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Microbial Carbon Substrate Utilization Differences among High- and Average-Yield Soybean Areas

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Academic Editor: Eric C. Brevik

Received: 23 March 2017; Accepted: 24 May 2017; Published: 30 May 2017

Abstract: Since soybean (*Glycine max* L. (Merr.)) yields greater than 6719 kg ha⁻¹ have only recently and infrequently been achieved, little is known about the soil microbiological environment related to high-yield soybean production. Soil microbiological properties are often overlooked when assessing agronomic practices for optimal production. Therefore, a greater understanding is needed regarding how soil biological properties may differ between high- and average-yielding areas within fields. The objectives of this study were to (i) evaluate the effects of region on soil microbial carbon substrate utilization differences between high- (HY) and average-yield (AY) areas and (ii) assess the effect of yield area on selected microbiological property differences. Replicate soil samples were collected from the 0–10 cm depth from yield-contest-entered fields in close proximity that had both a HY and an AY area. Samples were collected immediately prior to or just after soybean harvest in 2014 and 2015 from each of seven geographic regions within Arkansas. Averaged across yield area, community-level carbon substrate utilization and Shannon's and Simpson's functional diversity and evenness were greater ($p < 0.05$) in Region 7 than all other regions. Averaged across regions, Shannon's functional diversity and evenness were greater ($p < 0.05$) in HY than in AY areas. Principal component analysis demonstrated that a greater variety of carbon substrates were used in HY than AY areas. These results may help producers understand the soil microbiological environment in their own fields that contribute to or hinder achieving high-yielding soybeans; however, additional parameters may need to be assessed for a more comprehensive understanding of the soil environment that is associated with high-yielding soybean.

Keywords: carbon substrate; soybean; high-yield; Arkansas; principal component analysis

1. Introduction

Microorganisms exist in nearly all environments, and since microbes occupy the base of the food chain, microbes are the first organisms to react to changes in the environment [1]. Microbes contribute to soil nutrient levels, plant processes and functions, and overall crop health and productivity [2,3]. Furthermore, soil microbial communities are affected by inherent soil properties and current conditions, crop management approaches, and aboveground vegetation presence and type [2,3]. Therefore, microorganisms are often a precursor to changes in the health of an environment as a whole [4], particularly the soil environment.

The assessment of microbial communities may provide greater insight into ecosystem roles than isolation of specific community members [5]. Chemoheterotrophic bacteria utilize specific organic sources of carbon (C) and energy for growth, and the ability of species to use diverse substrates can be used to identify and characterize cultures and communities [6]. Although community-level

physiological profiling (CLPP) involves inoculating plates with mixed cultures of microbes, where only a small percentage are culturable, CLPP is effective at detecting spatial and temporal changes in soil communities, is widely used, and provides information regarding functional aspects of soil communities [2–4,6,7]. Konopka et al. [6] and Haack et al. [7] noted that while substrate oxidation patterns may not correlate directly to microbial growth, number of species, or species richness, patterns are sometimes nonlinear, with a lag phase, log phase, and stationary phase common to bacterial logistic growth curves.

The Biolog EcoPlate™ (Biolog, Inc., Hayward, CA, USA) is a system of three replications of wells, where each well contains one of 31 of the most utilized C sources for soil microbial, primarily bacterial, community analysis [4,8]. The EcoPlate™ approach offers a more rapid evaluation of metabolic profile diversity of a microbial community [9] than more traditional molecular assay approaches, such as phospholipid fatty-acid analysis (PFLA) or denaturing/temperature gradient gel electrophoresis (DGGE/TGGE) [10]. Evaluating substrate use using the EcoPlate™ allows for functional diversity to be assessed and systematically compared among microbial communities from environmental samples [8–10]. However, challenges may arise when working with EcoPlates™. For example, oligotrophs, organisms that can survive with low nutrient concentrations, may produce an all-negative response in microplates, and, additionally, color that develops in the blank well may occur as a result of spore formation or cell lysis [7]. Furthermore, a strong correlation exists between inoculum cell density and color development rate in the EcoPlate™, which can lead to mistaking community differences for total populations [7]. Therefore, for proper analysis and sound results, it is crucial that metabolically active cells be inoculated and the same amount is inoculated across wells [2,6,7]. Haack et al. [7] demonstrated a lack of similarity in replicates, due to heterogeneity in the soil samples that were collected. However, EcoPlates™ have been shown to be more effective at distinguishing minute changes in the environment compared to other methods [2], such as PFLA or DGGE/TGGE [4,10], and are quicker, simpler, and less labor intensive and costly than culturing [6].

Legume crops, such as soybean, are agriculturally important, not only for keeping up with the food demand caused by increased world populations and greater individual incomes, but by having the ability to form symbiotic relationships with bacteria and fungi [11]. The broad range of rhizosphere microbial species in soil, which affect plant-soil interactions and likely contribute both directly and indirectly to yield, have not been well-characterized. With estimates of 6000 to 10,000 species (g soil)⁻¹, ecological population interactions and functional redundancy complicate the understanding of which taxa contribute to ecological functioning under dynamic environmental conditions. However, soil microorganism diversity is an important soil quality indicator [12,13]. The EcoPlate™ enables the comparison of the actual overall microbial community function in different samples based on cells being active enough to utilize the specific C sources in the plate [14].

Certain soil environments have been shown to produce ultra-high crops yields (i.e., >6719 kg ha⁻¹ or 100 bu ac⁻¹), particularly for soybean [15]. However, it is unknown to what extent soil microbiological properties contribute to yield differences between areas with ultra-high yields and areas with average yields within the same production field or in adjacent fields with similar soil physical properties. Yield contests have been used by various crop industries in the past few decades as an incentive for individual agricultural producers to push the limits of crop production, at least on a small parcel of land. Consequently, state-wide yield contests offer a unique opportunity for paired comparisons of the soil physical, chemical, and particularly the under-studied biological environment associated with ultra-high and average yields. Therefore, the objectives of this study were to (i) evaluate the effects of region (i.e., geographically isolated area for yield-contest purposes) on soil microbial C substrate utilization differences between high- and average-yielding areas and (ii) assess the effect of yield area (i.e., high- versus average-yield area) on selected microbiological properties. It was hypothesized that the substrate utilization rate and both Shannon's and Simpson's functional diversity indices would be greater in the high-yield areas as a result of more intensive agronomic management practices.

