Differences in Soil Microbial Communities in Dryland Forage Production Systems in Semi-arid Texas High Plains

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ABSTRACT

The declining water supply for irrigation in the semi-arid Texas High Plains is encouraging some growers to transition their continuous cotton (*Gossypium hirsutum* L.) land to dryland production of forages for grazing cattle and hay. These changes can modify soil water dynamics, which might affect soil microbial communities. The objective of this study was to determine changes in soil microbial communities following the transition from cotton to dryland annual forages at 0–5 cm and 5–15 cm over time. Soil water content was greater ($P \leq 0.05$) in fall 2018 than in fall 2016 at both depths. Soil microbial biomass carbon (MBC) was greater ($P < 0.001$) at 0–5 cm, but not significant ($P = 0.10$) at 5–15 cm in fall 2016 (518 mg kg^{-1} at 0–5 cm and 311 mg kg^{-1} at 5-15 cm) compared to fall 2018 (301 mg kg^{-1} at 0-5 cm and 233 **mg kg^{-1} at 5-15 cm). In contrast, fall 2018 had greater soil microbial biomass nitrogen (MBN) than fall 2016 at both depths, but not significantly ($P \geq 0.15$). Soil microbial community structure showed no sampling-time effect on total fatty acid methyl esters (FAMEs) nor on total fungal and bacterial populations at both depths. Soil microbial communities were generally at least maintained in dryland annual forage production systems even after several years of transitioning from irrigated cotton to annual dryland forages.**

KEY WORDS: Annual forages, conventional cotton, cover crop, soil microbial biomass, soil microbial community, pasture soil health

INTRODUCTION

Some growers in the Texas High Plains are converting irrigated cotton (*Gossypium hirsutum* L.) cropland to dryland production of annual forage crops because of a decline in groundwater supply for irrigation from the Ogallala Aquifer. Changes in land use and management may significantly alter soil water dynamics, which would have strong effects on microbial community size, composition, and activities (Suseela et al. 2012; Bhandari, West, et al. 2020). The typical practice of continuous cotton culture with conventional tillage in this region adds less organic inputs to the soil when compared to rotation with forage crops with no tillage (Acosta-Martínez et al. 2004; Allen et al. 2008). The soil in the Texas High Plains has low soil organic matter (SOM), and continuous cultivation of cotton with intensive tillage and low residue return further decreases SOM (Cotton et al. 2013). The size, composition, and activities of soil microbial communities are relevant in assessing potential soil quality enhancement (Acosta-Martinez, Bell, et al.

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2010) as soil microbial communities are the primary source of soil enzymes which mediate soil organic matter transformation (Tabatabai 1994). Fungi constitute a major proportion of the soil microbial biomass and support ecological sustainability due to their key roles in C sequestration, organic matter formation, aggregate stability, and nutrient cycling. Thus, such changes in microbial community size, including fluctuations in bacterial and fungal populations, can significantly impact organic matter transformations and soil water relations (Cano et al. 2018). Previous studies have reported that soil microbial biomass and enzyme activities involved in N, C, S, and P cycling were enhanced by changing cultivated cropland to perennial grassland in association with reduced fluctuations in soil water content in grassland (Acosta-Martínez et al. 2004; Karlen et al. 1999; Potter et al. 1999).

Integrating perennial pasture grasses into crop-livestock systems protects against soil-surface erosion (Troeh et al. 2004) and conserves soil nutrients through recycling and decomposition of plant residues (Patra et al. 2005). Perennial grass pastures had greater soil microbial biomass carbon (MBC) and fungal abundances than continuous cotton (Acosta-Martinez et al. 2004, 2010b; Fultz et al. 2013) on a Texas High Plains site. In a recent study at the same site, integrating the perennial grass 'WW-B.Dahl' Old World bluestem [*Bothriochloa bladhii* (Retz) S.T. Blake] into a forage-livestock system improved soil health indicators including microbial communities (Bhandari, West, et al. 2020; Bhandari, West, Acosta-Martinez, et al. 2018). Building soil organic C (SOC) is of increasing importance in the semi-arid Texas High Plains because it improves the water holding capacity of soil (Cano et al. 2018); however, the slowness of SOC changes against a large background obscures effects of management on soil C storage. Soil MBC can provide information about short-term changes in microbial community structure and function as affected by management practices. Additional measurements that are relatively sensitive in the short term to changes in soil health include profiling of fatty acid methyl ester (FAME) and specific markers of bacteria and fungi.

