

## Effect of Nitrogen Fertilization and Cover Cropping Systems on Sorghum Grain Characteristics

R. C. Kaufman,<sup>†</sup> J. D. Wilson,<sup>\*,†</sup> S. R. Bean,<sup>†</sup> D. R. Presley,<sup>‡</sup> H. Blanco-Canqui,<sup>§</sup> and M. Mikha<sup>||</sup>

<sup>†</sup>USDA-ARS Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, Kansas 66502-2736, United States

<sup>‡</sup>Department of Agronomy, 2004 Throckmorton Plant, Sciences Center, Kansas State University, Manhattan, Kansas 66506, United States

<sup>§</sup>Department of Agronomy and Horticulture, 202 Keim Hall, University of Nebraska, Lincoln, Nebraska 68583-0915, United States

<sup>||</sup>USDA-ARS Central Great Plains Research Station, 40335 County Road GG, Akron, Colorado 80720-9471, United States

**ABSTRACT:** Cover crop treatments and nitrogen (N) fertilization rates were investigated for their impact on sorghum grain quality attributes. Sorghum was planted in field plots treated with differing cover cropping systems and fertilization rates. The size (weight and diameter) and hardness of the kernels were influenced by both the cover crop and N rates. The protein content increased as the N rate increased and also with the addition of cover crops to the system. The protein digestibility values and starch granule size distributions were not affected by N rate or the cover cropping treatments. Soil properties were tested to determine relationships with grain quality attributes. The utilization of cover crops appears to increase the protein content without causing a deleterious effect on protein digestibility. The end-product quality is not hampered by the use of beneficial cropping systems necessary for sustainable agriculture.

**KEYWORDS:** sorghum, nitrogen fertilization, cover crop, protein, hardness

### ■ INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth leading cereal grain produced worldwide. Because sorghum is tolerant to heat and drought conditions, it is commonly grown under nonirrigated conditions in semiarid parts of the United States, such as Kansas, Oklahoma, and Texas. In 2003, 25% of U.S. sorghum acreage was grown under a cultural practice known as no tillage, with estimates of 34% utilization of no tillage by 2009 or an annual increase of 1.5%.<sup>1</sup> No tillage systems typically use herbicides rather than mechanical cultivation for weed control and seedbed preparation, thus providing benefits to the soil by reducing erosion and increasing soil organic matter content.

Recently, cover crops have been added to no till cropping systems. Cover crops do not produce a marketable product, but they have many benefits, including increasing organic matter content, providing residue cover, preventing or reducing soil erosion, cycling nutrients, reducing nitrate leaching, suppressing weeds, and adding diversity to crop sequences.<sup>2</sup>

Nitrogen (N) fertilization effect on grain quality has been studied extensively in many cereal grains. Numerous studies in wheat have shown that increasing N levels leads to an increase in wheat protein content.<sup>3–5</sup> Similar effects have been found in triticale<sup>6</sup> and maize.<sup>5</sup> In addition to fertilization, crop rotation or cropping systems also affect the grain quality in wheat. Galantini et al.<sup>7</sup> found that wheat grown in a rotation with a legume was higher in protein content as well as higher yielding than wheat grown in a rotation with another grass. The effect of tillage has also been investigated for impact on the grain quality. No tillage systems produced wheat with lower protein content than conventional tillage systems.<sup>8</sup> However, long-term studies have shown that this effect was caused by an increase in N immobilization and can be alleviated with increased N fertilization rates.<sup>5</sup>

Prior work on crop rotation and soil treatment effects on sorghum has shown grain test weight, hardness, and protein content increases with increases in available soil N. Kaye et al.<sup>9</sup> investigated different sources of N on a conventionally tilled soil with differing preceding crops grown on specific plots. Virtually no research has been conducted to determine how N rates as well as tillage practices impact sorghum grain composition and quality. Therefore, the objectives of this study were (1) to determine the effect of cover crops and N fertilization on the physical grain characteristics of sorghum, and (2) to determine the protein and starch quality of sorghum grown under different cropping systems.