2. Materials and Methods

2.1. “Grow for the Green” Yield Contest

In 1999, “Grow for the Green”, an annual soybean yield contest, was initiated in Arkansas by the Arkansas Soybean Promotion Board together with the Arkansas Soybean Association [16]. In 2013, Arkansas was split into seven geographic regions (Figure 1), and an eighth, statewide, non-genetically-modified-organism category for yield-contest purposes. The seven regions are as follows: Division (1): Northeast Delta; Division (2): Northeast; Division (3): White River Basin; Division (4): Central and Grand Prairie; Division (5): East Central Delta; Division (6): Southeast Delta; and Division (7): Western (Figure 1).

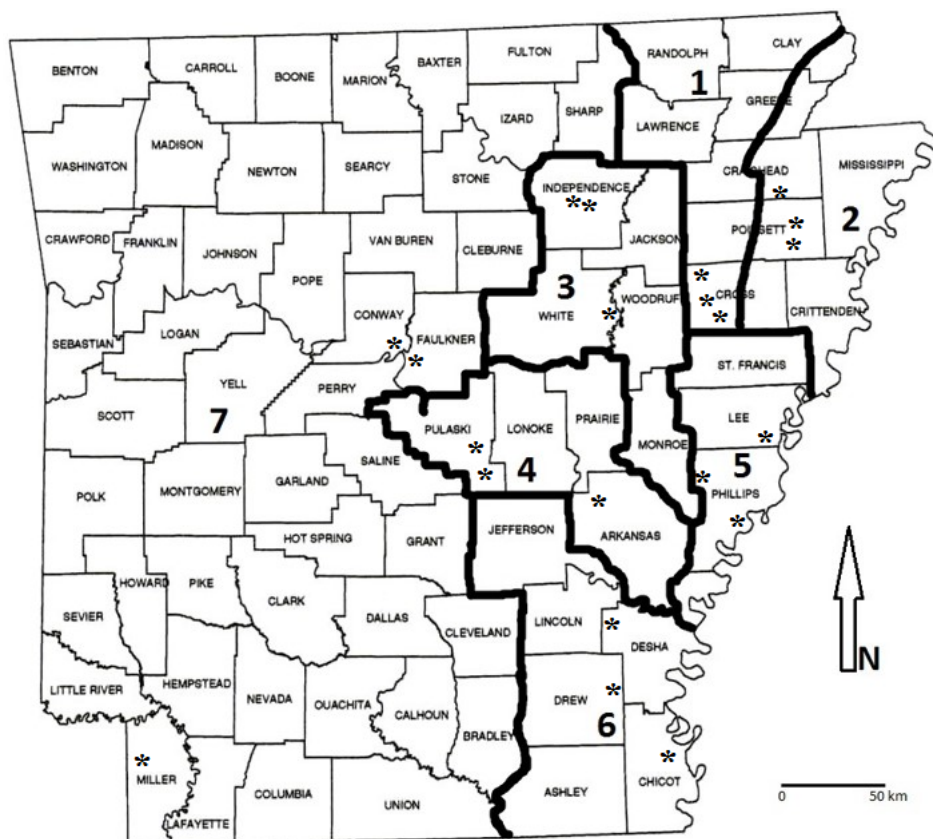


Figure 1. Seven regions for the “Grow for the Green” contest sponsored by the Arkansas Soybean Promotion Board together with the Arkansas Soybean Association. Division 1: Northeast Delta; Division 2: Northeast; Division 3: White River Basin; Division 4: Central and Grand Prairie; Division 5: East Central Delta; Division 6: Southeast Delta; Division 7: Western. Asterisks denote sampling sites in 2014 and 2015. County names are indicated within each county area.

2.2. Study Area Descriptions

In late summer to early fall 2014, two producers who had a field entered into the 2014 yield contest as well as an average-yielding area (AY) within the same field or in an adjacent field in each of the seven state-wide regions were identified as willing cooperators (Table 1). The location of the AY area was based on each producer’s qualitative and quantitative, historic knowledge of the productivity of their own fields and areas within fields. Average-yield areas were selected with cooperation from the landowner to be within 0.25 km of the HY area, have the same soil series present as the HY area (Table 1), and excluded areas with known historic problems due to sub-surface compaction, weeds, and/or poor irrigation coverage.

Table 1. Summary of participating grower sites in the 2014 and 2015 “Grow for the Green” yield contest sponsored by the Arkansas Soybean Promotion Board.