Little is known about the changes in soil microbial communities after many years of transitioning from irrigated cotton production to dryland annual forage. Annual forages accommodate producers' flexible decision-making on diversification into livestock production by providing quick forage productivity on part of the cropland while slow-growing perennial forages become established. Annual forages are also beneficial as grazed cover crops to recoup their costs of establishment. Teff [*Eragrostis tef* (Zucc.) Trotter] is a relatively drought-tolerant, warm-season annual forage grass that may be an alternative to traditional summer forages that require more water (Saylor 2015). This grass has potential for forage production in the Texas High Plains thanks to its low input requirements and ease of establishment (Baxter et al. 2017). Including rye (*Secale cereale* L.) as a cool-season cover crop has enhanced plant-available water supply, rooting depth, and yield in cotton (Marshall et al. 2016). Acosta-Martinez, Bell, et al. (2010) found that cotton fields rotated with rye had greater soil microbial biomass N (MBN) and enzyme activities compared to cotton with forage sorghum [*Sorghum bicolor* (L.) Moench], indicating benefits of rye for promoting soil organic matter and nutrient cycling. The objective of this research was to monitor changes in soil microbial community size and structure over a recent time period at 13–15 years after transition from irrigated cotton to dryland production of annual forages in the Texas High Plains.

MATERIALS AND METHODS

Description of Study Site. Research was conducted at the New Deal Research Farm of Texas Tech University located in northeast Lubbock County (33°45' N, 101°47' W; 993 m elevation) near Lubbock, TX. The soil is almost level (0–1% slope) and classified as Pullman clay-loam (fine, mixed, thermic Torrertic Paleustolls) with 38% clay, 28% silt, and 34% sand. The soil contained a 60 to 120 cm deep root-restricting caliche (calcium carbonate) layer. The site was managed for long-term continuous, furrow-irrigated cotton before 2003. Subsequently, the site was subdivided into experimental fields for inclusion in a series of grazing trials (cropping and fertilizer history provided in Table 1). The forage crops planted after 2003 were not irrigated. Forage sorghum was grown in 2004. In 2005–2010, dryland cotton and foxtail millet (*Setaria italica* L.) were rotated annually except that forage sorghum replaced foxtail millet in 2008. Rye was no-till planted in the fall of 2012 as a winter cover crop. The summer-annual forage grass teff was no-till planted from 2013–2016. Rye was again no-till planted in the fall of 2016. Cattle grazed during the summers of 2014–2016 as part of a grazing trial (Baxter et al. 2017; Bhandari, West, Longing, et al. 2018), but not during fall 2016–2018. There were three replicate fields of annual forages, within which soil was sampled.

Table 1. Management history of the pastures sampled for soil microbial community size and structure.

Year of activity	Crop planted	Other management practices
Prior to 2003	Continuous cotton with furrow irrigation	Not available
2004	Forage sorghum	Not available
2005–2007	Cotton-foxtail millet rotation	Not available
2008	Forage sorghum	Not available
2009–2010	Cotton-foxtail millet rotation	Not available
2011	No crop due to drought	Not available
2012	Rye planted in fall	No fertilizer; no grazing
2013	Teff planted in spring	No fertilizers; no grazing; teff harvested for hay
2014	Teff planted in spring	No fertilizers; cattle grazed; teff harvested for hay
2015	Teff planted in spring	65 kg N/ha applied; cattle grazed; teff harvested for hay
2016	Teff planted in spring; rye planted in fall	15 kg N/ha, 34 kg P/ha, 7 kg K/ha, 9 kg S/ha applied; no grazing
2017	Pearl millet planted in summer; fallow in fall	No fertilizers; no cattle grazing
2018	Teff planted in spring; fallow in fall	No fertilizers; no cattle grazing

