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Chemistry and Microbial Functional Diversity Differences in Biofuel Crop and Grassland Soils in Multiple Geographies

Lidia S. Watrud

United States Environmental Protection Agency, watrud.lidia@epa.gov

Jay R. Reichman

United States Environmental Protection Agency

Michael A. Bollman

United States Environmental Protection Agency

Bonnie M. Smith

United States Environmental Protection Agency

E. Henry Lee

United States Environmental Protection Agency

See next page for additional authors

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Authors

Lidia S. Watrud, Jay R. Reichman, Michael A. Bollman, Bonnie M. Smith, E. Henry Lee, Julie D. Jastrow, Michael D. Casler, Harold P. Collins, Steven Fransen, Robert B. Mitchell, Vance N. Owens, Brent Bean, William L. Rooney, Donald D. Tyler, and George A. King

Chemistry and Microbial Functional Diversity Differences in Biofuel Crop and Grassland Soils in Multiple Geographies

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Abstract We obtained soil samples from geographically diverse switchgrass (*Panicum virgatum* L.) and sorghum (*Sorghum bicolor* L.) crop sites and from nearby reference grasslands and compared their edaphic properties, microbial gene diversity and abundance, and active microbial biomass content. We hypothesized that soils under switchgrass, a perennial, would be more similar to reference grassland soils than sorghum, an annual crop. Sorghum crop soils had significantly higher NO_3^- -N, NH_4^+ -N, SO_4^{2-} -S, and Cu levels than grassland soils. In contrast, few significant differences in soil chemistry were observed between switchgrass crop and

grassland soils. Active bacterial biomass was significantly lower in sorghum soils than switchgrass soils. Using GeoChip 4.0 functional gene arrays, we observed that microbial gene diversity was significantly lower in sorghum soils than grassland soils. Gene diversity at sorghum locations was negatively correlated with NO_3^- -N, NH_4^+ -N, and SO_4^{2-} -S in C and N cycling microbial gene categories. Microbial gene diversity at switchgrass sites varied among geographic locations, but crop and grassland sites tended to be similar. Microbial gene abundance did not differ between sorghum crop and grassland soils, but was generally lower in

L. S. Watrud (✉) · J. R. Reichman · M. A. Bollman · B. M. Smith · E. H. Lee
United States Environmental Protection Agency,
200 SW 35th Street,
Corvallis 97333, OR, USA
e-mail: watrud.lidia@epa.gov

J. D. Jastrow
Argonne National Laboratory Biosciences Division,
9700 South Cass Avenue,
Argonne 60439, IL, USA

M. D. Casler
United States Department of Agriculture, -ARS, US Dairy Forage
Research Center, 1925 Linden Drive,
Madison 53706, WI, USA

H. P. Collins
United States Department of Agriculture-ARS, Vegetable and
Forage Crops Research Unit, 24106 N. Bunn Road,
Prosser 99350, WA, USA

S. Fransen
Washington State University Irrigated Agriculture Research and
Extension Center, 24106 N. Bunn Road,
Prosser 99350, WA, USA

R. B. Mitchell
United States Department of Agriculture-ARS, University of
Nebraska, 35 Keim Hall,
Lincoln 68583, NE, USA

V. N. Owens
Plant Science Department, South Dakota State University,
1110 Rotunda Lane North, 244C SNP,
Brookings, Box 2140C, 57007, SD, USA

B. Bean
Texas Agrilife Research and Extension Center, Texas A & M
University, 6500 Amarillo Blvd. West,
Amarillo 79106, TX, USA

W. L. Rooney
Department of Soil and Crop Sciences, Texas A & M University,
Foundation Seed Bldg., 405 Turk Road,
College Station 77843, TX, USA

D. D. Tyler
West TN Research and Education Center, University Tennessee,
605 Airways Blvd,
Jackson 38301, TN, USA

G. A. King
Dynamac Corporation, 200 SW 35th Street,
Corvallis 97333, OR, USA

switchgrass crop soils compared to grassland soils. Our results suggest that switchgrass has fewer adverse impacts on microbial soil ecosystem services than cultivation of an annual biofuel crop such as sorghum. Multi-year, multi-disciplinary regional studies comparing these and additional annual and perennial biofuel crop and grassland soils are recommended to help define sustainable crop production and soil ecosystem service practices.

Keywords Switchgrass · Sorghum · GeoChip · Functional gene array · Soil ecosystem services

Introduction

Second-generation lignocellulosic biofuel sources such as dedicated biomass crops, forestry and agricultural wastes are of interest as renewable sources of energy [1–6]. They are also of interest to decrease our national dependence on foreign petrochemicals and to mitigate global climate change effects [7]. Since many of the proposed lignocellulosic biofuel species are non-food perennial grasses, economic savings are anticipated to result from the lower input costs for tillage, fertilizers, and other crop chemicals, as compared to annual food crops such as corn. The lower inputs for perennial grasses as compared to annual crops are anticipated to provide long-term ecological benefits relating to biodiversity of plants and soil microbes and their associated ecosystem services, including increased soil organic matter content, reduced soil erosion, and improved surface and groundwater quality [8–14]. The primary objectives of our study were to identify the chemical and biological characteristics of soils associated with cultivation of the biofuel crop candidates switchgrass (*Panicum virgatum* L.) and sorghum (*Sorghum bicolor* L.) in diverse geographic locations in the USA, and to compare them to nearby perennial grassland reference soils. Availability of such information will help inform land management decisions to select and maximize biofuel crop production practices and to sustain or enhance desired ecosystem services such as carbon sequestration, pollutant degradation, the functional and taxonomic diversity of beneficial soil biota, and availability of soil nutrients to plants.

Our multidisciplinary approach involved the use of microbiological, chemical, and molecular methods to analyze soil microbial community responses to land use change [15–22]. We chose to evaluate microbial functional gene diversity rather than taxonomic diversity since multiple microbial taxa often carry out the same ecological functions. We employed a combination of standard chemical and microscopy-based biological methods and a functional gene array (FGA) approach to identify potential soil chemical and

physical factors and microbial genes involved in key soil ecosystem services such as C utilization, N cycling, P cycling, and S utilization. The FGA approach we used [23] contrasts with high throughput rRNA sequencing approaches that can result in the identification of taxa [18, 24–26] and also with mRNA-enriched environmental functional gene microarrays that can be used to profile gene transcripts [27]; it allowed us to identify potential differences in functional diversity as well as relative abundance of microbial functional genes in regional crop soil and nearby non-crop perennial grassland soil samples. Differences in the abundance and diversity of functional genes were proposed to serve as a means of identifying potential indicators of key ecosystem services such as C and N cycling in soil that might be impacted by the growth of annual and perennial biofuel crops such as sorghum and switchgrass.

Our field survey design included sampling replicate fields or plots in diverse geographic growing areas for the two types of crops, as well as in nearby perennial generally non-cultivated grassland reference areas, that are referred to hereafter as “grasslands.” This was by intent a design distinct from studies in which sampling occurs within a given geographic locale or region on one type of soil, rather than in multiple geographic areas that may contain diverse types or series of soils. We proposed that if differences were similar between crop and grassland areas in multiple geographies and latitudes on multiple soil series, the findings would be of broader interest and potential applicability than observations that were unique to or based on a given locale or soil series. The major objectives of these studies were to test the hypotheses that (1) soil from a perennial crop field such as switchgrass would tend to be more similar to nearby grassland soils than would be soil from an annual crop such as sorghum and (2) that trend would be observed in multiple geographies and types of soils.

Materials and Methods

Collection of Soil Samples

Six sets of soil samples were taken from switchgrass fields and associated nearby grasslands in IL, WI, NE, SD, WA, and in the case of TN, from long-term pastures derived from historic oak-hickory forest (Tables 1 and 2). Three additional sets of soil samples were taken from sorghum fields and nearby grasslands in WA, the TX panhandle, and East TX. As shown in Table 2, agronomic practices such as tillage, irrigation, and the amounts and types of N fertilizer varied widely by crop and geographic location. At all locations, switchgrass had been grown as a perennial biofuel crop for the prior 2–8 years, while sorghum was grown in an annual rotation with various grain, legume, or other food, feed, or