Year	Region [†]	Site	MLRA [‡]	Dominant Soil Series (Taxonomic Description) in High-Yielding Area [§]	Soil Surface Texture [§]
2014	1	1	131A	Dundee (Typic Endoaqualfs)	Fine sandy loam
		2	131A	Dundee (Typic Endoaqualfs)	Silt loam
	2	1	134	Arkabutla (Fluventic Endoaquepts)	Silt loam
		2	134	Crowley (Typic Albaqualfs)	Silt loam
	3	1	131A	Bosket (Mollic HapludalFs)	Fine sandy loam
		2	131A	Wiville (Ultic HapludalFs)	Fine sandy loam
	4	1	131B	Hebert (Aeric Epiaqualfs)	Silt loam
		2	131B	Rilla (Typic HapludalFs)	Silt loam
	5	1	131A	Commerce (Fluvaquentic Endoaquepts)	Silt loam
		2	134	Dubbs (Typic HapludalFs)	Silt loam
	6	1	131B	Rilla (Typic HapludalFs)	Silt loam
		2	131B	Hebert (Aeric Epiaqualfs)	Silt loam
	7	1	131B	Gallion (Typic HapludalFs)	Silt loam
		2	131C	Bossier (Aeric Epiaquerts)	Clay
2015	1	1	131A	Dubbs (Typic HapludalFs)	Silt loam
		2	131A	Dundee (Typic Endoaqualfs)	Silt loam
	2	1	134	Arkabutla (Fluventic Endoaquepts)	Silt loam
		2	134	Crowley (Typic Albaqualfs)	Silt loam
	3	1	131A	Bosket (Mollic HapludalFs)	Fine sandy loam
		2	131A	Wiville (Ultic HapludalFs)	Fine sandy loam
	4	1	131B	Portland (Chromic Epiaquerts)	Silty clay
		2	131D	Rilla (Typic HapludalFs)	Silt loam
	5	1	131A	Henry (Typic Fragiaqualfs)	Silt loam
		2	134	Dundee (Typic Endoaqualfs)	Silt loam
	6	1	131B	Dundee (Typic Endoaqualfs)	Silt loam
		2	131A	Hebert (Aeric Epiaqualfs)	Silt loam
	7	1	131B	Gallion (Typic HapludalFs)	Silt loam
		2	131B	Roxana (Aeric Epiaquerts)	Silt loam

[†] The regions are as follows Region 1: Northeast Delta; Region 2: Northeast; Region 3: White River Basin; Region 4: Central and Grand Prairie; Region 5: East Central Delta; Region 6: Southeast Delta; Region 7: Western. [‡] Major Land Resource Area [17]: 131A: Southern Mississippi River Alluvium; 131B: Arkansas River Alluvium; 131C: Red River Alluvium; 131D: Southern Mississippi River Terraces; 134: Southern Mississippi Valley Loess. [§] Data obtained from [18,19].

Considering the HY areas were entered into a yield contest, landowners necessarily used additional management practices and/or amendments that were not used in the AY areas. The soybean variety planted was the same between yield areas in three of the seven regions (i.e., Regions 1, 2, and 4), but differed in the other four regions (i.e., Regions 3, 5, 6, and 7). However, the same maturity group soybean was planted for both yield areas in every region. Planting date was the same for both yield areas in some regions, but not in others, though planting date did not differ by more than 1 week between yield areas in any region. Within all regions, row spacing and irrigation management were similar between yield areas, but seeding density was unknown for all yield areas. Though there were differences between yield areas, this study was conducted to determine if additional, site-specific management that was conducted on a small area to boost soybean yields sufficiently altered soil biological properties and C-substrate utilization to warrant further research.

The contest-verified yield was used for the high-yield area, while the actual yield harvested in the field containing the average-yield area was used for the average-yield area to confirm yield differences between the two areas each year. For the 2015 growing season, this process was repeated (Table 1). Of the producers identified to cooperate in 2015, seven were the same producers who were cooperators in

2014; however, different high-yield (HY) and AY areas were used each year. The two areas (i.e., HY and AY) per producer within each region were used for subsequent soil sampling purposes in both years. Table 1 further summarizes the soil series, surface texture, and Major Land Resource Area (MLRA) of the sites sampled in 2014 and 2015. Region 7 includes Major Land Resource Area (MLRA) 131C (Red River Alluvium), which consists mainly of Vertisols, Entisols, Alfisols, and Inceptisols with 37% of the land area in row-crop agriculture [20] (Table 1). Major Land Resource Areas 131A (Southern Mississippi River Alluvium) and 131B (Arkansas River Alluvium) have 70% of their land area under row-crop agricultural production, consist mainly of Alfisols, Vertisols, Inceptisols, and Entisols, and occupy portions of Regions 1, 2, 3, 5, and 6 and Regions 4 and 6, respectively [20]. In MLRAs 131D (Southern Mississippi River Terraces) and 134 (Southern Mississippi Valley Loess), which consist mainly of Alfisols, Entisols, Inceptisols, and Ultisols and occupy portions of Regions 4 and 6 and Regions 2 and 5, respectively, nearly 40% of the land area is under row-crop agriculture [20] (Table 1).

Annual precipitation varies slightly across Arkansas, with annual amounts in counties sampled ranging from 1225 mm in Craighead and Cross Counties [21] in the northern portion of Arkansas (Figure 1) to 1363 mm in Chicot and Desha Counties [21] in the southern portion of Arkansas. Similar to precipitation, average air temperature varies slightly across the state. The lowest average January temperature (2.1 °C) and the lowest average annual temperature (15.1 °C) are both in Craighead County [21]. The greatest average July air temperature of the counties sampled (28.1 °C) occurs in Philips, Chicot, and Desha Counties [21], while the greatest average annual air temperature (17.7 °C) occurs in Miller County [21] in the southwestern portion of Arkansas (Figure 1).

2.3. Sample Collection and Processing

In 2014 and 2015, immediately before or just after soybean had been harvested in each HY and AY area, three sample points were established in a planted row approximately 62 m apart from one another. At each point, soil samples were collected from the 0- to 10-cm depth interval using a beveled, 4.8-cm diameter, stainless steel core chamber. Samples were oven-dried at 70 °C for 48 h, weighed for bulk density (BD) determinations, and ground to pass a 2-mm mesh sieve. Soil organic matter (SOM) concentration was determined by weight-loss-on-ignition at 360 °C for 2 h and total C (TC) and N (TN) concentrations were determined by high-temperature combustion using a VarioMax CN analyzer (Elementar Americas Inc., Mt. Laurel, NJ, USA). Measured BD and SOM, TC, and TN concentrations (mg kg^{-1}) were used to determine SOM, TC, and TN contents. Using measured soil contents, C:N ratio, and C (C_{frac}) and N (N_{frac}) fractions of soil OM were calculated.