Soil Sampling. Soil samples were collected at 0–5 and 5–15 cm in late fall (Dec 6) of 2016 and again in fall (Oct 29) of 2018 to compare the effect of time (sampling date) on soil microbial communities. In 2016, samples were taken after rye was planted. In 2018, samples were collected when fields were fallow, and the field was partly covered by weeds. Soil samples were collected from three field replications. Five samples were collected from

0–5 cm and 5–15 cm depths from each of three replications. At least 30 m distance was maintained between adjacent soil samples. All samples from each depth within a field were thoroughly mixed, and a single composite sample was made per depth and per date for a total of 12 samples. All composite samples were immediately transferred into air-tight storage bags, which were stored in an iced cooler in the field and transported to the laboratory on the same day. Samples were passed through a 4.75 mm sieve within 48 h and stored at 4 °C until further analyses.

Determination of soil microbial community size. Determination of soil MBC and MBN was performed according to a chloroform-fumigation extraction method on 15 g of field-moist soil samples as described by Vance et al. (1987) and Brookes et al. (1985), respectively. In brief, organic C and N were extracted from fumigated (24 h) and non-fumigated (control) soil samples using 0.5 M K_2SO_4, and then extracted organic C and N were quantified with a CN analyzer (Shimadzu Model TOC-V/CPH–TN, Shimadzu Corp., Kyoto, Japan). The values obtained from non-fumigated samples were subtracted from those obtained from fumigated samples. The calculation of MBC and MBN was done using a k_{EC} factor of 0.45 (Wu et al. 1990) and k_{EN} factor of 0.54 (Jenkinson 1988), respectively. Duplicate analyses were performed for each soil sample, and results are expressed on a dry-weight basis. Soil samples were dried at 105 °C for 48 h to determine soil gravimetric water content.

Soil microbial community structure. The ester-linked fatty acid methyl esters (EL-FAMEs) method as described by Schutter and Dick (2000) was used to analyze soil microbial community structure. In summary, three steps of methylation, neutralization, and extraction were used. In methylation, 3.5 g field-moist equivalent soil was added to each test tube (20 x 150-mm Teflon-lined screw-cap), followed by 15 mL of 0.2 M KOH in methanol. Tubes were heated in a 37 °C water bath for 60 min during which each tube was vortexed for 5 s every 15 min (four times total) and then cooled to ambient temperature for about 5 min. During neutralization, 3 mL of 1.0 M acetic acid was added to each test tube, and then vortexed for 5 s. During extraction, 3 mL of 100% hexane was added to each tube, inverted five times by hand, and then centrifuged at 2200 rpm for 8 min. The top organic phase was transferred to test tubes (13 x 100 mm Teflon-lined, screw-cap), dried by heating at 37 °C for 20 min, followed by addition of 100 µL of standard hexane. After transferring to vials, FAME fractions were analyzed on a 6890 GC series II gas chromatograph (Hewlett Packard, Wilmington, DE, USA). The FAME marker 16:1ω5c was used to describe arbuscular mycorrhizal fungi (AMF), and the FAME markers 18:3ω6c, 18:1ω9c, 18:2ω6c, 18:1ω7c, 18:1ω6c, 18:4ω3c, and *i*18:0 were used to describe saprophytic fungi (Frostegard and Baath 1996; Olsson et al. 1995; Zelles 1997). Gram positive (G+) bacteria were indicated by using markers *a*14:0, *i*14:0, *a*15:0, *i*15:0, *a*16:0, *i*16:0, *a*17:0, *i*17:0, and *i*19:0; and Gram negative (G−) bacteria were described by cy17:0, *i*17:0 3OH, cy19:0, and cy19:0ω7c (Zelles 1999). The FAME markers 10Me 16:0, 10Me 17:0, 10Me 18:0, 10Me 17:1ω7c, 10Me 18:1ω7c, and 10Me 19:1ω7c were used to indicate actinomycetes. Fungal and bacterial markers were added to calculate the total FAMEs.

Statistical Analyses. A randomized complete block layout was used in analysis of variance to analyze the data with sampling times as repeated measure and depth as a strip plot within sampling time. Proc Mixed in SAS 9.4 (Littell et al. 2006) was used to analyze the data in which replicate was set as a random effect. Means were compared using LSMEANS

procedure. Differences between sampling times in each sampling depth were considered significant at $P \leq 0.05$. Pearson correlation coefficients between soil water content and major soil microbial variables at two sampling depths were calculated (n = 12) to explore relationships between soil water content and soil variables.

