab]	5.59
CC 2020	48.50 [a]	2.89	54.00 [a]	2.94	41.00 [b]	0.81	47.00 [a]	1.83	31.25 [c]	2.75	39.00 [b]	6.86
Cotton	30.75 [a]	4.71	33.63 [a]	4.21	30.25 [a]	4.43	33.63 [a]	4.63	30.63 [a]	3.81	34.88 [a]	5.91
CC 2021	39.75 [a]	4.34	21.25 [a]	1.71	36.00 [a]	4.96	19.00 [a]	1.42	34.75 [a]	4.35	20.00 [a]	0.82

CC: cover crop, CT: conventional tillage, NT: no tillage; same letter indicates no significant difference among means by LSD comparison of means at α = 0.05. Letters are within carbon amendments and tillage. avg = mean and sd = standard deviation for the mean of treatment replicates.

Cotton was planted after the harvest of CCs in the year 2020. Significant differences were observed in AMF root associations between plots receiving CC treatment and plots not receiving CC treatment (Table 2). AMF root association for cotton was significantly greater under CC plots relative to NCC plots for both depths 0–15 and 15–38 cm. The AMF

root colonization was increased by 6 and 3.92% by cover cropping for cotton at depths of 0–15 and 15–38 cm, respectively.

3.2. Root Architecture

Corn root architecture was observed to 40 cm below the soil surface. No significant differences in corn root length were observed for either depth (Table 5). However, numerically greater root length was seen in biochar-treated soil compared to composted biosolid and the control treatment (Table 6). Carbon amendments significantly affected the root angle only at depths of 20–30 cm. Likewise, tillage did not affect the corn root length or angle. Average corn root angles for biochar-treated soil at depths of 20–30 cm were 104.51 and 122.67 degrees, respectively, for CT and NT plots.

Table 5. ANOVA *p* values for the effects of carbon amendments, tillage, and their interactions for corn root length (cm) and root angle (°) at 0–10, 10–20, 20–30, and 30–40 cm depths.

Source	df	0–10 cm		10–20 cm		20–30 cm		30–40 cm	
		RL	RA	RL	RA	RL	RA	RL	RA
C amendment	2	ns	ns	ns	ns	ns	0.0012	ns	ns
Tillage	1	ns	ns	ns	ns	ns	ns	ns	ns
C amendment * tillage	2	ns	ns	ns	ns	ns	0.0109	ns	ns

C amendment: carbon amendment, RL: root length, RA: root angle.

Table 6. Mean separation of root length (cm) and root angle (°) for corn with carbon amendments and tillage at 0–10, 10–20, 20–30, and 30–40 cm depths.

Depth (cm)	Root Character	Biochar				Biosolid				Control			
		CT		NT		CT		NT		CT		NT	
		Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
0–10	RL	13.56	2.31	10.28	2.8	10.46	5.76	8.05	2.031	7.57	0.83	9.51	3.99
	RA	71.41	10.18	102.03	6.89	56.11	7.31	56.01	10	89.72	6.18	84.32	30.11
10–20	RL	12.27	1.81	9.14	3.38	10.34	2.46	8.08	0.69	7.8	0.24	8.73	2.43
	RA	92.16	55.49	74.8	5.61	73.12	16.21	90.12	9.59	72.91	4.29	80.21	25.51
20–30	RL	12.23	1.68	10.09	1.24	10.55	0.21	11.95	1.59	7.59	0.2	9.05	4.48
	RA	104.51	8.62	122.67	15.62	93.55	2.01	52.24	12.83	81.37	2.72	68.87	3.06
30–40	RL	13.37	1.14	8.53	2.84	11.43	2.29	10.94	1.04	8.95	0.39	9.4	2.64
	RA	77.68	13.99	117.36	9.49	78.74	0.94	72.25	1.09	72.41	5.41	93.07	9.98

RL: root length, RA: root angle, CT: conventional tillage, NT: no tillage, avg = mean and sd = standard deviation for the mean of treatment replicates.

Carbon amendments did not show any significant differences for either RL or RA for CCs in the years 2020 and 2021 (Table 7). However, tillage influenced RL for the CC 2020 sample in mustard, oat, and pea. Average RLs for mustard, oat, and pea for CT were 20.53, 57.53, and 75.09 cm, respectively, and for NT, 26.50, 62.88, and 56.01 cm, respectively (Table 8). Furthermore, tillage affected the RA of mustard only. The average RAs for mustard for CT and NT were 53.31 and 65.59°, respectively. Clear differences in root length were observed for CC species. The average root length was greater for pea compared to oat and mustard. Greater root length was also measured for mustard and oat under CT in the year 2020. No interaction effect was seen for RL and RA in the year 2020.