### ■ MATERIALS AND METHODS

**Cover Crop System.** The grain samples were obtained from a long-term cover crop experiment located near Hesston, KS (38°8'24" N, 97°25'48" W). The soil is a Geary silt loam (fine-silty, mixed, superactive, mesic Udic Argiustoll) with <3% slope and is deep and moderately well-drained. The study region receives 874 mm of annual precipitation and has a mean annual temperature is 14.4 °C. The study was initiated in 1995 with a winter wheat [*Triticum aestivum* (L.)] and grain sorghum [*Sorghum bicolor* (L.)] crop rotation. From 1995 to 2000 hairy vetch (*Vicia villosa* Roth) was planted as a winter cover crop between the wheat and sorghum crops, and the whole site was managed with reduced tillage. From 2000 to 2002, no cover crop was planted, and the entire site was planted to winter wheat. From 2002 to 2009, the cover crop treatments were none, late soybean (*Glycine max* L.), or Sunn hemp (*Crotalaria juncea* L.) under no-till management. The experimental design was randomized complete block with

Received: March 19, 2013

Revised: May 20, 2013

Accepted: May 26, 2013

Published: May 27, 2013

a factorial design. There were three cover crop treatments (none, late soybean, and Sunn hemp) and four N rates (0, 33, 66, and 100 kg N/ha), replicated four times, for a total of 48 plots. The cover crops were planted during the summer after wheat crop was harvested, and terminated in early autumn. In 2009, sorghum grain samples were harvested from the center two rows using a mechanical plot combine. The plot size was  $6 \times 13.5 \text{ m}^2$ . For a detailed description of complete field operations during the history of the experiment, refer to two previously published papers by Blanco-Canqui et al.<sup>10,11</sup> on the effects of the management practices on soil properties and crop yields, respectively.

**Soil Testing.** Soil samples were collected from 0 to 7.5 cm of each plot in early spring 2010 from the center, nontrafficked row of each plot.<sup>11</sup> The samples were air-dried and ground to pass through a 250- $\mu\text{m}$  sieve to determine the total organic C and N concentration by the dry combustion method of Nelson and Sommers.<sup>12</sup>

**Grain Processing.** A sample of grain from each of the 48 field plots was used for chemical analysis. An UDY mill (Udy Corporation, Fort Collins, CO) equipped with a 0.5 mm screen was used to produce whole grain meal used in protein analysis techniques. For starch isolation, grain samples were first decorticated (20% removal) using a TADD (Venables Machine Works), and then decorticated grain meal was produced similarly to the whole grain meal.

**Grain Hardness and Sizing.** The physical attributes of the sorghum kernels (hardness, diameter, and weight) were measured using a single kernel characterization system (SKCS 4100, Perten Instruments) controlled by SKCS for Windows software (Version 2.1.0.1) using 100 kernels per sample.<sup>13</sup>

**Chemical Analysis of Sorghum Meal.** Total protein content of the whole grain was determined using a N combustion method (AACC method 46–30)<sup>14</sup> using a LECO FP-528 Nitrogen Determinator (St. Joseph, MI). Nitrogen values were converted to protein by multiplying by 6.25. Protein digestibility was determined using the modified pepsin method described by Mertz et al.<sup>15</sup> with the residues analyzed by N combustion.

Kafrins were extracted for analysis as described in Bean et al.<sup>16</sup> The extracted kafrins were analyzed via reversed phase high performance liquid chromatography (RP-HPLC) as described in Bean et al.<sup>16</sup> using an Agilent 1100 HPLC system (Agilent, Palo Alto, CA) equipped with a Poroshell 300SB-C8 column.