An additional set of 10 soil samples were collected from the 0- to 10-cm depth interval using a 2-cm diameter push probe and mixed for one composite soil sample per sampling point from within 0.5 m in both directions along the row from the three sample points. Samples were immediately put on ice and stored for approximately five to eight months in a refrigerator at 4 °C for biological property determinations.

For soil biological properties, a series of dilutions (i.e., 10^{-1} , 10^{-2} , 10^{-3}) were prepared for each soil sample and were chemically flocculated with 0.85% sodium chloride (NaCl) to remove suspended clays. An aliquot of 150 μL of the 10^{-3} dilutions was dispensed by pipette into each of the 96 wells of Biolog EcoPlates™, similar to procedures in Yu et al. [22], and the plates were incubated in the dark at 20 °C for 6 days. Table 2 summarizes the various C sources used in the EcoPlates™ for soil microbial, primarily bacterial, community analysis. A Synergy HT microplate reader (Biotek Instruments, Inc., Winooski, VT, USA), set to a wavelength of 590 nm, was used to read the plates immediately and at intervals of 24, 36, 48, 60, 72, 96, 120, 144 h after incubation. Similar to Yu et al. [22], average well-color development (AWCD) was determined after subtracting the absorbance in the control well for each substrate and setting all negative readings to zero to normalize data. Absorbance values were plotted against time to calculate the three-parameter logistic equation for community level substrate utilization rates (Equation (1)):

$$\text{AWCD (abs day}^{-1}\text{)} = (\theta_1)/(1 + \theta_2 * e^{\theta_3 * X}) \quad (1)$$

where θ_1 was the asymptote, θ_2 was the utilization rate, θ_3 was the inflection point of the curve, and X was the hour of measurement (i.e., 72). The 72 h mark represented the time when the majority of the inflection points occurred for the logistic curve. Shannon's diversity (Equation (2)) and evenness (Equation (3)) and Simpson's diversity (Equation (4)) and evenness (Equation (5)) based on the 72 h AWCD results based on the C substrate utilization patterns were used to calculate functional diversity on EcoPlates™ [23]. The equation for Shannon's diversity (H) was:

$$H = -\sum p_i \ln(p_i) \quad (2)$$

where p_i was the proportion of species i relative to the total number of species (i.e., substrates, 31 total) [24,25], while the equation for Shannon's evenness (E_H) was:

$$E_H = H/\ln(31) \quad (3)$$

where 31 was the number of substrates on the EcoPlates™. The equation for Simpson's diversity (D) was:

$$D = 1/\sum p_i^2 \quad (4)$$

where p_i was the proportion of species i relative to the total number of species [26,27], and the equation for Simpson's evenness (E_D) was:

$$E_D = D/31 \quad (5)$$

Table 2. Carbon substrates represented on EcoPlates™ [4,28].

Well Number	Compound Type	Compound	
7	Carbohydrates	D-cellobiose	
8		α -D-lactose	
9		β -methyl-D-glucoside	
10		D-xylose	
11		i-erythritol	
12		D-mannitol	
13		N-acetyl-D-glucosamine	
15		Glucose-1-phosphate	
16		D,L- α -glycerol phosphate	
17		D-galactonic acid γ -lactone	
31		Amines and amides	Phenylethylamine
32			Putrescine
2		Carboxylic and ketonic Acids	Pyruvic acid methyl ester
18	D-galacturonic acid		
21	γ -hydroxybutyric acid		
22	Itaconic acid		
23	α -ketobutyric acid		
24	D-malic acid		
19	Phenolics	2-hydroxy benzoic acid	
20		4-hydroxy benzoic acid	
3	Polymers	Tween 40	
4		Tween 80	
5		α -cyclodextrin	
6	Amino acids	Glycogen	
25		L-arginine	
26		L-asparagine	
27		L-phenylalanine	
28		L-serine	
29		L-threonine	
30	Glycyl-L-glutamic acid		

2.4. Statistical Analyses

A two-factor analysis of variance (ANOVA), assuming a completely randomized design, was conducted using SAS (version 9.3, SAS Institute, Inc., Cary, NC, USA) to evaluate the effects of region (i.e., seven different regions in Arkansas) and yield area (i.e., HY and AY), and their interaction on AWCD rate, H , E_H , D , and E_D based on measured color development in EcoPlates™. Year (i.e., 2014 or 2015) was initially treated as a fixed effect, but was non-significant as a main or interaction effect. Therefore, the model was simplified by treating year as a random effect. Significance was judged at $p < 0.05$ and, when appropriate, means were separated by least significant difference at $\alpha = 0.05$. In addition, color development in wells of the EcoPlates™ was used to perform principal component analysis (PCA) using JMP (version 12 Pro, SAS Institute, Inc., Cary, NC, USA), where HY and AY areas were evaluated separately in order to elucidate differences in substrate utilization patterns. Linear correlation analyses were also conducted in JMP to evaluate the relationships between SOM, TC, TN, C:N ratio, C_{frac} , and N_{frac} and H and D combined across regions and yield areas. A one-factor ANOVA was also conducted to evaluate yield differences between the two yield areas.

3. Results and Discussion

3.1. Yield Differences

Across both years of yield results, soybean yields were greater ($p < 0.001$) from the HY than from the AY areas. The mean yield for all AY areas was 4701 kg ha^{-1} , which was 1409 kg ha^{-1} greater than the Arkansas state average from 2015 and 804 kg ha^{-1} greater than the Nebraska state average in 2015, in which Nebraska was the most productive soybean state in the United States in 2015 [29]. The mean yield for all HY areas was 5498 kg ha^{-1} . These results confirm adequate selection of yield areas that produced significantly different yields.