Table 7. ANOVA *p* value for the effects of carbon amendments, tillage, and their interactions on root length (cm) and root angle (°) of mustard, oat, and pea in the years 2020 and 2021.

Year	Source	Df	Mustard		Oat		Pea	
			RL	RA	RL	RA	RL	RA
CC 2020	C amendment	2	ns	ns	ns	ns	ns	ns
	Tillage	1	0.0083	0.0024	0.0008	ns	0.0498	ns
	C amendment * tillage	2	ns	ns	ns	ns	ns	ns
CC 2021	C amendment	2	ns	ns	ns	ns	ns	ns
	Tillage	1	ns	ns	ns	ns	ns	ns
	C amendment * tillage	2	ns	ns	ns	0.0259	ns	ns

C amendment: carbon amendment, RL: root length, RA: root angle.

Table 8. Mean root length (cm) and angle (°) analysis results for mustard, oat, and pea with carbon amendments and tillage at depths of 0–10 cm for the years 2020 and 2021.

Crop	Root Character	Biochar				Biosolid				Control			
		CT		NT		CT		NT		CT		NT	
		Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd	Avg	Sd
						Year 2020							
Mustard	RL	19.51	7.93	24.83	2.85	22.46	5.45	24.04	4.07	19.62	3.54	30.64	6.09
	RA	54.4	12.93	63.49	2.85	55.19	5.45	67.49	4.09	50.33	13.45	66.09	6.09
Oat	RL	59.51	4.3	63.96	8.16	59.38	5.05	62.82	14.09	53.69	7.16	61.87	8.99
	RA	57.29	3.36	68.13	7.52	53.21	3.02	46.49	10.33	50.07	4.54	55.82	7.26
Pea	RL	65.35	22.64	67.4	13.21	80.42	16.26	61.74	8.41	79.49	27.19	51.87	3.1
	RA	55.8	5.93	54.01	4.9	64.03	5.34	56.64	4.53	60.55	5.03	57.39	5.55
						Year 2021							
Mustard	RL	24.36	7.92	25.83	8.58	22.28	5.8	26.54	3.12	32.04	8.28	25.18	10.45
	RA	57.79	7.92	57.28	8.58	55.4	5.8	62.71	3.12	56.73	8.28	53.73	10.45
Oat	RL	63.82	8.16	53.55	7.52	62.82	14.09	62.82	14.09	61.87	8.99	62.81	10.06
	RA	60.34	7.52	55.74	6.29	55.45	10.33	46.49	10.33	48.84	7.26	59.84	6.94
Pea	RL	67.4	13.21	59.9	6.6	61.74	8.41	59.99	7.18	51.57	3.1	50.12	10.61
	RA	54.01	4.9	54.01	4.9	56.64	4.53	55.89	5.21	57.39	5.55	57.39	7.34

RL: root length; RA: root angle, CT: conventional tillage, NT: no tillage avg = mean and sd = standard deviation for the mean of treatment replicates.

Minirhizotrons were also inserted next to cotton plant rows but no cotton root was visible. Therefore, no results for the RL and RA of cotton are available to present.

4. Discussion

Several studies have indicated that AMF root colonization could be significantly increased by applying biochar to soil [21,23,59,60]. Our study found that the biochar can increase AMF percentage in corn by 6% and by 20.5% in CCs, while IIA [61] observed that AMF colonization percentage can decrease by 27% with the addition of biochar at a rate of 10 g biochar kg^{-1} of soil in corn. A previous study observed that AMF colonization decreased by 48% and 73% when biochar was applied at rates of 2 and 4% *w/w* basis [62]. Moreover, Warnock [19] proposed four mechanisms to explain AMF colonization with biochar. (i) Alternation of soil physio-chemical properties: biochar addition increases the bioavailability of P and other metal ions. It also alters soil pH, increases cation exchange capacity, and decreases bulk density. (ii) Alteration of soil micro-organisms: biochar alters mycorrhization helper bacteria and phosphate solubilizing bacteria, which have benefits for

AMF. (iii) Alteration of plant–fungus signaling processes: biochar inhibits AMF colonization by adsorbing signaling compounds or adsorbing compounds toxic to AMF. (iv) Serving as a refuge for AMF colonization: hyphae of AMF colonized in biochar may be protected against soil predators, such as protozoans, nematodes, mites, etc. Biochar effects were stronger in the first year after application than in the second year.