The Megazyme Total Starch Assay kit (K-TSTA, Megazyme International, Wicklow, Ireland) with the DMSO pretreatment (AACC Method 76-13)<sup>17</sup> was used to determine the total starch content of the whole milled sorghum. Starch content was corrected to a dry basis content. Starch was isolated from the decorticated sorghum meal by the sonication method of Park et al.<sup>18</sup>

Starch granule size distributions were measured using a single wavelength Beckman Coulter LS 13 320 Particle Size Analyzer (Miami, FL) with the Universal Liquid Module (ULM) for liquid-based measurements. Data were calculated as volume percent measurements and binned according to common size groupings: A-type ( $>15 \mu\text{m}$ ), B-type ( $5\text{--}15 \mu\text{m}$ ), and C-type ( $<5 \mu\text{m}$ ).

Whole grain sorghum meal samples were analyzed for mineral concentration by Ward Laboratories (Kearney, NE). Samples were analyzed for Ca, P, K, Mg, Zn, Fe, Mn, Cu, S, and Na concentrations.

## RESULTS AND DISCUSSION

**Soil Properties.** Soil organic C and total N concentration in the 0–7.5-cm soil depth were both significantly affected by the long-term N rate and cover crop treatments (Table 1). The mean values for SOC and total N gradually increased with higher N rates. The late soybean and Sunn hemp treatments had statistically similar SOC and total N levels, and were significantly greater than the plots where cover crops were not grown. An extensive evaluation of soil properties was reported for the same experiment by Blanco-Canqui et al.<sup>11</sup> Organic matter is an extremely complex substance, and is largely composed of SOC and total N. Therefore, increasing SOC and

**Table 1. Soil Properties after a Long Term Cover Crop System and N Fertilization Rate Study**

	soil organic carbon, g/kg	total N, g/kg
N Rate		
0	13.92c <sup>a</sup>	1.37b
33	15.61bc	1.5ab
66	17.25ab	1.69a
100	18.18a	1.61a
LSD	1.75	0.21
Cover Crop		
none	13.66b	1.32b
late soybean	16.9a	1.63a
Sunn hemp	18.16a	1.67a
LSD	1.52	0.18

<sup>a</sup>Means with identical letters within each variable and study factor are not different ( $P < 0.05$ ). Data was adapted from Blanco et al.<sup>11</sup>

total N gradually over a period of several years has led to an increase in soil organic matter, which is considered a critical part of building healthy soils and is the foundation of sustainable agriculture.<sup>19</sup> It is important to remember that the study was established in 1995, and the soil samples were collected in early spring 2010; thus, these changes in SOC and total N are a cumulative effect of many years of management.

**Whole Kernel Properties.** The effect of N rate on the single kernel measurements is reported in Table 2. The

**Table 2. Physical Characteristics of Sorghum Kernels Grown under Differing N Fertilization Rates and Cover Crop Systems**

	hardness unit	weight, mg	diameter, mm	test weight, kg/(m <sup>3</sup> )	grain yield, kg/ha
N Rate					
0	68.7b <sup>a</sup>	23.6b	2.16b	687.3a	5110.9b
33	70.0b	24.0a	2.21a	714.3a	6609.6b
66	76.2a	24.4a	2.21a	720.7a	7763.5a
100	77.3a	23.3b	2.18b	727.2a	7964.2a
LSD	3.1	0.6	0.03		464.1
Cover Crop					
none	69.7b	23.4b	2.16b	713.0a	6302.4c
late soybean	74.7a	23.9a	2.21a	705.3b	6741.3b
Sunn hemp	74.6a	24.0a	2.21a	718.1a	7537.7a
LSD	2.7	0.5	0.03	6.4	376.3

<sup>a</sup>Means with identical letters within each variable and study factor are not different ( $P < 0.05$ ).

hardness values appear to increase as the level of N increases across all cover crop treatments. The resultant hardness of the grain from N applications of 0 and 33 kg/ha is significantly lower than from 66 and 100 kg/ha of N applications. The utilization of a cover crop system also increased the SKCS hardness value while there was not a significant difference in hardness of the grain between the late soybean (74.7) and Sunn hemp (74.6) cover crop system. Hardness index was higher in plots with than without cover crops.