RESULTS

Environmental parameters and soil microbial biomass C and N. Minimum and maximum temperatures on the sampling date (Dec 6) in 2016 were –1.3 °C and 15.5 °C, respectively. For the previous week of sampling date in 2016, mean minimum and maximum temperatures were –1.0 °C and 12.3 °C (overall mean 4.7 °C). Similarly, 6.4 °C and 22.1 °C were the minimum and maximum temperatures on the sampling date (Oct 29) in 2018. Weekly minimum and maximum temperatures for the previous week of sampling date in 2018 were 5.9 °C and 20.3 °C (overall mean 12.3 °C). Total rainfall was 16 mm in November in 2016. On Dec. 2, 2016, a rain event of 5 mm occurred. In October 2018, total rainfall was 112 mm. Rain events of 22 mm and 12 mm occurred on Oct 19 and Oct 25 of 2018, respectively, before the 2018 sampling date. The total amount of rain in the six months before soil sampling in 2016 was 199 mm, and 330 mm in the six months before sampling in 2018. The soil water content was greater ($P \leq 0.04$) in fall 2018 than in fall 2016 at both 0–5 cm and 5–15 cm depths (Table 2). In fall 2016, soil samples were taken after rye was planted, which may have caused some soil water loss due to disturbances from the planting operation, although such disturbances would have been minimal because of the absence of tillage. In fall 2018, soil samples were collected from the fallow field. The greater soil water content in fall 2018 than in fall 2016 was at least partially explained by higher amounts of rainfall during the fall, and the field was fallow for about five months in each fall of 2017 and 2018. Soil MBC was greater ($P < 0.001$) in fall 2016 than fall 2018 at 0–5 cm depth (Table 2). At 5–15 cm, fall 2016 had greater ($P = 0.10$) MBC than fall 2018, but not significantly. Soil MBN was greater in fall 2018 than fall 2016, but significantly only at 5–15 cm depth (Table 2).

Table 2. Changes in gravimetric soil water content and soil microbial biomass C and N during two years of annual forage production systems (n =3).

Sampling time	Soil water	MBC†	MBN†
	g g^{-1}	- - - - -- - - - - mg kg^{-1} - - - - -	
		0–5 cm	
Fall 2016	0.132 b	518 a	26.8
Fall 2018	0.152 a	301 b	36.1
Time effect	$P = 0.052$	$P < 0.001$	$P = 0.19$
		5–15 cm	
Fall 2016	0.102 b	311	12.5
Fall 2018	0.174 a	233	23.0
Time effect	$P < 0.001$	$P = 0.10$	$P = 0.15$

† MBC= microbial biomass carbon; MBN = microbial biomass nitrogen
SOC = soil organic carbon

Soil microbial community structure. Soil microbial community structure, according to EL-FAMEs analysis, demonstrated that total FAMEs were not significantly different between fall 2016 and fall 2018, but were numerically greater in fall 2016 than fall 2018 at 0–5 cm and numerically greater in fall 2018 than fall 2016 at 5–15 cm (Table 3). Similar trends were found for total fungal and bacterial populations. Among the Gram– markers, *i*17:0 3OH and cy19:0 were detected only in 2016, whereas marker cy19:0ω7c was detected only in 2018 (data not shown). Some additional saprophytic fungal markers (18:3ω6c, 18:1ω7c, 18:1ω6c, 18:4ω3c, and *i*18:0) were detected only in fall 2018 (data not shown). Similarly, Gram+ bacterial markers *a*14:0, *i*14:0, *a*16:0, *i*16:0, and *i*19:0, Gram–marker cy19:0ω7c, and actinomycete markers 10Me 17:1ω7c, 10Me 18:1ω7c, and 10Me 18:1ω7c were detected only in 2018 (data not shown). Although not significant, the fungi:bacteria ratio was greater in 2016 than in 2018 at the 0–5 cm depth. Lower fungi relative to bacteria in fall 2018 may be due to lower residue return during the fallow period in fall 2018. In contrast, the ratio was greater in fall 2018 than in fall 2016 at 5–15 cm. In all cases, the fungi:bacteria ratio was less than 1.0, indicating that total bacterial populations exceeded total fungal populations. Soil water content was not significantly positively correlated with microbial biomass and other microbial groups (data not shown).