3.2. Treatment Effects

For the 2014 and 2015 soybean growing seasons, across all regions and yield areas, soil microbiological properties measured on EcoPlates™ from samples collected from the top 10 cm varied in range and magnitude (Table 3). For both yield areas, rates of AWCD from community level physiological profiles based on utilization of 31 C substrates ranged from 0.023 in an AY area to $0.082 \text{ Abs day}^{-1}$ in a HY area (Table 3), which were both measured in Region 7. Region 7 encompasses the entire western portion of Arkansas (Figure 1), and is characterized by many different soil characteristics. Averaged across yield areas, AWCD rate differed ($p < 0.05$; Table 4; Figure 2) among regions and was greatest in Region 7, which did not differ ($p > 0.05$) from that measured in Region 4. Averaged across yield area, AWCD rate in Region 4 also did not differ ($p > 0.05$; Figure 2) from that measured in Regions 1, 2, and 6, which did not differ ($p > 0.05$) from that measured in Regions 3 and 5. Deng et al. [30] reported that enhanced levels of soil nutrients, as well as rhizosphere secretions, may result in greater growth rates of plants, which leads to increased nutrient cycling rates. In addition, these rates are not measures of microbial growth per se, but rather AWCD development, which is linked to cellular respiration of the specific C substrates on the EcoPlates™ [23].

Table 3. Summary of minimum and maximum values for soil microbiological properties measured on EcoPlates™ [4] averaged across high- and average-yield areas and years (2014 and 2015) for the seven regions in the “Grow for the Green” yield contest across Arkansas. Values are rounded.

Property †	Region						
	1	2	3	4	5	6	7
AWCD rate	0.036–0.061	0.042–0.061	0.039–0.063	0.033–0.070	0.037–0.065	0.042–0.062	0.023–0.082
Shannon’s diversity	2.37–2.99	2.42–2.97	2.35–2.94	2.22–3.02	2.21–3.25	2.65–2.92	2.49–3.14
Shannon’s evenness	0.69–0.87	0.70–0.86	0.68–0.85	0.65–0.88	0.64–0.95	0.77–0.85	0.73–0.92
Simpson’s diversity	6.66–17.1	6.43–16.2	6.87–16.0	6.22–18.9	7.52–21.5	11.1–15.6	6.79–20.1
Simpson’s evenness	0.21–0.55	0.21–0.52	0.22–0.52	0.20–0.61	0.24–0.69	0.36–0.50	0.22–0.65

† Units and abbreviations are as follows: AWCD (Average well color development) rate (absorbance day⁻¹).

Table 4. Analysis of variance summary of the effects of region (i.e., 1 through 7 of Arkansas’ “Grow for the Green” yield contest), yield area (i.e., high- and average-yield), and their interaction on microbiological growth rate and Shannon’s and Simpson’s diversities measured on EcoPlates™ [4] averaged across years (2014 and 2015).

Variable	Region	Yield Area	R x YA
Growth rate †	<0.001	NS ‡	NS
Shannon’s diversity	0.043	0.004	NS
Shannon’s evenness	0.043	0.004	NS
Simpson’s diversity	0.036	NS	NS
Simpson’s evenness	0.036	NS	NS

† Units are absorbance day⁻¹. ‡ Effects and interactions that are not significant at the 0.05 level are represented by NS (i.e., $p > 0.05$).

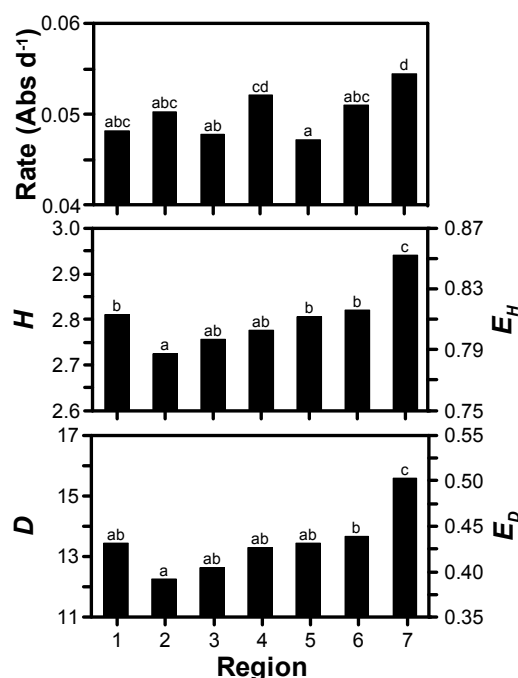


Figure 2. Microbial substrate utilization rate (absorbance day⁻¹), Shannon’s diversity (H) and evenness (E_H), and Simpson’s diversity (D) and evenness (E_D), averaged across yield areas, measured in the seven regions of the “Grow for the Green” yield contest across Arkansas in 2014 and 2015. Bars with different letters are significantly different (i.e., $p \leq 0.05$).

Considering the EcoPlates™ approach's sensitivity to differences in microbial biomass and/or population size, results obtained in this study may be slightly biased, as the potential differences in microbial biomass and/or population size were unknown and not specifically controlled for in these experiments. Consequently, if the assumption of similar microbial biomass and/or population size between AY and HY areas was not valid, AWCD may have slightly over- or under-represented actual functional diversity captured in the soil samples. However, it is likely that microbial biomass and/or population size were within the same order of magnitude between AY and HY areas, since soil sampling of all sites occurred at the same time in the growing season and would have all been reflective of end-of-the-growing-season management when potential differences in nutrient availabilities and soil moisture would be minimal.

Shannon's diversity ranged from 2.21, which corresponded to an E_H of 0.64, to 3.25, which corresponded to an E_H of 0.95, both of which were measured in Region 5 (Table 3). Region 5 is in the middle portion of the Mississippi River Delta (Figure 1), and consists of mainly alluvial and some loess soils. Measured H values are below those reported by Yu et al. [22], where H averaged 3.5 across treatments in mulberry (*Morus* spp.) production on a clay-loam soil in China. However, Janniche et al. [14] reported measured H across treatments in agriculturally affected groundwater (2.97 to 3.04) which was within the range measured in this study. Averaged across yield area, H and E_H were greater ($p < 0.05$; Figure 2) in Region 7 than in all other regions. Shannon's diversity and E_H were greater ($p < 0.05$) in Regions 1, 5, and 6 than in Region 2, which did not differ ($p > 0.05$; Figure 2) from H and E_H in Regions 3 and 4.