The kernel size also significantly responded to both the N fertilization and cover crop systems. The 0 and 100 kg/ha rate had kernels of similar diameter and weight. The kernel size for the two intermediate N levels (33 and 66 kg/ha) was larger and heavier than the 0 and 100 kg/ha levels. Batey and Reynish<sup>3</sup> observed a reduction in grain size of wheat grown on increasing levels of N fertilization. They hypothesized that the decline in

**Table 3. Protein Analysis of Sorghum Grown under Differing N Fertilization Rates and Cover Crop Systems**

	protein content, % D.B.	protein digestibility, %	$\gamma$ -kafirin PA <sup>a</sup> , mAU	non- $\gamma$ -Kafirin PA, mAU	total PA, mAU	$\gamma$ -kafirin PA/PC <sup>b</sup>	$\gamma$ -kafirin, % TPA
N Rate							
0	8.1c <sup>c</sup>	73.6a	3810b	52 268c	56 078c	472a	6.82a
33	8.5bc	74.5a	3917b	55 197bc	59 114bc	461ab	6.65a
66	8.9b	73.3a	3948b	56 063b	60 011b	441b	6.57a
100	9.5a	73.0a	4323a	61 844a	66 168a	456ab	6.55a
LSD	0.45	1.88	271	3510	3718	28	0.3
Cover Crop							
none	8.2c	74.2a	3811b	52 956c	56 767c	464a	6.75a
late soybean	9.2a	73.4a	4171a	59 823a	63 994a	452a	6.53a
Sunn hemp	8.8b	73.2a	4017ab	56 250b	60 267b	457a	6.67a
LSD	0.39	1.6	235	3040	3220	24	0.26

<sup>a</sup>PA = peak area. <sup>b</sup>PC = protein content. <sup>c</sup>Means with identical letters within each variable and study factor are not different ( $P < 0.05$ ).

grain size was due to additional tiller survival, and therefore more kernels per plant to divide the carbohydrate produced through photosynthesis. The significantly higher number of heads/plant observed in the 100 kg/ha treatment versus the lower N rates could explain the reduction in kernel size of the 100 kg/ha treatment. Cover crop plots produced kernels that were significantly heavier and larger than plots without cover crop. The cover cropped plots had kernels that were numerically similar to that of the intermediate N rates for both kernel diameter and weight. Cover crop and intermediate N levels exhibited a range in heads/plant from 1.33 to 1.40, suggesting that this range is the optimum value for heads/plant to maximize the kernel size.

**Protein Properties.** The protein content across increased with higher rates of N fertilization, from 8.1% protein (0 kg/ha) to 9.1% protein (100 kg/ha) (Table 3). The 33 kg/ha rate (8.5% protein) did not differ from 0 kg/ha rate, but the 66 kg/ha rate (8.9%) was different from both the 0 and 100 kg/ha rates. This result is in agreement with prior research that showed that protein of cereal grains is related to the N fertilization level. Batey and Reynish<sup>3</sup> demonstrated that increasing N fertilization rates led to an increase in grain protein content in wheat. In triticale, Lestingi et al.<sup>6</sup> found not only that increasing N fertilization increased grain protein content, but also that tillage systems affected grain quality parameter including protein content. The cover cropped plots produced sorghum with a higher protein content than that of the noncover cropped control. The plots cover cropped with soybean had the highest protein content at 9.2%, followed by the Sunn hemp plots that produced grain with 8.8% protein while the control had a protein content of 8.2%. Galatini et al.<sup>7</sup> showed that wheat grown in a wheat–legume rotation exhibited an increase in protein content in the grain as well as an increase in production.

RP-HPLC was used to quantify kafirin subclass composition of the samples. The 100 kg/ha treatment exhibited a greater peak area for the  $\gamma$ -kafirins than the other fertilizer levels; however, the proportion of  $\gamma$ -kafirins peak area to total peak area exhibited no differences among N levels. A similar result was found with the cover crop treatments. The plots utilizing soybean as a cover crop had a greater  $\gamma$ -kafirin peak area than the plots without a cover crop. The relative percentage of  $\gamma$ -kafirin did not statistically differ across the three cover crops.