Table 3. Changes in total fatty acid methyl esters (FAMEs), fungal and bacterial groups, and their ratio during two years of dryland production of annual forage grasses (n =3).

Sampling time	Total FAMEs	AMF Fungi	Saprophytic fungi	Total fungi	Gram+ bacteria	Gram– bacteria	Actino-bacteria	Total bacteria	Fungi: bacteria
	nmol g^{-1}								
					0–5 cm				
Fall 2016	322	4.9	65.1	69.9	37.9	10.1	23.2	71.2	0.95
Fall 2018	236	3.7	53.9	57.6	38.0	7.1	20.6	65.7	0.87
Time effect	*P* = 0.28	*P* = 0.22	*P* = 0.53	*P* = 0.51	*P* = 0.99	*P* = 0.32	*P* = 0.49	*P* = 0.69	*P* = 0.53
					5–15 cm				
Fall 2016	145	2.4	23.2	25.7	17.9	9.1	10.7	37.7	0.69
Fall 2018	152	2.7	32.5	35.1	24.6	5.4	15.5	45.5	0.75
Time effect	*P* = 0.93	*P* = 0.82	*P* = 0.60	*P* = 0.61	*P* = 0.45	*P* = 0.15	*P* = 0.21	*P* = 0.58	*P* = 0.63

Total fungi: AMF (16:1w5c), saprophytic fungi (18:3ω6c, 18:1ω9c, 18:2ω6c, 18:1ω7c, 18:1ω6c, 18:4ω3c, and *i*18:0); total bacteria: Gram+ (*a*14:0, *i*14:0, *a*15:0, *i*15:0, *a*16:0, *i*16:0, *a*17:0, *i*17:0, and *i*19:0), Gram– (cy17:0, *i*17:0 3OH, cy19:0, and cy19:0ω7c) and actinomycetes (10Me 16:0, 10Me 17:0, 10Me 18:0, 10Me 17:1ω7c, 10Me 18:1ω7c, and 10Me 19:1ω7c)

CONCLUSION AND DISCUSSION

This study provided a scenario of changes in soil microbial communities in a cropping system that involved the dryland production of annual forages for grazing cattle and hay. The decrease in soil MBC from fall 2016 to fall 2018 may be associated with the lesser residue returned during the fall fallow periods in 2017 and 2018. This decrease in soil MBC over 2 yr suggests a benefit to avoiding fallow. The numerical increase in soil MBN in fall 2018 indicated that N is released even during the fallow period. This study was challenged by the fact that it did not monitor changes in soil microbial community since the transition from continuous cotton from before 2003 to annual forages, rather, it was restricted to 2016 and 2018, a mere 2-yr change in soil microbial community. Therefore, there was no original baseline of soil measures to compare to. Also, the only treatment comparison was between two sampling dates that were 13 and 15 yr after the transition from cotton, which limited the scope of the study to the effect of sampling dates on soil microbial communities. While soil microbial communities decreased over 2 yr at the shallower depth, the increases in those communities at the deeper depth indicated a positive effect on soil health. Similarly, more-abundant soil microbial communities (particularly at deeper depth) from fallow fields compared to soil from a recently planted field with no tillage suggests that soil microbial communities can be enhanced or at least maintained in dryland forage production systems with no tillage in the Texas High Plains.