Table 1 Site locations and soils

Code	Site type	City, state	Latitude	Longitude	Elevation (m)	Soil texture	Soil series
TX-Ac1	Sorghum	Bushland, TX	35.204883	-102.045098	1,164	Silty clay loam	Pantex
TX-Ac2	Sorghum	Wildorado, TX	35.146440	-102.202568	1,176	Clay loam	Pullman
TX-Ac3	Sorghum	Canyon, TX	35.027687	-101.936193	1,110	Clay loam	Pullman
TX-An1	Native	Bushland, TX	35.214075	-102.046675	1,157	Silty clay loam; clay loam	Pantex; Pullman
TX-An2	Native	Wildorado, TX	35.145782	-102.194188	1,176	Clay loam	Pullman
TX-An3	Native	Canyon, TX	35.012035	-101.936298	1,106	Clay loam	Pullman
TX-Cc1	Sorghum	College Station, TX	30.549433	-96.439083	69	Clay	Belk
TX-Cc2	Sorghum	College Station, TX	30.549483	-96.431817	68	Clay	Belk
TX-Cc3	Sorghum	College Station, TX	30.540367	-96.423250	67	Clay	Ships
TX-Cn1	Native	College Station, TX	30.533350	-96.420000	68	Clay	Belk
TX-Cn2	Native	College Station, TX	30.528483	-96.411650	66	Clay	Ships
TX-Cn3	Native	College Station, TX	30.553917	-96.428583	67	Clay	Ships
WA-Oc1	Sorghum	Othello, WA	46.799700	-119.041717	371	Silt loam	Shano
WA-Oc2	Sorghum	Othello, WA	46.789567	-119.039467	367	Silt loam	Shano
WA-Oc3	Sorghum	Othello, WA	46.789283	-119.038883	365	Silt loam	Shano
WA-On1	Native	Othello, WA	46.782033	-119.031167	362	Silt loam	Shano
WA-On2	Native	Othello, WA	46.781200	-119.031067	361	Silt loam	Shano
WA-On3	Native	Connell, WA	46.702283	-119.011233	290	Silt loam	Prosser-Starbuck
WA-Pc1	Switchgrass	Paterson, WA	45.940781	-119.496528	128	Loamy sand	Quincy
WA-Pc2	Switchgrass	Paterson, WA	45.940373	-119.496847	129	Loamy sand	Quincy
WA-Pc3	Switchgrass	Paterson, WA	45.939782	-119.497413	126	Loamy sand	Quincy
WA-Pn1	Native	Paterson, WA	45.938915	-119.498461	127	Loamy sand	Quincy
WA-Pn2	Native	Paterson, WA	45.938757	-119.497789	126	Loamy sand	Quincy
WA-Pn3	Native	Paterson, WA	45.938372	-119.496857	127	Loamy sand	Quincy
WIc1	Switchgrass	Arlington, WI	43.29963	-89.35562	317	Silt loam	Plano
WIc2	Switchgrass	Arlington, WI	43.30054	-89.34676	316	Silt loam	Plano
WIc3	Switchgrass	Sauk City, WI	43.34632	-89.73866	269	Silt loam	Richwood
WIIn1	Native	Arlington, WI	43.34399	-89.30884	311	Fine sandy loam	Sisson
WIIn2	Native	Wyocena, WI	43.45897	-89.33047	284	Fine sandy loam	Wyocena
WIIn3	Native	Sauk City, WI	43.29248	-89.78944	242	Loamy sand	Gotham
ILc1	Switchgrass	Batavia, IL	41.841983	-88.229850	226	Silt loam	Grays
ILc2	Switchgrass	Batavia, IL	41.841367	-88.229350	226	Silt loam	Grays
ILc3	Switchgrass	Batavia, IL	41.840350	-88.230850	226	Silt loam	Grays
ILn1	Native	Batavia, IL	41.851850	-88.211733	222	Silt loam	Markham
ILn2	Native	Batavia, IL	41.850800	-88.211817	221	Silty clay loam	Drummer
ILn3	Native	Batavia, IL	41.861667	-88.252483	226	Silt loam	Mundelein
ILr1	Restored	Batavia, IL	41.841017	-88.245217	226	Silt loam	Grays
ILr2	Restored	Batavia, IL	41.843067	-88.267300	231	Silt loam	Ozaukee
ILr3	Restored	Batavia, IL	41.840500	-88.274000	233	Silt loam	Wauconda
SDc1	Switchgrass	Colman, SD	44.025589	-96.844843	518	Silty clay loam	Wentworth-Egan
SDc2	Switchgrass	Bristol, SD	45.275761	-97.837151	520	Loam	Buse-Barnes
SDn1	Native	Colman, SD	44.027352	-96.850980	518	Silty clay loam/loam	Egan-Ethan
SDn2	Native	Bristol, SD	45.329295	-97.837024	528	Loam/silty clay loam	Forman-Buse-Parnell
TNc1	Switchgrass	Milan, TN	35.944008	-88.711956	114	Silt loam	Providence
TNc2	Switchgrass	Milan, TN	35.942097	-88.706908	113	Silt loam	Collins
TNc3	Switchgrass	Milan, TN	35.925647	-88.716914	128	Silt loam	Grenada
TNn1	Pasture	Milan, TN	35.897522	-88.719364	140	Silt loam	Lexington
TNn2	Pasture	Milan, TN	35.871639	-88.731769	138	Silt loam	Lexington
TNn3	Pasture	Milan, TN	35.901983	-88.731911	122	Silt loam	Falaya

Table 1 (continued)

Code	Site type	City, state	Latitude	Longitude	Elevation (m)	Soil texture	Soil series
NEc1	Switchgrass	Mead, NE	41.153117	-96.447567	354	Silt loam	Fillmore
NEc2	Switchgrass	Mead, NE	41.168217	-96.411983	351	Silty clay loam	Yutan
NEn1	Native	Lincoln, NE	40.869350	-96.806717	400	Clay loam	Pawnee

Soil texture and series are from the NRCS Web Soil Survey: <http://websoilsurvey.nrcs.usda.gov/app/>

fiber crops. For all locations, nine soil cores (approximately 2.5 cm wide and 15 cm deep) were to be collected from each of three spatially distinct biofuel feedstock (switchgrass or

sorghum) sites preferably greater than 0.2 ha in size, and also from three nearby grassland sites that contained some native plants and had similar types of soils. The samples

Table 2 Agronomic practices

Code	Crop	Cultivar	Crop rotation history	Fertilization/hectare	Irrigation
TX-Ac1	Sorghum	Forage	Sorghum>>wheat>>wheat>> fallow	33,626 kg manure every 2 years, 140 kg N as NH ₃	Full
TX-Ac2	Sorghum	Forage	Wheat>>sorghum>>fallow	See TX-Ac1	Furrow; full
TX-Ac3	Sorghum	Forage	Wheat>>sorghum>>fallow	See TX-Ac1	Row water
TX-Cc1	Sorghum	Forage	Sorghum/cotton rotation	168 kg 10–34–0 preplant plus 4 kg zinc; 112 kg 32–0–0 side dressed	Furrow; limited to pre-flowering
TX-Cc2	Sorghum	Forage	Sorghum/cotton rotation	See Tx-Cc1	See TX-Cc1
TX-Cc3	Sorghum	Grain	Sorghum/cotton rotation	See Tx-Cc1	See TX-Cc1
WA-Oc1	Sorghum	Sugar Drip	2008: flax and potatoes; 2009: wheat and beans	2010: 224–168–168	Rill; 70 % ET
WA-Oc2	Sorghum	Sugar Drip	2008: flax and potatoes; 2009: wheat and beans	2010: 224–168–168	Rill; 50 % ET
WA-Oc3	Sorghum	Sugar Drip	2008: flax and potatoes; 2009: wheat and beans	2010: 224–168–168	Rill; 30 % ET
WA-Pc1	Switchgrass	Shawnee	Leveled in 2006 for irrigation center pivot, leaving the subsoil as the new surface; 2007: wheat; 2008: switchgrass (perennial)	112 kg N/year; 336 kg P ₂ O ₅ /year; 168 kg SO ₄ /year; 336 kg K ₂ O/year	Center pivot ~91 cm/year
WA-Pc2	Switchgrass	Shawnee	See Wa-Pc1	See WA-Pc1	See WA-Pc1
WA-Pc3	Switchgrass	Shawnee	See Wa-Pc1	See WA-Pc1	See WA-Pc1
Wlc1	Switchgrass	Shawnee	Corn/soybean, 7 years switchgrass	336 kg NH ₄ NO ₃ per year	None
Wlc2	Switchgrass	Hiawatha	4 years grass, 2 years soybean, 5 years switchgrass	336 kg NH ₄ NO ₃ per year	None
Wlc3	Switchgrass	Cave-in-Rock	Corn/soybean, 7 years switchgrass	112 kg NH ₄ NO ₃ per year	None
ILc1	Switchgrass	Cave-in-Rock	First cultivated mid-1800s; converted from row crop to c3 pasture grasses in 1971 but not grazed after 1972 or 1973; switchgrass planted in 2008	67 kg N/year as urea beginning June 2009	None
ILc2	Switchgrass	Cave-in-Rock	See ILc1	See ILc1	None
ILc3	Switchgrass	Cave-in-Rock	See ILc1	See ILc1	None
SDc1	Switchgrass	NE-28	Third year switchgrass	2005–present: no fertilizer	None
SDc2	Switchgrass		2005–2007: soybean; 2008–present: switchgrass	See SDc1	None
TNc1	Switchgrass	Alamo	7 years switchgrass	67 kgN/year	None
TNc2	Switchgrass	Alamo	7 years switchgrass	67 kgN/year	None
TNc3	Switchgrass	Alamo	7 years switchgrass	67 kgN/year	None
NEc1	Switchgrass	Shawnee	Seeded in 2006	67 kgN in spring 2010	None
NEc2	Switchgrass	Cave-in-Rock	Seeded in 1998	112 kgN/year	None

were collected during the 2010 growing season around the time of crop flowering. For fields >0.2 ha, soil cores were collected at 5-m intervals along two perpendicular transects that intersected at their centers; for smaller sampling areas, a central transect and shorter sampling intervals were used. Soil cores were kept on ice during the collection day and also during shipping to the United States Environmental Protection Agency Laboratory in Corvallis, OR. Corers were cleaned with disposable disinfecting wipes (Clorox Company, Oakland, CA) between sampling sites; alternatively, fresh corers were used at each sampling location.