Since the primary utilization of sorghum in the United States is for animal feed, the digestibility of sorghum proteins can be an important end-use quality trait. The N and cover crop treatments did not display any significant difference in the digestibility

(Table 3). The utilization of cover crops or various N fertilization levels does not create any deleterious effect on the proportion of protein that is digestible.

**Starch Properties.** The total starch content of the whole milled sorghum did not show any effect of N fertilization levels. The 0 kg/ha fertilization level had a total starch content of 75.3% whereas the 100/kg/ha level had a total starch content of 73.9%. The intermediate N levels had total starch contents in between the aforementioned values; however, there were no significant differences among the fertilization levels. The plots with Sunn hemp as a cover crop were significantly higher in total starch content than the plots with soybean. The noncover crop treatments were not significantly different from the two cover crop treatments with total starch content of 74.9%. In addition to the total amount of starch present, the granular architecture (size distribution) of the starches is also related to its functionality.

Starch granules are commonly organized into three size types (A, B, and C). The A-type granules (>15  $\mu\text{m}$ ) make up the largest proportion of the total volume of starch, followed by the B-type granules (5–15  $\mu\text{m}$ ). The C-type (<5  $\mu\text{m}$ ) has the smallest proportion of volume, but typically outnumbers the other types numerically. The ratio of granule types can be found in Table 4. In the A-type granules only the 100 kg/ha N

**Table 4. Starch Properties of Sorghum Grown under Differing N Fertilization Rates and Cover Crop Systems**

	total starch, % D.B.	A-type granule volume, %	B-type granule volume, %	C-type granule volume, %
N Rate				
0	75.3a <sup>a</sup>	52.3a	38.9b	8.8a
33	74.2a	52.6a	38.6b	8.8a
66	74.7a	50.4ab	40.4ab	9.3a
100	73.9a	48.7b	41.9a	9.4a
LSD	1.92	3.31	2.62	0.72
Cover Crop				
none	74.9ab	50.4a	40.3a	9.3a
late soybean	73.4b	52.1a	39.1a	8.7a
Sunn hemp	75.2a	50.4a	40.3a	9.3a
LSD	1.66	2.87	2.27	0.62

<sup>a</sup>Means with identical letters within each variable and study factor are not different ( $P < 0.05$ ).

rate produced lower proportion statistically than 0 and 33 kg/ha levels. The 66 kg/ha was not statistically separated from neither higher nor lower N treatment levels. Conversely, the highest N

**Table 5. Mineral Concentrations of Sorghum Grown under Differing N Fertilization Rates and Cover Crop Systems**

	Ca, %	P, %	K, %	Mg, %	Zn, ppm	Fe, ppm	Mn, ppm	Cu, ppm	S, %	Na, %
N Rate										
0	0.0367a <sup>a</sup>	0.3625a	0.4658a	0.1542a	16.28a	89.08a	13.00a	2.73a	0.0917c	0.0258ab
33	0.0375a	0.3658a	0.4700a	0.1567a	16.96a	99.08a	14.17a	2.67a	0.0975b	0.0292a
66	0.0367a	0.3575ab	0.4641a	0.1550a	16.33a	97.58a	13.50a	2.89a	0.1008ab	0.0250b
100	0.0333a	0.3442b	0.4567a	0.1542a	18.00a	75.75a	13.42a	2.83a	0.1033a	0.0267ab
LSD	0.0079	0.0179	0.0221	0.0074	2.19	40.13	2.77	0.37	0.0048	0.0040
Cover Crop										
none	0.0388a	0.3594a	0.4681a	0.1519a	16.19a	83.94a	13.31a	2.63a	0.0931c	0.0269a
late soybean	0.0356a	0.3550a	0.4606a	0.1550a	17.24a	99.31a	13.63a	2.95a	0.1038a	0.0263a
Sunn hemp	0.0338a	0.3581a	0.4638a	0.1581a	17.24a	87.88a	13.63a	2.76a	0.0981b	0.0269a
LSD	0.0068	0.0155	0.0191	0.0064	1.90	34.76	2.40	0.32	0.0041	0.0035

<sup>a</sup>Means with identical letters within each variable and study factor are not different ( $P < 0.05$ ).