Simpson's diversity ranged from 6.22, which corresponded to an E_D of 0.20, measured in Region 4 to 20.1, which corresponded to an E_D of 0.65, measured in Region 7 (Table 3). Region 4 includes the Grand Prairie region of Arkansas, normally associated with rice (*Oryza sativa* L.) production [31]. Similar to H and E_H , D and E_D were greater ($p < 0.05$) in Region 7 than in all other regions. Simpson's diversity and E_D were greater ($p < 0.05$) in Region 6 than in Region 2, but did not differ ($p > 0.05$; Figure 2) from D and E_D in Regions 1, 3, 4, and 5.

Shannon's diversity index mathematically weights rare species (i.e., substrate utilization) more than common ones, while Simpson's diversity index mathematically weights common species more and rarer species relatively less [14]. Therefore, the two diversity indices are not meant to be equivalent in magnitude. Because richness was a constant of 31 substrates utilized, both E_H and E_D are simple calculations based on their respective diversities. Therefore, it was expected that the same differences that occurred among regions with diversity would occur for evenness as well. As expected, both diversity indices and evennesses produced similar results among regions (Figure 2). Both diversities and evennesses were significantly greater in Region 7 compared to the other six regions (Figure 2). Both diversities and evennesses were numerically smallest in Region 2, while no differences in diversities and evennesses occurred among the other four regions (Figure 2). Consequently, both Shannon's and Simpson's indices appear to similarly capture the diversity of the microbial communities associated with soils under cultivated, irrigated soybean production in Arkansas. In addition, it appears that there are few to no extreme differences in rare and/or common species associated with microbial communities under cultivated, irrigated soybean production in Arkansas that would lead to differing interpretations of microbial diversity depending on which diversity index was used.

The boundaries of the regions of the "Grow for the Green" yield contest are somewhat arbitrary and contain large variations in soil-forming factors (i.e., differences in soil parent material, climate, topography, etc.) within and across regions. Therefore, regional differences may be random, and perhaps do not reflect consistencies within and among regions. Considering no previous studies have evaluated differences among regions in the "Grow for the Green" yield contest, these results may provide a framework for characterizing the major soybean-yield-influencing soil microbial parameters across regions in Arkansas.

As hypothesized, across the 2014 and 2015 soybean growing seasons and averaged across regions of the "Grow for the Green" yield contest, H and E_H were greater ($p < 0.05$; Table 4; Figure 3) in HY than

in AY areas. Differences that occurred between yield areas with diversity also occurred for evenness. Since it can be assumed that management for contest purposes included practices likely to benefit soil health and fertility, this may explain the greater diversity in HY areas. Soil microbial diversity and community structure are affected by different management practices [32], and the influence of fertilizer applications on diversity is complex, perhaps related to fertilizer type, application rate and placement, and soil texture [22]. Studying mulberry growth in a clay-loam soil in China, Yu et al. [22] stated that the soil physio-chemical characteristics (e.g., SOM, soil nutrient content, and pH) governed microbial functional diversity. Yu et al. [22] also cited studies that reported appropriate fertilizer management, including the application timing, type, and quantity, can increase microbial functional diversity; thus, functional diversity may induce changes in resulting substrate utilization. However, Girvan et al. [32] reported that biodiversity can decrease from excessive application of fertilizers and pesticide use and that community compositions are determined primarily by the soil environment rather than different management practices. Degens et al. [12] measured microbial catabolic diversity by directly adding organic compounds to the soil and measuring respiration; however, although this approach was prescribed by Nannipieri et al. [13], it would have been impractical in the present study due to the wide geographic separation among research sites.

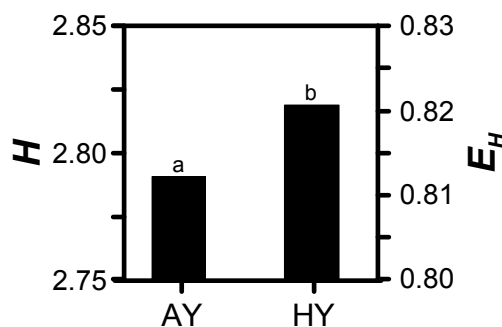


Figure 3. Shannon's diversity (H) and evenness (E_H), averaged across regions, measured in the average- (AY) and high-yield (HY) areas of the "Grow for the Green" yield contest across Arkansas in 2014 and 2015. Bars with different letters are significantly different (i.e., $p \leq 0.05$).

Multiple potential reasons may explain the lack of differences ($p > 0.05$) in AWCD rate between yield areas. One explanation is that the substrate utilization rates measured in this study are culture-based; thus, the organisms are extracted from their natural environment and may not be culturable or are inactive [8,32]. Furthermore, rates are tested under well-defined conditions that do not mimic in-situ conditions [23]. Additionally, the substrate utilization rates measured in this study are "community" rates based on all substrates on the EcoPlates™, and some species may antagonize or synergistically interact with each other [8,23]. Some specific substrates may be more informative than others, and the combination of several approaches (i.e., other ways to evaluate the data such as principal component analysis; PCA) may be necessary [19,23,32].