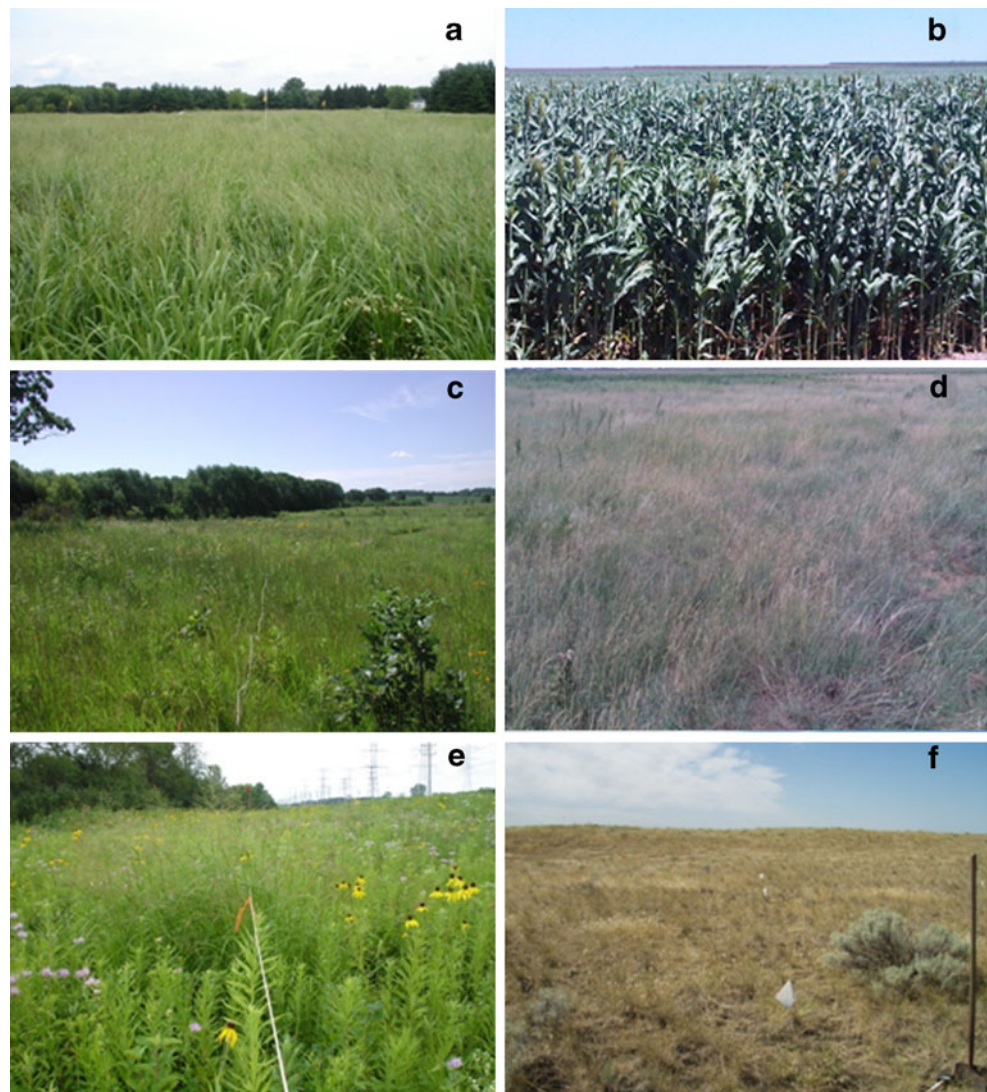
The soil series at each of the regional sampling locations are shown in Table 1. Figure 1 shows switchgrass and sorghum crop sites that were sampled in IL and TX (Fig. 1a, b), as well as reference grassland sites that were sampled such as an oak savannah in WI (Fig. 1c) and short grass prairie in TX (Fig. 1d) near switchgrass and sorghum sites. The broad range of grasslands that were sampled is further illustrated in (Fig. 1e) a tall grass prairie in the upper

Midwest and (Fig. 1f) sagebrush steppe in the Pacific Northwest. Cropping history and agronomic practices for sorghum and switchgrass cultivated crop sites are summarized in Table 2. Edaphic properties are provided in Tables 1, 3, 4, and 5.

Compositing and Subsampling of Soil Samples

Following their arrival in Corvallis, nine cores from each crop and reference grassland site were homogenized by mixing for 5 min with a Hobart mixer (Model D300, Hobart Corp., Troy, OH); the Hobart mixing bowl was sanitized with a disinfecting wipe following homogenization of each group of nine soil cores. Compositated samples were divided into portions for moisture determinations (5 g), soil chemical analyses (250 g), DNA extraction (two 10-g aliquots), and for determinations of active bacterial and fungal biomass (250 g). Samples for DNA analyses were stored at -80°C ; samples for chemical, soil bacterial, and fungal

Fig. 1 Photographs taken at times of soil sampling in 2010 at switchgrass (a) and sorghum (b) crop sites, respectively, in IL and TX. Grassland sampling sites are shown for an oak savannah site in WI (c) a short grass prairie site in the TX panhandle (d) a remnant tall grass prairie site in IL (e) and a sagebrush steppe site in WA (f)



biomass were stored at 4 °C until delivery (within 48 h of receipt in Corvallis) to local testing labs. Soil moisture determinations were made gravimetrically, based on differences in the fresh and dry weights of samples dried at 105 °C in a Blue M oven (Model POM-326E, Thermal Product Solutions, New Columbia, PA).

Soil Chemical Analyses

Soil chemical analyses were conducted by the Central Analytical Laboratory at Oregon State University [28] on soil samples that were air-dried at room temperature (22 °C) for approximately 1 week. A subsample of each composited soil sample was extracted with a DTPA extraction solution [0.05 M DTPA (diethylenetriaminepentaacetic acid), 0.1 M triethanolamine, and 0.01 M CaCl₂], and concentrations of the elements (B, Ca, Cu, Fe, K, Mg, Mn, and Zn) were determined using a Perkin Elmer Optima 3000DV inductively coupled plasma optical emission spectrometer with a diode array detector. Similarly, ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N), following extraction with 2 M potassium chloride (KCl), and P, following Bray P1 extraction, were analyzed on an AlpKem RFA300, and total %C and %N were analyzed on a Leco CNS-2000 Macro Analyzer. Sulfate concentration was determined using a Dionex IC 2000 following extraction with calcium dihydrogen phosphate (Ca(H₂PO₄)₂·H₂O). Soil particle size distributions (percent sand, percent silt, and percent clay) were estimated by using

data from the United States Department of Agriculture Natural Resources Conservation Service (<http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm>) for the soil series at each of the regional crop and reference grassland sampling sites.

Active Bacterial and Fungal Biomass

Active bacterial and fungal biomass was determined by Soil Foodweb, Inc. Corvallis, OR. Extracts of soil stored at 4 °C were stained with fluorescein diacetate, a substrate that binds and fluoresces to the metabolically active bacteria and fungi [29, 30]. Brightfield microscopy was used to enumerate bacteria and to measure the widths and lengths of fungal hyphae. Calculated biovolumes were then used to estimate active bacterial and fungal biomass values [31].

Soil Community DNA Isolation

Soil community DNA extractions for GeoChip 4.0 [32] FGA analyses were carried out at the United States Environmental Protection Agency laboratory in Corvallis, OR. Genomic DNA was extracted from 4–5 g of each soil sample using PowerMax Soil DNA Isolation Kits (MO BIO Laboratories Inc., Carlsbad, CA). The manufacturer's protocol was followed with the following exceptions: (a) the centrifugal spin speed was increased to 4000×g for the ethanol wash steps, (b) an additional wash step using 10 ml 95 %

Table 3 Soil pH, carbon, nitrogen, and soil physical properties (mean±SE) at sampling locations

Location	Land use	pH	%OM	%C	%N	Soil moisture (%)	Sand (%)	Silt (%)	Clay (%)
TX-A	Sorghum	7.8±0.1	2.8±0.3	1.1±0.2	0.12±0.02	13.2±2.7	27	39	34
	Grassland	7.4±0.3	2.9±0.2	1.1±0.1	0.11±0.01	9.0±0.6	29	37	34
WA-O	Sorghum	6.7±0.4	1.7±0.0	0.5±0.0	0.07±0.01	9.3±1.9	22	71	8
	Grassland	7.2±0	1.8±0.1	0.6±0.1	0.07±0.01	2.4±0.1	26	67	8
TX-C	Sorghum	8.3±0.1	2.7±0.3	2.1±0.1	0.10±0.01	14.9±2.0	15	27	58
	Grassland	8.2±0.0	3.0±0.3	2.6±0.3	0.14±0.01	19.4±9.5	12	24	64
WI	Switchgrass	6.5±0.1	4.3±0.3	2.9±0.3	0.21±0.03	25.9±1.4	9	70	21
	Grassland	6.6±0.4	2.7±0.7	1.9±0.8	0.11±0.04	12.0±2.4	73	18	9
WA-P	Switchgrass	7.1±0.1	1.1±0.1	0.4±0.0	0.03±0.01	8.9±0.9	80	16	4
	Grassland	7.4±0	0.9±0.0	0.4±0.0	0.03±0.00	0.9±0.1	80	16	4
IL	Switchgrass	5.9±0.1	4.3±0.0	2.4±0.0	0.22±0.00	20.3±1.0	9	70	21
	Grassland	6.5±0.1	4.1±0.2	2.5±0.1	0.21±0.01	18.4±1.4	9	70	21
	Native Grassland	7.3±0.2	7.3±2.6	5.8±1.4	0.37±0.14	27±6	9	65	26
SD	Switchgrass	7.1±1.0	3.6±1.1	2.9±0.5	0.13±0.03	18±7	23	50	27
	Grassland	7.6±0.0	4.8±0.6	2.9±0.1	0.24±0.02	25±5	23	50	27
TN	Switchgrass	6.5±0.2	2.5±0.2	1.0±0.1	0.10±0.01	22.2±1.4	11	78	12
	Grassland	6.0±0.1	3.4±0.3	1.3±0.1	0.15±0.01	19.8±1.8	11	70	19
NE	Switchgrass	6.1±0.1	3.3±0.1	1.7±0.1	0.17±0.02	23.8±0.8	9	61	30
	Grassland (n=1)	6.1	5.1	2.9	0.24	25.5	34	32	34