Our study found that AMF root colonization was greater in NT relative to CT. Oehl and Koch [63] proposed that mechanical disturbances in soil rupture the extra-radical mycelium and, thereby, reduce the viability of potential propagules. Similarly, Mozafar et al. [32] observed greater and more rapid AMF colonization in NT soil compared to CT.

Several studies have reported the positive effects of different CC species on AMF root colonization in subsequent crops, such as corn [37,38]. Cover crops also have been shown to increase the abundance of spores available to colonize the subsequent crop [18]. This study also found greater AMF colonization in cotton following CCs. A meta-analysis was conducted across five continents to understand tillage and cover crop effects on AMF and found that reduced tillage and winter CCs increased the AMF colonization in subsequent summer crops by 30% [64].

Root development and distribution in the soil profile play a vital role in crop growth and yield through the uptake of water and nutrients [65]. Previous researchers have found that crop roots were more densely distributed in topsoil [66,67]. This could be due to higher plant nutrient availability in topsoils and greater soil resistance in deeper soil horizons. This study analyzed total root length at top 10 cm depths and failed to find differences in either RL or RA with the addition of carbon amendments in corn. In contrast, Liu et al. [68] found that RL was significantly increased by 46.1% relative to control treatments in corn when biochar was applied at 20 Mg ha^{-1}. They spread the biochar on the soil surface and then mixed it with the top 15 cm of soil by hand. The amount of biochar applied was greater compared to our experiment and organic compounds of biochar might have stimulated root growth in biochar applied soil. Similarly, other researchers observed comparatively longer and bigger roots in biochar-amended soil in the Loess Plateau [69,70]. The longer roots facilitate more nutrient and water uptake from deeper in the soil.

The current study did not find any significant effect of tillage on either root length or angle in corn (2020) but did find a significant effect on CCs. Previous studies have also found that average corn root length was not affected by tillage practices [71]. This study found that significantly greater root lengths were observed under NT systems for mustard and oat in 2020. In contrast, significantly higher root lengths were observed under CT for pea. Similarly, a greater root angle was observed for pea under NT practices than CT practices. More fine and taller roots were observed in corn plants under NT than CT [72]. The authors also found that the NT system resulted in 5.5 and 4.8% more roots with diameters of 0.1–0.2 mm size at depths of 0–5 and 5–10 cm, respectively, compared to CT. This could have been due to higher organic carbon in NT improving the soil structure and facilitating plant root growth under NT. In addition, higher microbial activity under the NT system would improve plant nutrient uptake, thereby increasing the plant roots. Moreover, higher soil water content is often observed under NT compared with CT, indicating that different availabilities of water could influence plant roots [73]. Research has shown that increased bulk density under the NT system could impact the RL and RA of crops [74]. Significant differences in RA between CT and NT were reported, and the average root angles for the wheat crop grown under CT and NT were 106.8° and 102.8°, respectively [75]. In this study, corn root angles under CT and NT were 93.14° and 81.26°, respectively, at 20–30 cm. Wide variation in RAs was observed in the current study. Previous researchers have documented that shallow root angles increase the acquisition of soil P in crops such as maize and bean [76,77]. Availability of P content affects AMF colonization in plant roots. In contrast, steep root angles in plants such as rice, wheat, and bean enhance water acquisition and subsoil exploration [78,79].

5. Conclusions

This study showed that carbon amendments, NT, and cover cropping positively affected AMF colonization. Native AMF colonization was observed under field conditions without the addition of commercial strains. Biochar-treated soil promoted greater AMF colonization throughout the two-year crop sequence. AMF colonization was found to be higher after CCs than with NCC as CCs act as a habitat for AMF. This study highlights the importance of including NT and CCs in agronomic practices as they favor AMF colonization in the system. Farmers could take advantage of AMF benefits, including improvements to soil structure and nutrient acquisition, through the adoption of NT and CCs. Cotton root systems were far less prolific than corn or CC root systems. Additionally, AMF colonization was lower in the cotton plant compared to the corn plant. Instead of minirhizotron, another technique capable of getting closer to the root system of cotton is required to study the cotton root architecture.

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