3.3. Principal Component Analysis

Principal component analysis is a statistical method for reducing redundancy in data sets, where each principal component characterizes the data, while also capturing as much of the variation within the original data as possible. For HY areas combined across 2014 and 2015, principal components (PCs) 1 and 2 explained 19.4 and 8.1% (Table 5), respectively, of the variation in the EcoPlates™ results, which corresponded to eigenvalues of 6.01 and 2.53, respectively. A carbohydrate, α -D-lactose, with coefficient 0.2899 (Table 5), received the greatest weight in the PC1. The relative sizes of the component coefficients, often called loadings, suggest the univariate contribution of an individual factor to a given PC, forming an eigenvector as a whole. Other substrates that had coefficients greater than 0.25 for PC1 were the amino acid glycl-L-glutamic acid, the carbohydrate D-xylose, the carboxylic and ketonic acids

α -ketobutyric acid and γ -hydroxybutyric acid, and the phenolic compound 2-hydroxy benzoic acid. For PC2, the greatest coefficient (0.3350; Table 5) occurred at the ketonic pyruvic acid methyl ester, followed by the carbohydrates D-cellobiose, *N*-acetyl-D-glucosamine, glucose-1-phosphate, and glycogen, a polymer, with coefficients greater than 0.25. Every compound group represented (Tables 2 and 5) on EcoPlates™, except the amines and amides, contributed to the PCs that explained the most variation in substrate utilization for HY areas. Chakraborty et al. [33] reported fertilization reduced amine and amide use with long-term fertilization in jute (*Corchorus* spp.)-rice-wheat (*Triticum* spp.) rotation in tropical soil.

Table 5. Principal components (PCs) 1 and 2, their respective eigenvalues (including percentage of variation explained), substrates loaded in each PC, substrate type, and respective eigenvector for substrate in each PC derived from measurements on EcoPlates™ [4] from high-yield and average-yield areas across Arkansas in 2014 and 2015.

Yield Area	Component	Eigenvalue	Substrate	Type †	Coefficient ‡
High-yield	PC 1	6.01 (19.4%)	α -D-lactose	C	0.2899
			Glycl-L-glutamic acid	A	0.2747
			D-xylose	C	0.2688
			α -ketobutyric acid	K	0.2675
			2-hydroxy benzoic acid	P	0.2660
			γ -hydroxybutyric acid	K	0.2632
	PC 2	2.53 (8.1%)	Pyruvic acid methyl ester	K	0.3350
			D-cellobiose	C	0.3208
			<i>N</i> -acetyl-D-glucosamine	C	0.3004
			Glucose-1-phosphate	C	0.2898
			Glycogen	O	0.2790
Average-yield	PC 1	6.89 (22.2%)	D-xylose	C	0.3089
			α -D-lactose	C	0.3052
			L-threonine	A	0.2921
			i-erythritol	C	0.2829
			α -cyclodextrin	O	0.2676
			Glycl-L-glutamic acid	A	0.2667
	PC 2	2.37 (7.6%)	2-hydroxy benzoic acid	P	0.2648
			D-cellobiose	C	0.4162
			D-mannitol	C	0.3680
			Glucose-1-phosphate	C	0.3353
			β -methyl-D-glucoside	C	0.3110
			Tween 80	O	0.2557

† C is carbohydrates, A is amino acids, K is carboxylic and ketonic acids, O is polymers, and P is phenolics. ‡ Values are rounded. Only substrates with a coefficient > 0.25 are reported.

For AY areas combined across 2014 and 2015, 22.2% and 7.6% (Table 5) of the variation in substrate utilization on EcoPlates™ were explained by PCs 1 and 2, respectively, which corresponded to eigenvalues of 6.89 and 2.37, respectively. D-xylose, a carbohydrate, had the greatest coefficient (0.3089) for PC1, and carbohydrates α -D-lactose and i-erythritol, amino acids L-threonine and glycl-L-glutamic acid, the polymer α -cyclodextrin, and the phenol 2-hydroxy benzoic acid all had coefficients greater than 0.25 (Table 5). For PC2, the substrates with the greatest coefficient (0.4162; Table 5) were D-cellobiose, a carbohydrate, followed by the carbohydrates D-mannitol, glucose-1-phosphate, β -methyl-D-glucoside, and the polymer Tween® 80, with coefficients greater than 0.25. In contrast to the factors contributing to PCs 1 and 2 in the HY areas, the greatest factors contributing to PCs 1 and 2 in the AY areas were mainly carbohydrates, except for two amino acids (i.e., L-threonine and glycl-L-glutamic acid), one phenolic (2-hydroxy benzoic acid), and two polymers (α -cyclodextrin and Tween® 80; Table 5). The AY areas did not have a large proportion of variation on EcoPlates™ explained by carboxylic and ketonic acids and, similar to the HY areas, amines and amides.

Although carbohydrates are the most represented substrate group on EcoPlates™ (i.e., 10 of 31), only two (D,L- α -glycerol phosphate and D-galactonic acid γ -lactone) did not have coefficients greater than 0.25 for PCs 1 and 2 for HY and AY areas. Furthermore, both of the amines and amides, half of the carboxylic and ketonic acids (D-galacturonic acid, itaconic acid, and D-malic acid), one of two phenolics (4-hydroxy benzoic acid), one polymer (Tween® 40), and four of six amino acids (L-arginine, L-asparagine, L-phenylalanine, and L-serine) did not have coefficients greater than 0.25 for PCs 1 and 2 for HY and AY areas (data not shown). Factors with coefficients greater than 0.25 in common to PCs 1 and 2 for HY and AY areas (Table 5) were α -D-lactose, glycl-L-glutamic acid, D-xylose, 2-hydroxy benzoic acid, D-cellobiose, and glucose-1-phosphate. However, factors with coefficients greater than 0.25 present only in PCs 1 or 2 for HY areas (Tables 2 and 5) included one carbohydrate, three carboxylic and ketonic acids, and one polymer, while those factors present only in PCs 1 or 2 for AY areas (Tables 2 and 5) included three carbohydrates, one amino acid, and two polymers.