Sand, silt, and clay content based on USDA Natural Resources Conservation Service data (<http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm>)

Table 4 Mean ± SE values for soil macronutrients at sampling locations

Location	Land use	NO ₃ ⁻ -N (ppm)	NH ₄ ⁺ -N (ppm)	Bray-P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	SO ₄ ²⁻ -S (ppm)
TX-A	Sorghum	63±49	2.0±0.3	61±25	610±85	3,100±500	514±26	15±8
	Grassland	1.8±1.2	2.2±0.1	19±5	430±21	2,500±300	496±51	2.6±0.4
WA-O	Sorghum	110±46	26±6	36±3	250±64	1,300±163	190±21	38±30
	Grassland	2.3±0.8	3.7±0.5	23±3	300±44	847±17	170±10	1.5±0.0
TX-C	Sorghum	4.4±0.3	1.7±0.3	5±2	280±41	5,700±670	340±38	6±3
	Grassland	8.6±2.7	12±9	6±3	390±55	6,000±810	260±71	16±10
WI	Switchgrass	11.4±2.5	3.6±1.2	25±7	150±42	1,500±91	430±32	3.2±1.0
	Grassland	3.3±2.8	5.6±2.0	19±9	70±14	810±390	250±129	2.4±0.4
WA-P	Switchgrass	1.7±0.2	2.2±0.5	33±3	192±14	640±81	111±5	4.6±0.6
	Grassland	4.3±0.6	1.4±0.1	17±1	202±2	662±22	101±6	2.6±0.1
IL	Switchgrass	8.1±1.3	5.8±0.4	5±0	102±10	1,328±30	384±20	3.0±0.1
	Grassland	3.2±0.4	2.4±0.1	6±2	82±6	1,410±120	410±53	2.9±0.4
	Native grassland	6.5±2.6	3.0±0.2	3±0	200±76	2,800±444	800±119	6.3±0.7
SD	Switchgrass	4.2±2.7	2.4±0.0	34±17	170±98	3,800±1930	370±17	2.3±0.6
	Grassland	4±3	2.6±0.1	5±2	160±50	3,700±780	700±440	5.1
TN	Switchgrass	2.7±0.5	2.3±0.5	16±5	84±16	1,110±117	77±4	2.5±0.1
	Grassland	6.3±1.4	6.4±0.8	7±2	99±16	1,260±197	200±50	4.8±0.9
NE	Switchgrass	4.3±0.3	5.2±2.9	9±3	320±47	2,100±360	440±152	2.1±0.2
	Grassland (n=1)	5.4	6.3	6	338	1,825	478	2.5

ethanol was added, and (c) after the ethanol wash steps and the final spin, DNA was eluted in 5 ml sterile nuclease-free PCR grade water. Genomic DNA was dried in a DNA120 SpeedVac Concentrator (Thermo Inc. Asheville, NC) and

was resuspended in 200 µl Solution C6. DNA concentrations were determined using a NanoDrop ND-1000 Spectrophotometer (Thermo Inc., Waltham MA). The DNA sample concentrations were adjusted to 20 ng/µl.

Table 5 Mean±SE values for soil micronutrients and sodium at sampling locations

Location	Land use	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Na (ppm)
TX-A	Sorghum	0.5±0.0	0.6±0.0	19±5	12±3	2.0±0.6	91±29
	Grassland	0.5±0.0	0.6±0.0	24±5	19±3	1.4±0.3	23±2
WA-O	Sorghum	0.2±0.0	0.9±0.1	15±6	10±6	2.4±0.5	18±2
	Grassland	0.3±0.0	0.7±0.0	6±1	3±0	<0.1	11±1
TX-C	Sorghum	0.6±0.1	10.8±0.8	68±16	53±7	<0.1	62±23
	Grassland	1.0±0.5	12.6±1.8	100±43	65±14	4.4±4.3	26±11
WI	Switchgrass	0.3±0.0	2.7±0.3	90±6	26±4	4.3±1.2	11±2
	Grassland	0.2±0.1	2.3±0.3	43±7	27±7	2.7±0.3	6±0
WA-P	Switchgrass	0.1±0.0	0.3±0.0	22±2	3±0	0.7±0.1	22±1
	Grassland	0.1±0.0	0.3±0.0	14±1	3±0	0.6±0.0	15±0
IL	Switchgrass	0.5±0.0	3.0±0.0	131±5	20±3	4.0±0.0	21±0
	Grassland	0.4±0.1	3.0±0.0	70±6	19±2	3.3±0.3	22±1
	Native grassland	0.7±0.2	3.7±0.3	70±31	18±3	31±28	23±1
SD	Switchgrass	0.4±0.1	1.1±0.3	60±54	15±9	4.9±1.8	39±0
	Grassland	0.7±0.2	1.1±0.2	42±13	15±6	0.9±0.8	120±66
TN	Switchgrass	0.1±0.0	0.5±0.0	69±4	42±9	0.8±0.0	17±0
	Grassland	0.1±0.0	0.5±0.0	120±35	26±5	1.2±0.2	21±2
NE	Switchgrass	0.5±0.1	0.9±0.1	53±14	24±2	< 0.1	30±1
	Grassland (n=1)	0.5	0.8	57	15	1.1	27

Microarray Analyses with GeoChip 4.0

To measure potential differences in microbial abundance and functional diversity for soil ecosystem services, we utilized GeoChip 4.0, a functional microbial gene array [17, 33]. GeoChip 4.0 provides a broad representation of probes for genes involved in many microbial processes [23]; it contains over 83,000 probes distributed among 15 categories of functional genes. The GeoChip probes are designed based on sequences available in the GenBank database that are derived from both unculturable and culturable soil microorganisms. The 15 gene categories represented on the GeoChip 4.0 include antibiotic resistance, bacteriophage, carbon cycling, metabolic energy, fungal functions, metal resistance, nitrogen cycling, organic remediation, phosphorus utilization, stress tolerance, sulfur utilization, other genes, soil benefit, soil pathogens, and virulence. The soil benefit category contains microbial genes shown to be beneficial to plant growth by protecting plants from pathogens, enhancing water or nutrient uptake, or stimulating growth; examples of genes in the benefit category include those involved in antibiotic, plant hormone, and siderophore production.

DNA preprocessing, hybridizations to GeoChip 4.0, and microarray data collection were carried out at Glomics Inc., Norman, OK using methods that were similar to those previously described [23]. Briefly, the preprocessing steps for soil genomic DNA samples included amplification of 4 µg of whole community genomic DNA, followed by labeling of 2 µg of amplified DNA with Cy-3 using random primers and the Klenow fragment of DNA polymerase I [34]. Sample tracking control (NimbleGen, Madison, WI, USA) was added to samples after drying, washing, and rehydration. The hybridization buffer contained Cy5-labeled common oligo reference standard (CORS) target and Cy3-labeled alignment oligo (NimbleGen). The CORS probes were placed randomly throughout the GeoChip 4.0 array and were used for signal normalization [23, 35]. The quality of microarray spots was assessed, and low-quality spots were removed prior to statistical analysis [36]. The spots were scored as positive if the signal-to-noise ratio was >2.0 and the coefficient of variability of the background was <0.8. Singlet genes (detected in only one sample) were removed [23]. Data used in our analyses of microbial gene functional diversity and abundance were further restricted to those genes that were detected in at least two out of the three replicate sampling sites for individual crop or grassland geographic locations. The number of positive genes within each category (diversity) was normalized to the mean across samples for that category. Mean signal intensity values of microbial genes (relative abundance) were calculated by dividing the total signal intensity per gene category for a sample by the number of positive genes detected within that

category for the sample. Signal intensities were transformed to reflect relative gene abundance per gram dry weight soil for each sample.

Statistical Methods

Univariate and multivariate analyses of variances (ANOVAs) were conducted for the FGA, chemistry, and biological data to test for differences between soil obtained from sorghum fields, switchgrass fields, and from perennial reference grasslands. A log or square root transformation was applied as needed to the soil chemistry and microarray gene variables in order to stabilize their variances prior to ANOVA. Linear discriminant analysis (LDA) was used to classify the plots into mutually exclusive groups for switchgrass crop, switchgrass grassland, sorghum crop, and sorghum grassland based on soil chemistry data. All calculations were performed using JMP 6.0 (SAS Institute, Cary, NC) and SAS GLM procedures (SAS Institute Inc., Cary, NC).

Probability values <0.05 were considered statistically significant. Pearson correlations among soil FGA, chemistry, and biological data were used to measure the linear association among these diverse soil indices. Under the assumption of zero correlation, the test statistic, $t = r\sqrt{(N-2)/\sqrt{(1-r^2)}}$ where r =Pearson correlation and N =sample size, follows a Student's t distribution with $N-2$ degrees of freedom. Correlation analysis was performed using an application within the microarray data analyses pipeline at the University of Oklahoma Institute for Environmental Genomics (<http://ieg.ou.edu/>).

Results

Edaphic properties, microbial gene diversity and abundance, and active microbial biomass varied by geographic location, crop type, and/or land use, (i.e., crop or reference grassland). In particular, differences between crop and grassland soils varied by location and crop as indicated by significant land use × location interactions in the ANOVAs. These land use differences in microbial biomass, gene diversity, and abundance reflect the regional differences in soil types and properties between geographic locations and differences in agronomic practices between switchgrass, a perennial biofuel crop, and sorghum, an annual biofuel crop.

Soil Texture, Chemistry, and Biology

Soil series and texture varied between the geographically diverse locations; however, with the exception of the SE Texas (TX-C) samples, which were clays, the majority of

the remaining samples were loamy soils (Table 1). Sand content ranged from 9 to 80 %. Likewise, soil chemistry varied widely between locations (see Tables 3, 4, and 5). For example, soil pH values ranged from acidic (pH 5.9) to basic (pH 8.3), organic matter values ranged from 0.9 to 7.3 %, and nitrate from 2 to 114 ppm (Tables 3, 4, and 5).