The greater soil water content in fall 2018 than in fall 2016 was a consequence of greater rainfall and a fallow period prior to soil sampling in fall 2018. Rye was planted prior to soil sampling in 2016, which may have caused some soil water loss due to the disk openers of the no-till planter. In contrast, the field was fallow in the fall before soil sampling, and sparse weeds were present in the field at the time of sampling in fall 2018. Teff was not grazed in 2013, and was grazed intermittently during the summers of 2014-2015, but not during 2016-2018. Teff was harvested for hay during 2013–2014, but not harvested during 2015-2018. These practices may have contributed to greater soil water content in fall 2018. Despite greater soil water content in fall 2018 at both sampling depths, lower MBC in fall 2018 (numerically lower at 5–15 cm depth) than in fall 2016 suggests that MBC may not be affected by soil water content. A previous study at the same soil site (Acosta-Martinez, Burow, et al. 2010) reported that low levels of MBC were associated with low amounts of crop residue returned to the soil, intensive tillage, and the exposure of soil to wind during fallow periods in the winter. Potential changes in soil quality and C sequestration are associated with increase in fungal:bacterial ratio because fungi can assimilate more C than bacteria (de Vries et and Bardgett 2012; Fierer et al. 2009). Increased MBC is associated with increased soil quality and C sequestration in agriculture systems focusing on minimizing fallow periods (Acosta-Martínez et al. 2004; Karlen et al. 1999; Moore et al. 2000). The lower MBC in fall 2018 than fall 2016 in the current study was probably due in part to less crop residue return and sparse surface cover during the fall fallow periods of 2017 and 2018 and lower fungal:bacterial ratio in fall 2018.

Our finding of lower MBC in fall 2018 than fall 2016 agrees with the finding by Acosta-Martinez, Bell, et al. (2010) in which MBC in a wheat (*Triticum aestivum* L.)–fallow–rye–cotton system was lower in summer 2007 than in summer 2004, despite greater soil moisture in summer 2007. Greater MBC and MBN at 0–5 cm than at 5–15 cm on both sampling dates in the current study are in line with previous findings in long-term perennial pastures (grasses growing with and without alfalfa (*Medicago sativa* L.) conducted at the same site in summer and fall of 2016 (Bhandari, West, Acosta-Martinez, et al. 2018) and

in wheat-fallow-rye-cotton systems conducted in fall 2002 and summer 2003 (Acosta-Martinez et al. 2004). The reason behind greater soil MBN (numerically greater at 0–5 cm) in fall 2018 than fall 2016 is not clear, but release of N during decomposition of organic matter in the fallow periods during fall 2017 and 2018 may have provided N-rich substrate in the soil, resulting in greater MBN in fall 2018. Acosta-Martinez, Burow, et al. (2010) reported higher MBN (but lower MBC) in irrigated, N-fertilized pasture than in ungrazed land in the conservation reserve program (CRP, planted to perennial grasses and receiving no inputs), indicating that higher MBN in pasture may be associated with the greater N availability.

Two trends related to soil microbial community structure were observed. Total FAMEs and total fungal and bacterial populations decreased from fall 2016 to fall 2018 at 0–5 cm, whereas those populations increased at 5–15 cm. The study by Acosta-Martinez, Bell, et al. (2010) conducted on rye–cotton–wheat–fallow found that total fungal and bacterial populations decreased from summer 2004 to fall 2006 at 0–5 cm. Those results agree with the current results of decreased fungal and bacterial populations from fall 2016 to fall 2018 at 0–5 cm. The contributing factors governing the increase in total FAMEs and total fungal and total bacterial populations from 2016 to 2018 (particularly at 5–15 cm) are not known. Presence of some additional saprophytic fungal markers (18:3ω6c, 18:1ω7c, 18:1ω6c, 18:4ω3c, and *i*18:0), Gram+ bacterial markers (*a*14:0, *i*14:0, *a*16:0, *i*16:0, and *i*19:0), and actinomycete markers (10Me 17:1ω7c, 10Me 18:1ω7c, and 10Me 19:1ω7c) only in fall 2018 but not in fall 2016 agrees with previous studies at the same site conducted on perennial pastures in fall 2016 (Bhandari, West, Acosta-Martinez, et al. 2018; Table 4) and in spring 2018 (Bhandari, West, et al. 2020; Table 3). The differences in markers are difficult to explain beyond being associated with differences in sampling time.

ACKNOWLEDGEMENT

Funding for this research was partially provided by the Southern Sustainable Agriculture Research and Education Program (SARE) (Project No. LS14-261) and USDA Project No. 2016-68007-25066, through the National Institute for Food and Agriculture's Agriculture and Food Research Initiative, Water for Agriculture Challenge Area. Project website: ogallalawater.org. We acknowledge Veronica Acosta-Martinez and Jon Cotton for their laboratory assistance. Technical assistance by Philip Brown and Paul Green is gratefully acknowledged.

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