The carbohydrate with a relatively large coefficient (i.e., >0.25) in PC 2 from only HY areas was N-acetyl-D-glucosamine, a carbohydrate that polymerizes into chitin, which exists in many fungi and in the exoskeleton of many invertebrates [34]. The ketonic acid that had a large coefficient in PC 1 from HY areas was α -ketobutyric acid, one of the products of the catabolism of threonine, which is an amino acid that is another substrate on EcoPlates™ [35]. Another pathway for α -ketobutyric acid is eventually entering the citric acid cycle [35]. One carboxylic acid that had a large contribution to PC 1 from HY areas was γ -hydroxybutyric acid, which is a derivative of butyric acid, also known as butanoic acid or BTA, and a product of anaerobic fermentation [36]. The other carboxylic acid that had a large contribution to PC 2 from HY areas was pyruvic acid methyl ester, the ester of pyruvic acid, which is also an intermediate in the citric acid cycle [35]. The polymer that had a relatively large coefficient in PC 2 from HY areas was glycogen, a polysaccharide of glucose and main storage entity of glucose in fungi [37].

Carbohydrates that had a relatively large coefficient appearing only in PCs 1 or 2 in AY areas were α -D-lactose, D-mannitol, and β -methyl-D-glucoside. The disaccharide of glucose and galactose is α -D-lactose, while D-mannitol is the sugar alcohol of the simple sugar D-mannose [35]. One variant of methyl glucoside, β -methyl-D-glucoside, is a monosaccharide and a variation of the product of the reaction of glucose and methanol [38]. The amino acid that had a large coefficient in PC 1 in AY areas was L-threonine, which is used in the synthesis of proteins and also synthesized from *Escherichia coli* [39]. The polymers that had a large coefficient occurring only in PCs 1 or 2 in AY areas were α -cyclodextrin, an oligosaccharide [40] and Tween® 80, which is also known as polysorbate 80, a polymer of ethylene oxide [41].

Results indicated that soil microbial communities in HY areas were able to utilize a greater variety of substrate types, while communities in AY areas utilized mostly carbohydrates, and amino acids, phenolics, and polymers to a lesser extent. More carbohydrate and carboxylic acid utilization occurred in organic-inorganic fertilized soil, but amino acid utilization was greater in N fertilized soil in mulberry plantations where fertilization may have reduced diversity [22]. Greater levels of versatility indicate that soil microbial communities are more able to capitalize on various natural and anthropogenic compounds present in soil [42], and thus are potentially more active in HY areas. Although results of the substrate utilization rate analysis did not identify specific differences between yield areas, results of the PCA, combined with the diversity results, demonstrate that perhaps a greater abundance and relative proportion of certain bacterial and fungal individuals within soil microbial communities may contribute to greater yields. Additional analysis of the mechanisms underlying plant interactions with various soil microbes during growth in the field would perhaps further characterize the communities that contribute to greater soybean growth and resulting yield in the field.

3.4. Correlations

Combined across the 2014 and 2015 soybean growing season, SOM content was linearly correlated ($p < 0.05$; $n = 168$) with both H and D . Soil organic matter was weakly positively correlated with both

H ($p = 0.02$; $r = 0.18$) and D ($p = 0.03$; $r = 0.16$). Grayston et al. [43] reported that SOM was positively correlated with CLPP in 10 sites of a field study conducted in the United Kingdom. It may be intuitive that greater SOM contents are associated with greater functional diversity, as there is more substrate to support decomposers [44]. However, it can also be thought that greater diversities of microorganisms would be able to break down more SOM, since different soil microorganisms produce various enzymes to degrade different molecules and, hence, the microorganisms carry out specific processes during SOM mineralization [45]. These results illustrate the positive correlation between both H and D and SOM content in high- and average-yield soybean production systems across Arkansas. Total C and TN contents, C:N ratio, C_{frac} , and N_{frac} were not linearly correlated ($p > 0.05$) with either H or D .

Despite the measured microbial community differences and correlations among various soil properties and microbial community diversities, this study was not designed to determine cause and effect for the yield differences. Based on this preliminary study, it was not possible to ascertain if yield differences between HY and AY areas were a response to the associated microbial community, if the measured microbial community differences were a response to the associated plant productivity, or if plant productivity and microbial community diversity are both co-related to other abiotic factors not evaluated in this study. However, it is suspected that plant productivity in the high-yield environment and microbial community differences are complex and inter-related. Results of this study clearly demonstrated the need to further investigate cause and effect between yield and microbial community difference.

4. Conclusions

Averaged across the 2014 and 2015 soybean growing seasons, based on EcoPlates™ substrate utilization, soil microbiological properties differed between HY and AY areas and across soybean growing regions in Arkansas. Averaged across yield areas, substrate utilization rate and H , E_H , D , and E_D differed among regions. Additionally, averaged across regions, H and E_H were greater in HY than in AY areas. Results of PCA demonstrated a variety of C substrates contributed to the variation in overall substrate utilization, but amines and amides did not greatly contribute as reactive substrates in either yield area. In HY areas, a greater variety of C substrates was used, but in AY areas the greatest contributors were carbohydrates. Furthermore, across yield areas, SOM was positively correlated with both H and D .

Results revealed that the near-surface soil biological environment plays an important role in attaining large soybean yields. To meet the needs of an increasing global population and ensuing rise in food production efforts, continuous increases in yields are necessary to alleviate crop production expansion onto poorer quality soils, which may decrease land quality and threaten sustainability. Nevertheless, for a more complete comprehension of biological properties contributing to large yields, additional factors (i.e., genetic, agronomic, and/or environmental), beyond those evaluated in this field study, may need further assessment.

Acknowledgments: This study was supported by a grant from the Arkansas Soybean Research and Promotion Board.

Author Contributions: K. R. Brye partially conceived and designed the field experiment; T. C. Adams performed all field sampling, laboratory experiments, and data analyses; M. C. Savin provided guidance on the carbon substrate utilization portion of the laboratory experiment; J. A. Lee provided guidance on the principal component analyses; E. E. Gbur provided guidance on the overall experimental design of the field experiment.

Conflicts of Interest: The authors declare no conflicts of interest.

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