The LDA of soil chemistry data correctly differentiated 90 % (47/52) of the soil samples with regard to whether they were crop or grassland reference soils (Fig. 2). The sorghum crop and grassland sites were clearly different from the switchgrass crop and grassland sites. Further, the sorghum crop and grassland sites were different in soil chemistry, while the switchgrass crop and grassland groups overlapped. The top five soil chemistry factors that separated the sorghum, switchgrass, and grassland sites in the LDA were pH, NO₃⁻-N, K, Na, and %C.

Two-way ANOVAs were used to separately compare the sorghum crop versus grassland sites and switchgrass crop versus grassland sites. For the sorghum analyses, there was a significant land-use × geographic location interaction due to grassland differences; i.e., results for the Southeast TX (TX-C) site differed from those at the Texas panhandle, (TX-A), and Southeast WA, (WA-O), sites. The TX-A and Southeast WA (WA-O) crop soil samples were significantly higher in NO₃⁻-N (50±34 versus 1.4±0.7 ppm; *P*<0.001), NH₄⁺-N (14±6 versus 3.0±0.4 ppm; *P*=0.007), SO₄²⁻-S (13±9 versus 2.0±0.3 ppm; *P*=0.009), and Cu (0.71±0.08 versus 0.61±0.03 ppm; *P*=0.037) than the grassland samples. At all three sets of sorghum sites, Na concentrations were greater in the crop than the grassland soils (42±14 versus 17±3 ppm; *P*=0.005). Overall, there were few crop versus grassland differences among switchgrass sites. Soil pH tended to be higher in the grassland sites (TN excluded; 7.0±0.1 versus 6.5±0.2; *P*=0.062), while Fe was significantly higher in the crop sites (73±13 versus 49±8 ppm; *P*=0.035).

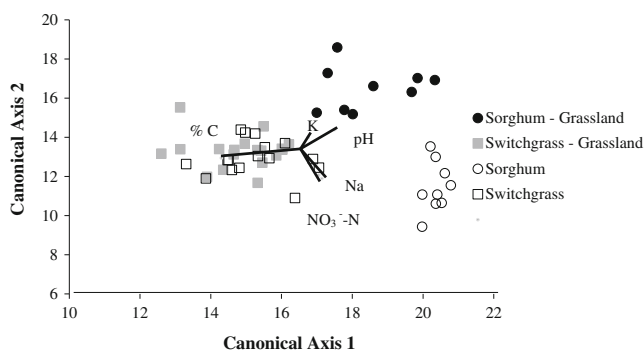


Fig. 2 Linear discriminant analysis of sorghum and switchgrass crop and grassland soil samples. Biplot rays are shown for the five most important soil chemistry variables used to create the discriminant function. The relative influence of the five variables in descending order was pH>NO₃⁻-N>K>Na>%C. Larger differences were observed between sorghum crop and grassland soils than between switchgrass crop and grassland soils

Both switchgrass crop and grassland sites had significantly higher active bacterial biomass content than the sorghum sites (25.3±1.9 versus 15.6±1.2 µg/g dry wt.; *P*=0.001), while no significant differences were observed for active fungal biomass (5.0±1.0 versus 4.8±1.4 µg/g dry wt.; *P*=0.926). Within the sorghum locations, crop and grassland sites did not differ significantly in terms of active bacterial biomass (15.1±1.8 versus 16.0±1.8 µg/g dry wt.; *P*=0.727) or active fungal biomass (4.1±1.7 versus 5.5±2.4 µg/g dry wt.; *P*=0.622). Likewise, the switchgrass crop and grassland sites did not differ significantly in active bacterial biomass (24.8±2.3 versus 25.8±3.1 µg/g dry wt.; *P*=0.253). However, four of the six switchgrass locations (IL, SD, WA-P, and WI) tended to have higher levels of active fungal biomass in the crop sites as compared to grassland sites (*P*=0.068).

FGA Data

We were able to collect GeoChip 4.0 FGA data that passed the signal to noise ratio criteria from 38,016 probes across all 15 gene categories with our samples. That represented 45 % of the total number of probes present on the microarray chips. The mean number of probes for which data were collected in all replicate sampling sites from individual crop and grassland sampling locations was 16,889±464 (SE). The mean number of probes by functional gene category detected in the soil community DNA samples were as follows: antibiotic resistance, 576±15; bacteriophage, 84±2; carbon cycling, 2,018±56; metabolic energy, 167±5; fungal functions, 659±20; metal resistance, 1,885±51; nitrogen cycling, 1,135±34; organic remediation, 4,065±125; phosphorus utilization, 241±6; stress tolerance, 3,399±91; sulfur utilization, 482±16; other genes, 305±9; soil benefit, 700±18; soil pathogens, 211±6; and virulence, 556±12. Preliminary analyses of the data down to the level of the gene name (for example gene category: nitrogen cycling; gene subcategory: denitrification; gene name *nirS*) indicated that the dominant patterns of difference among the samples were captured at the gene category tier (results not shown). Thereafter, further testing for changes affecting individual genes was not included for this study.

ANOVA results for the FGA data from switchgrass locations showed statistically significant interactions (*P*≤0.05) between geographic location and land use (crop or grassland). For example, upon inspection of the relative gene abundance sample means, an interaction was observed that was largely due to grassland versus crop differences for the TN samples, as compared to the other five regional switchgrass locations.

When data for sorghum and switchgrass locations were combined (Table 6), the number of positive genes detected among geographic locations, land use, and their interactions were each statistically significant (*P*<0.05) across the

Table 6 ANOVA results for sources of variation among GeoChip 4.0 number of positive genes (diversity) in each gene category

Source of variation	df	Antibiotic resistance	Bacteriophage cycling	Carbon cycling	Metabolic energy	Fungal function	Metal resistance	Nitrogen cycling	Organic remediation	Phosphorus utilization	Stress	Sulphur utilization	Other	Soil benefit	Soil pathogens	Virulence
All geographic locations	8	0.129	0.245	0.018*	0.051	0.019*	0.035*	0.041*	0.013*	0.020*	0.018*	0.063	0.004*	0.020*	0.007*	0.010*
Sorghum locations	2	0.018*	0.178	0.286	0.628	0.652	0.093	0.039*	0.042*	0.295	0.049*	0.269	0.067	0.011*	0.479	0.144
Switchgrass locations	6	0.263	0.327	0.044*	0.082	0.039*	0.083	0.106	0.045*	0.041*	0.056	0.112	0.018*	0.073	0.020*	0.032*
Switchgrass locations (Minus TN grassland)	5	0.013*	0.045*	0.002*	0.004*	0.002*	0.003*	0.005*	0.002*	0.002*	0.004*	0.004*	0.001*	0.004*	0.002*	0.003*
All land use	3	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Sorghum land use: sorghum vs. grassland	1	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Switchgrass land use: switchgrass vs. grassland	1	0.002*	0.020*	0.002*	0.003*	0.001*	0.002*	0.002*	0.001*	0.002*	0.002*	0.001*	0.008*	0.002*	0.001*	0.007*
Switchgrass land use: switchgrass vs. grassland (Minus TN grassland)	1	0.127	0.362	0.181	0.199	0.114	0.164	0.154	0.140	0.161	0.174	0.115	0.351	0.164	0.107	0.313
All locations × all land use	8	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Sorghum locations × sorghum land use	2	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Switchgrass locations × switchgrass land use	6	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Switchgrass locations × switchgrass land use (Minus TN grassland)	5	0.066	0.153	0.014*	0.020*	0.018*	0.026*	0.033*	0.011*	0.015*	0.035*	0.021*	0.011*	0.029*	0.015*	0.052

*P=<0.05

majority of 15 gene categories. For sorghum soils, microbial gene diversity was significantly affected by land use, but differences between crop and grassland sites varied by geographic location as indicated by a statistically significant interaction across all 15 GeoChip 4.0 gene categories. The normalized number of positive carbon cycling, nitrogen cycling, phosphorus utilization, and sulphur utilization genes are shown in Fig. 3. Crop versus grassland comparisons within four FGA gene categories (C, N, P, and S) were generally representative of the patterns seen across all gene categories for each sample location. Sorghum soils had consistently lower microbial gene diversity than grassland samples in the four gene categories (Fig. 3a). The difference was significant for all sorghum location × land use combinations except for sulphur utilization at the Eastern TX (TX-Cc and TX-Cn) sites.

For switchgrass soil samples, geographic location was a significant source of variation of microbial gene diversity (Table 6). This was true in seven GeoChip 4.0 gene categories. There were statistically significant interactions between geographic location and land use for all 15 gene categories for switchgrass. The switchgrass and grassland soil microbial gene diversity patterns were very similar across gene categories, but they were inconsistent among the sampling locations (Fig. 3b). At the WA-P and TN locations, we observed significantly lower gene diversity in switchgrass fields than in nearby grassland soils in all 15 gene categories. At the WA-P locations, the difference may be attributed to extensive recent deep land grading several years prior to planting of the switchgrass, which had removed much of the topsoil. The lower gene diversity at the TN locations may be explained by the greater disturbance of the sites due to land

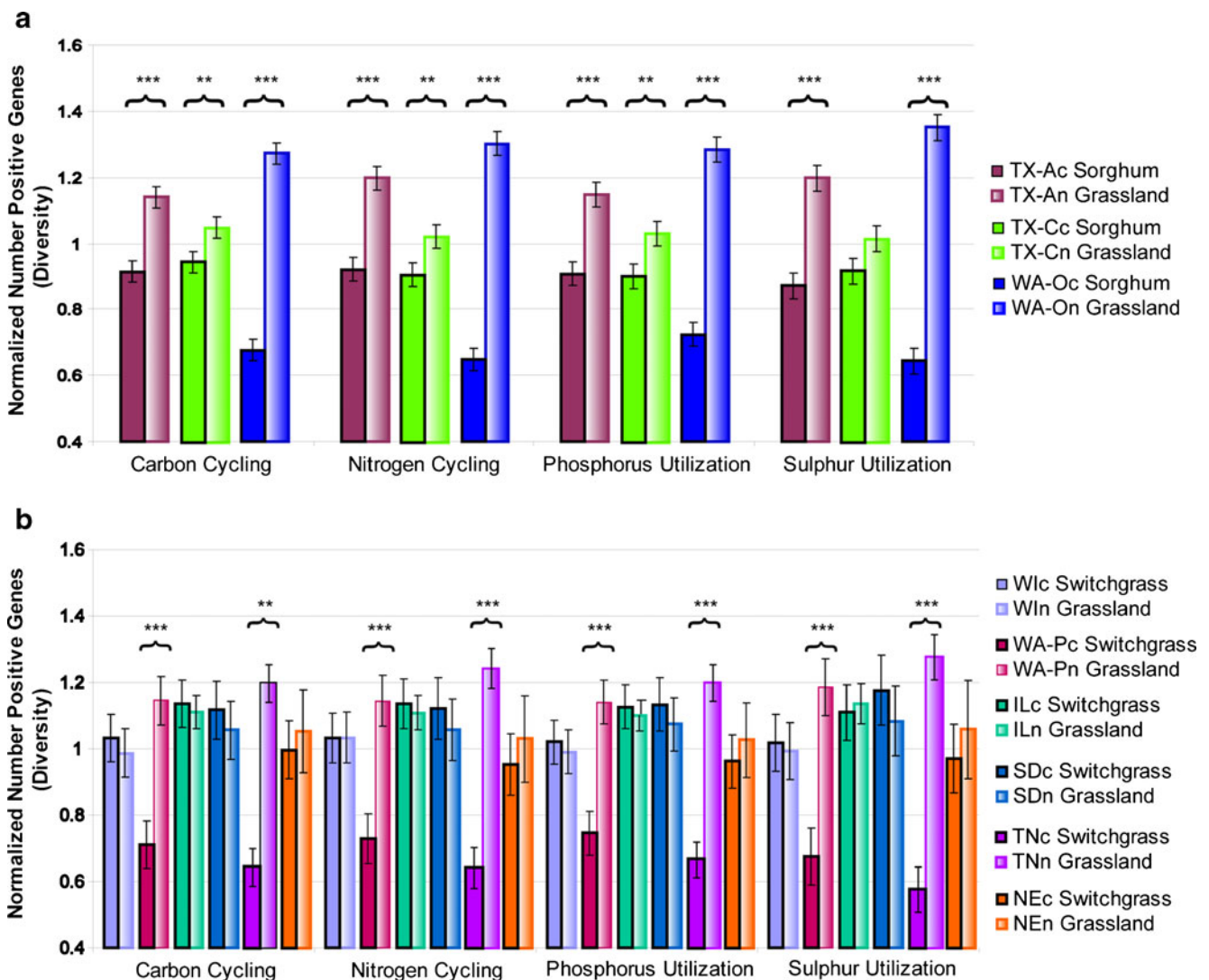


Fig. 3 Normalized number of microbial genes (diversity) in four representative functional gene categories found in **a** sorghum versus grassland samples and **b** in switchgrass and grassland samples.

Significance based on the least squares means test is shown above bracketed pairs of crop and grassland bars for the given geographic locations; ** $P \leq 0.05$ but > 0.01 ; *** $P \leq 0.01$

clearing of oak-hickory forests in the nineteenth century and to grazing of the long-term pastures for over 40 years. The remainder of our reference sites historically had been perennial grasslands, i.e., tall or short grass prairie or sagebrush steppe.

ANOVA results (Table 7) illustrate the sources of variation in the GeoChip 4.0 signal intensity data which we used to measure relative abundance of microbial genes per gram of dry soil. Differences in signal intensity data between geographic locations were statistically significant across all 15 GeoChip 4.0 gene categories when data for sorghum and switchgrass locations were combined. Crop versus grassland differences were statistically significant across 10 of 15 gene categories at the 0.05 level of significance. For sorghum soil samples, microbial gene abundances from different locations and from crop and grassland areas at the same geographic location were not statistically different. However, there was a trend of lower gene abundance in sorghum soils than grassland samples (Fig. 4a). Variation in gene abundance at switchgrass sites was dependent on geographic location and land use type in all FGA microbial gene categories. Significant interactions between switchgrass locations and land use were detected in all GeoChip 4.0 gene categories. Most switchgrass location \times land use combinations also had lower microbial gene abundance in crop soil than the grassland samples. This difference was statistically significant for the SD crop and grassland soils (Fig. 4b); the exception to this pattern was the TN location, where there was greater relative abundance of microbial genes in the crop soils, as compared to grassland (pasture) samples. Overall, the range of microbial gene abundance variation was less for sorghum sites than switchgrass sites.

Correlations Among Soil FGA, Chemistry, and Biological Data

Pearson correlation tests were run to see if FGA gene diversity and abundance across sorghum and switchgrass locations were significantly related to soil chemistry and biological data from those sites (see Table 8 Pearson correlation analyses). Gene diversity in at least four FGA categories at sorghum locations was negatively correlated with NO_3^- -N, NH_4^+ -N, and SO_4^{2-} -S. In contrast, gene diversity across six or more gene categories at switchgrass locations was positively correlated with NO_3^- -N, percent organic matter, and percent clay. Switchgrass gene diversity was negatively correlated to Bray-P. Interestingly, the relative microbial gene abundance at sorghum locations was positively correlated to percent sand. Gene abundance at switchgrass sites was positively correlated to Mn.

Discussion

Our data support the hypotheses that (1) soils from perennial switchgrass fields would be more similar to nearby grassland soils than soils from sorghum fields and (2) that trend would be observed in multiple geographies and different soils. For example, the levels of N, K, S, Na, and Ca as well as pH differed more between sorghum crop and grassland samples than between switchgrass crop soils and their nearby grassland sites. Our active microbial biomass data indicated that switchgrass soils were much more similar to grassland soils than were sorghum soils. Using the GeoChip FGA approach, we observed that microbial gene diversity was significantly lower in sorghum soils than nearby grassland soils. Microbial gene abundance did not differ between sorghum crop and grassland soils, but was generally lower in switchgrass soils as compared to nearby grassland soils.

The differences we observed in soil chemistry and microbial communities are likely linked to differences in land use, agronomic practices, and soil type [37–42]. Higher macronutrient levels in the sorghum sites than nearby grassland sites are likely the result of fertilizer additions during cultivation. These additions may have decreased the microbial gene diversity within the sorghum crop fields as the significant differences in gene diversity in neighboring sorghum crop and grassland soils were negatively correlated with concentrations of NO_3^- -N, NH_4^+ -N, and SO_4^{2-} -S. Allelochemicals released by sorghum roots into the soil and release of cyanide-containing compounds from leaves after frost may also have negatively impacted the diversity of plant-associated soil microbial communities in the sorghum fields [43–45].

The GeoChip data suggest that while sorghum agronomic practices decreased gene diversity compared to nearby grasslands, the overall gene abundance was not affected. In contrast, genetic diversity was similar between switchgrass fields and grasslands, while the abundance of the microbial genes was lower than in nearby grassland soils. These subtle but important differences present opportunities for future studies. They also suggest that the higher gene diversity in switchgrass (and perhaps other perennial) soils may foster higher sustainability of ecosystem services and greater resilience of plant-associated microbes and plants to environmental stressors.

Use of the GeoChip 4.0 additionally permitted exploration of the microbial community impacts of the differences in soil chemistry and structure within and across multiple gene categories that related to important ecosystem processes such as C, N, P, and S cycling. For instance, we observed some significant differences among the N-cycling genes for the sorghum crop and grassland sites for nitrogen fixation, nitrification, and denitrification. Interestingly, effects of N fertilization have been reported to impact the number of

Table 7 ANOVA results for sources of variation among GeoChip 4.0 signal intensities (relative abundance) in each gene category

Source of variation	df	Antibiotic resistance	Bacteriophage	Carbon cycling	Metabolic energy	Fungal function	Metal resistance	Nitrogen cycling	Organic remediation	Phosphorus utilization	Stress	Sulphur utilization	Other	Soil benefit	Soil pathogens	Virulence
All geographic locations	8	0.012*	0.027*	0.019*	0.022*	0.019*	0.016*	0.014*	0.014*	0.011*	0.017*	0.013*	0.015*	0.022*	0.023*	0.018*
Sorghum locations	2	0.598*	0.686	0.674	0.482	0.706	0.637	0.604	0.622	0.692	0.625	0.576	0.611	0.66	0.699	0.63
Switchgrass locations	6	0.001*	0.001*	0.001*	0.002*	0.001*	0.001*	0.001*	0.001*	<0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Switchgrass locations (Minus TN grassland)	5	<0.001	<0.001*	<0.001*	0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
All land use	3	0.026*	0.114	0.034*	0.032*	0.072	0.028*	0.029*	0.025*	0.054*	0.033*	0.052	0.033*	0.029*	0.051	0.04*
Sorghum land use: sorghum vs grassland	1	0.33	0.78	0.398	0.462	0.535	0.374	0.368	0.36	0.454	0.393	0.416	0.405	0.369	0.517	0.456
Switchgrass land use:	1	0.004*	0.008*	0.003*	0.003*	0.007*	0.003*	0.003*	0.003*	0.006*	0.004*	0.007*	0.004*	0.003*	0.005*	0.004*
switchgrass vs grassland	1	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Switchgrass land use:	1	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
switchgrass vs grassland (Minus TN grassland)	1	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
All locations × all land use	8	0.226	0.076	0.192	0.194	0.16	0.217	0.232	0.271	0.184	0.229	0.128	0.203	0.251	0.141	0.172
Sorghum locations × sorghum land use	2	0.943	0.752	0.922	0.952	0.903	0.912	0.951	0.938	0.904	0.929	0.914	0.9	0.925	0.9	0.921
Switchgrass locations × switchgrass land use	6	0.024*	0.003*	0.014*	0.016*	0.01*	0.021*	0.023*	0.029*	0.013*	0.021*	0.009*	0.022*	0.025*	0.01*	0.014*
Switchgrass locations × land use	5	0.227	0.061	0.176	0.159	0.147	0.213	0.238	0.246	0.171	0.209	0.128	0.239	0.238	0.129	0.155
Switchgrass locations × switchgrass land use (Minus TN grassland)	5	0.227	0.061	0.176	0.159	0.147	0.213	0.238	0.246	0.171	0.209	0.128	0.239	0.238	0.129	0.155

*P=<0.05

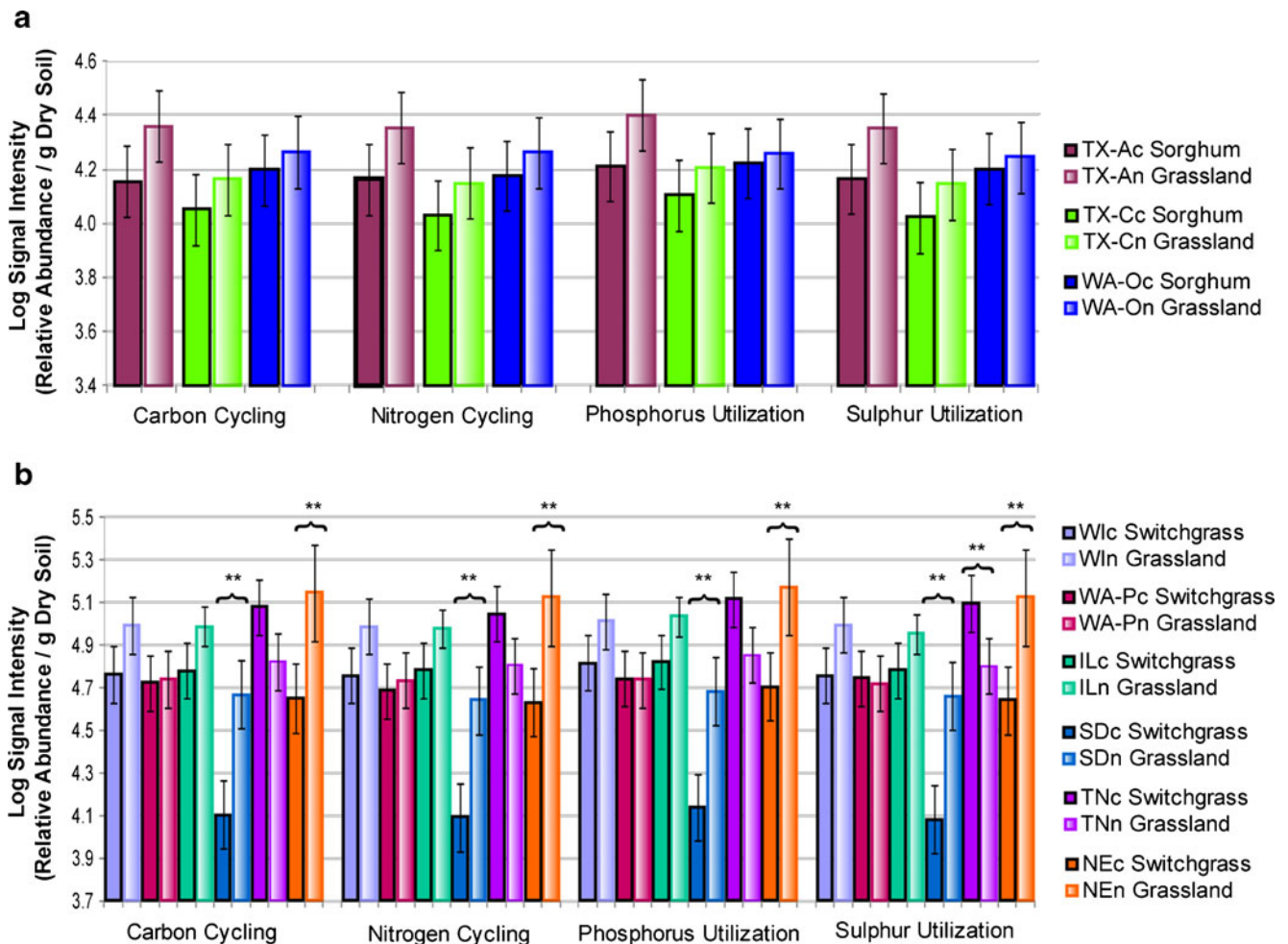


Fig. 4 Log signal intensity (relative gene abundance per gram of dry soil) of microbial genes in four representative functional categories found in **a** sorghum versus grassland samples and **b** in switchgrass and

grassland samples. Significance based on the least squares means test is shown above bracketed pairs of crop and grassland bars for the given geographic locations; ** $P \leq 0.05$ but > 0.01

copies of *nifH* genes in the rhizosphere of sorghum [46], numbers of ammonia-oxidizing bacteria denitrifiers [47], numbers of plant growth-promoting rhizobacteria in sorghum soils [48], and abundance of ammonia oxidizers and denitrifiers in maize soils [49]. The lower microbial gene diversity in sorghum soil samples was not restricted to genes within the carbon cycling, nitrogen cycling, phosphorus utilization, and sulphur utilization. Rather, it extended across multiple functional gene categories detected by the GeoChip 4.0. The fact that we did not observe significant differences in abundance or diversity for specific types of N or C cycling functional genes was surprising since one might have anticipated finding lower abundance or diversity of, e.g., nitrogenase or ammonia oxidase genes under higher levels of N fertilization, as in sorghum fields. However, quality control checks on the FGA chips and tests of quality and quantity of DNA in our soil samples did not reveal any technical problems. This suggests that any differences among our samples for individual genes were very minor.

Quantitative real-time PCR targeting specific functional genes could confirm these results, but this was beyond the scope of the current research. Use of additional, complementary molecular analytical methods may improve the limits of detection and maximize the information that can be derived from soil DNA samples.

Ecological Implications

We are not aware of other studies having looked at as broad a geographic comparison of crop and nearby reference grasslands, i.e., natural, minimally managed perennial grasslands, as we have presented here. For example, regional studies in Kansas and Wisconsin [50, 51] that compared various crops and much more intensively managed grasslands have reported shifts in microbial community composition that may occur with cultivation. In a Michigan study, da C. Jesus et al. [22] reported that soil attributes (principally pH, organic matter, and nutrients) had greater effects

Table 8 Pearson correlations and *P* values for Geochip 4.0 and soil environmental parameter data sets

Sorghum and grassland number of positive genes (diversity)									
Gene category	NO ₃ ⁻ -N (ppm)	NH ₄ ⁺ -N (ppm)	SO ₄ ²⁻ -S (ppm)	RFN	%N	%OM	%C	%Clay	
Antibiotic resistance	-0.437 (0.07)	-0.472 (0.048)*	-0.446 (0.063)	-0.471 (0.049) *					
Bacteriophage	-0.515 (0.029) *	-0.462 (0.054)	-0.421 (0.082)	-0.403 (0.097)					
Carbon cycling	-0.503 (0.033)*	-0.49 (0.039)*	-0.478 (0.045) *	-0.457 (0.057)					
Metabolic energy	-0.523 (0.026) *	-0.456 (0.057)	-0.475 (0.047) *	-0.461 (0.054)					
Fungal function	-0.536 (0.022) *	-0.443 (0.065)	-0.471 (0.049) *	-0.382 (0.118)					
Metal resistance	-0.461 (0.054)	-0.489 (0.04)*	-0.45 (0.061)	-0.446 (0.064)					
Nitrogen cycling	-0.451 (0.06)	-0.5 (0.035)*	-0.462 (0.054)	-0.47 (0.049) *					
Organic remediation	-0.444 (0.065)	-0.497 (0.036)*	-0.445 (0.064)	-0.462 (0.054)					
Phosphorus utilization	-0.408 (0.093)	-0.415 (0.087)	-0.419 (0.083)	-0.413 (0.088)					
Stress	-0.453 (0.059)	-0.482 (0.043)*	-0.446 (0.064)	-0.455 (0.058)					
Sulphur utilization	-0.421 (0.082)	-0.459 (0.056)	-0.435 (0.071)	-0.426 (0.078)					
Other category	-0.474 (0.047)	-0.501 (0.034)	-0.451 (0.061)	-0.451 (0.06)					
Soil benefit	-0.489(0.039)	-0.536 (0.022) *	-0.47 (0.049) *	-0.455(0.058)					
Soil-borne pathogen	-0.462(0.054)	-0.4 (0.1)	-0.428 (0.076)	-0.456(0.057)					
Virulence	-0.482(0.043)	-0.425 (0.079)	-0.427 (0.078)	-0.466(0.051)					
Switchgrass and grassland number of positive genes (diversity)									
Gene category	NO ₃ ⁻ -N (ppm)	Bray-P (ppm)	Cu (ppm)	Fe (ppm)	%N	%OM	%C	%Clay	
Antibiotic resistance	0.365 (0.034) *	-0.298 (0.088)	0.268 (0.125)	0.282 (0.106)	0.263 (0.133)	0.283 (0.105)	0.243 (0.167)	0.294 (0.092)	
Bacteriophage	0.288 (0.099)	-0.331 (0.056)	0.131 (0.460)	0.174 (0.325)	0.190 (0.283)	0.193 (0.275)	0.196 (0.267)	0.252 (0.150)	
Carbon cycling	0.392 (0.022) *	-0.381 (0.026) *	0.311 (0.073)	0.293 (0.092)	0.334 (0.054)	0.345 (0.045) *	0.324 (0.061) *	0.389 (0.023) *	
Energy process	0.359 (0.037) *	-0.364 (0.035) *	0.240 (0.172)	0.240 (0.172)	0.311 (0.073)	0.333 (0.054)	0.301 (0.083)	0.382 (0.026) *	
Fungal function	0.334 (0.053)	-0.398 (0.020) *	0.183 (0.301)	0.185 (0.295)	0.321 (0.065)	0.335 (0.053)	0.316 (0.069)	0.437 (0.010) *	
Metal resistance	0.385 (0.025) *	-0.353 (0.041) *	0.310 (0.074)	0.314 (0.071)	0.317 (0.068)	0.332 (0.055)	0.304 (0.080)	0.341 (0.049) *	
Nitrogen	0.387 (0.024) *	-0.336 (0.052) *	0.299 (0.086)	0.321 (0.064)	0.309 (0.075)	0.331 (0.056)	0.298 (0.087)	0.348 (0.044) *	
Organic remediation	0.389 (0.023) *	-0.362 (0.036) *	0.342 (0.048) *	0.307 (0.078)	0.325 (0.061)	0.338 (0.051)	0.318 (0.067)	0.355 (0.039) *	
Phosphorus	0.371 (0.031) *	-0.376 (0.028) *	0.289 (0.097)	0.316 (0.068)	0.314 (0.071)	0.329 (0.058)	0.306 (0.079)	0.350 (0.042) *	
Stress	0.373 (0.030) *	-0.358 (0.038) *	0.321 (0.065)	0.322 (0.063)	0.330 (0.057)	0.350 (0.043) *	0.316 (0.069)	0.370 (0.031) *	
Sulphur	0.372 (0.031) *	-0.357 (0.038) *	0.259 (0.140)	0.269 (0.124)	0.321 (0.064)	0.343 (0.047) *	0.310 (0.075)	0.374 (0.029) *	
Other category	0.404 (0.018) *	-0.324 (0.062)	0.331 (0.056)	0.346 (0.045) *	0.343 (0.047)*	0.367 (0.033) *	0.335 (0.053)	0.390 (0.023) *	
Soil-benefit	0.389 (0.023) *	-0.328 (0.058)	0.294 (0.092)	0.297 (0.088)	0.309 (0.075)	0.324 (0.061)	0.292 (0.093)	0.348 (0.044) *	
Soil-borne pathogen	0.347 (0.044) *	-0.441 (0.009) *	0.300 (0.085)	0.266 (0.128)	0.368 (0.032)*	0.374 (0.029) *	0.356 (0.039) *	0.413 (0.015) *	
Virulence	0.394 (0.021) *	-0.343 (0.047) *	0.342 (0.048) *	0.337 (0.052)	0.336 (0.052)	0.347 (0.045) *	0.300 (0.084)	0.348 (0.044) *	

Table 8 (continued)

Sorghum and non-crop gene signal intensity (abundance)

Gene category	%Sand
Antibiotic resistance	0.482 (0.043) *
Bacteriophage	0.415 (0.087)
Carbon cycling	0.459 (0.055)
Metabolic energy	0.469 (0.049) *
Fungal function	0.442 (0.066)
Metal resistance	0.486 (0.041) *
Nitrogen cycling	0.478 (0.045) *
Organic remediation	0.485 (0.041) *
Phosphorus utilization	0.472 (0.048) *
Stress	0.48 (0.044) *
Sulphur utilization	0.483 (0.042) *
Other category	0.461 (0.054)
Soil benefit	0.465 (0.052)
Soil-borne pathogen	0.444 (0.065)
Virulence	0.472 (0.048) *

Switchgrass and non-crop gene signal intensity (abundance)

Gene category	Mn (ppm)
Antibiotic resistance	0.523 (0.002) *
Bacteriophage	0.58 (<0.001) *
Carbon cycling	0.519 (0.002) *
Energy process	0.507 (0.002) *
Fungal function	0.544 (0.001) *
Metal resistance	0.512 (0.002) *
Nitrogen	0.501 (0.003) *
Organic remediation	0.51 (0.002) *
Phosphorus	0.528 (0.001) *
Stress	0.511 (0.002) *
Sulphur	0.543 (0.001) *
Other category	0.531 (0.001) *
Soil-benefit	0.511 (0.002) *
Soil-borne pathogen	0.528 (0.001) *
Virulence	0.523 (0.002) *

%OM % organic matter, *RFN* root-feeding nematodes* $P < 0.05$

on the diversity of bacterial rhizosphere communities than plant genus when analyzing samples from soybean, canola, sunflower, corn, and switchgrass. The authors noted higher bacterial diversity in the biofuel crop fields than in adjacent forest soils. The da C. Jesus et al. [22] results differ from our sorghum soil microbial diversity results, perhaps because of differences between the methods and designs of our studies. For example, except for the TN pasture sites that had been established on cleared oak-hickory forest land, our biofuel crop sites were paired with nearby perennial grasslands. Additionally, our soil samples were essentially bulk soil, rather than rhizosphere soil samples. Further, while the GeoChip 4.0 FGA we used includes bacterial probes, it additionally has oligonucleotide probes designed from archaea and fungi, two categories not evaluated in the da Jesus et al. [22] studies.

Overall, our molecular data support our hypothesis that switchgrass soils were much more similar to grassland soils than were sorghum soils. Using the GeoChip FGA approach, which is based on DNA probes for functional microbial genes rather than for specific taxa, we observed some significant differences among the N cycling genes for nitrogen fixation, nitrification, and denitrification. In addition to the known effects of N fertilization on soil microbial communities [46–49], other factors reported to influence microbial composition and activities in soils include plant species and genotype, soil type, soil structure and pH, and the interactions and feedback between plants and soils [37–42]. Use of the GeoChip 4.0 allowed us to look for microbial gene diversity and abundance differences among diverse geographic samples across multiple gene categories that related to important ecosystem processes such as C, N, P, and S cycling. When the microbial gene diversity of all samples was considered, sorghum samples were parsed by land use that also interacted significantly with geographic location. That is, microbial gene diversity in sorghum soil samples was lower than that in grassland soils, and there was lower microbial gene diversity in sorghum soils compared to grasslands. Our FGA results indicate that the most significant differences were at the microbial community level, rather than at the level of individual types of functional genes.

The very different centers of origin of the two biofuel crops may have impacted our findings of greater microbial gene diversity and abundance in switchgrass as compared to sorghum. That is, sorghum is not native to the USA; it originated in northeastern Africa, whereas switchgrass is a native plant found in the Great Plains, Upper Midwest and Eastern USA, but not in the Far West (WA, OR, and CA). Switchgrass has thus conceivably had a longer period of time to develop a more abundant and diverse indigenous rhizosphere and

soil microflora than has sorghum, in areas of the USA where switchgrass is a native species. The soil microbial functional gene diversity data support our hypotheses and are consistent with switchgrass, but not sorghum, being native to much of the USA. In samples from South Dakota, Wisconsin, Illinois, and Nebraska, gene diversity in the perennial switchgrass crop fields was similar to that in reference grasslands. In contrast, functional gene diversity was significantly lower in Washington and Tennessee switchgrass fields as compared to their respective reference grasslands. However, switchgrass is not indigenous to Washington, and although Tennessee is considered within the native range for switchgrass, it was introduced into previously cleared oak-hickory forest land. At all locations, soil gene diversity was lower in the annual crop sorghum fields than in reference grasslands.

While crop sorghum is not considered weedy, it hybridizes with its perennial relative, johnsongrass (*Sorghum halepense* L.), a noxious weed [52–54]. Thus gene flow from sorghum to johnsongrass, of genes selected or introduced for more rapid growth and abiotic or biotic stress tolerance, could conceivably result in more competitive, harder to control, johnsongrass. It is of interest to note that invasiveness of the noxious exotic weed *Centaurea maculosa* L. [55, 56] has been attributed in part to the lack of antagonistic or pathogenic soil microbiota that essentially act as biocontrol agents. Our observations of higher microbial gene diversity in switchgrass sites as compared to sorghum sites may thus suggest a lower potential for weediness of either feral cultivated switchgrass or crop-native switchgrass hybrids.

Conclusions

To help inform crop and land management decisions to ensure the sustainability of both biofuel crop production and soil ecosystem services, we used multidisciplinary analytical methods to compare soils obtained from switchgrass and sorghum crop fields from nine diverse geographic locations in seven states and compared them to nearby perennial grassland reference soils. Despite the large spatial variability in edaphic properties and the wide range of latitudes where soils were sampled, our results suggest that cultivation of switchgrass, a perennial, has less impact on edaphic properties and microbially mediated soil ecosystem services than sorghum, an annual crop. In future studies, it would be of interest to evaluate these and additional biofuel crops in multiple geographic growing areas where the crop soils can be compared longer term to common reference grassland and also to agronomic control sites, by the use of complementary chemical, biological, and molecular methods.

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