**Table 6. Pearson Correlation Analysis of Soil Properties to Sorghum Kernel Characteristics**

	hardness	diameter	wt	protein	digestibility	$\gamma$ -kafrin PA	non- $\gamma$ -kafrin PA	total PA	A-granules	B-granules	C-granules
total soil nitrogen	0.48	ns <sup>a</sup>	ns	0.40	ns	0.32	0.38	0.38	ns	ns	ns
soil organic carbon	0.51	ns	ns	0.48	ns	0.40	0.43	0.44	-0.29	0.29	ns

<sup>a</sup>ns = not significant ( $P < 0.05$ ).

level produced the highest proportion of B-type granules. The C-type experienced no differences across the N treatments. The starch granule size distribution was not affected by the cover cropping systems. There were no statistical separations in any size grouping (Table 4). Since there were very minimal differences in the total content and the granule size distributions, the functionality of the starch would most likely not be affected by the cropping systems.

**Mineral Analysis.** The mineral concentrations of the sorghum meal were not affected by the N treatments except for phosphorus (P) and sulfur (S) (Table 5). The concentration of P decreased as the level of N fertilizer increased. The decrease in P is similar to that found in a study by Zebarth et al.<sup>20</sup> on wheat. The S concentration exhibited the largest differences due to treatments for both the N fertilization rate and the cover crop. The S concentration increased 9.3% from the 0 kg/ha (0.0917% S) to 100 kg/ha (0.1033% S) treatments. A similar increase was seen in the cover crop treatments with soybean (0.1038% S) having the highest concentration followed by the Sunn hemp (0.0981% S) and the no cover crop (0.0931% S) plot the lowest concentration. Sulfur fertilization studies in wheat have shown that as the S concentration increases in the grain, the composition of the grain proteins changed, thus affecting the flour's functionality in dough mixing.<sup>4</sup> However, the increasing S concentration resulting from the fertilization and cover cropping did not appear to alter the digestibility of the proteins. A future study is needed to determine the mechanism for the increasing S concentration.

**Relationships with Soil Properties.** The soil properties that were studied showed some significant correlations to both physical and biochemical grain characteristics. Correlations can be seen in Table 6. Grain hardness was positively correlated with both the total soil N ( $r = 0.476$ ) and soil organic carbon ( $r = 0.509$ ) tests. The protein content and composition were also positively correlated with the soil properties; thus, improvements made in soil quality will enhance the grain properties. The total starch content was not correlated to the soil parameters, but the granule size distribution was related to the soil organic carbon content. A-type granules were negatively correlated ( $r = -0.288$ ) whereas B-type granules were positively

correlated ( $r = 0.292$ ). The overall functionality of the sorghum starch appears not to be as affected by soil properties as do the protein components.

In summary, the N fertilization and cover cropping systems appeared to enhance the soil fertility by increasing both total soil N and soil organic carbon. The cover crop systems provided an increase in the agronomic effect as well as overall sustainability of the production system without causing deleterious effects on the end product quality. Physical grain characteristics were influenced by the N rates and the utilization of a cover crop. The treatments also increased the amount of protein in the grain without reducing any digestibility, thus allowing for a greater digestible protein yield. The type of cover crop used also exhibited slight differences; therefore, more research is needed to examine this finding. Since cover cropping appears to provide both agronomic and end product quality benefits, increased utilization of this cropping system could be useful for developing sustainable agricultural systems.

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: jeff.d.wilson@ars.usda.gov. Phone: 785-776-2763. Fax: 785-537-5534.

### Notes

U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer, and all agency services are available without discrimination.

Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

The authors declare no competing financial interest.

## REFERENCES

- (1) Horowitz, J.; Ebel, R.; Ueda, K. "No-till" farming is a growing practice. *United States Department of Agriculture Economic Information Bulletin* 2010, 70, 1–22.

(2) Roozeboom, K.; Holman, J.; Jennings, J. Cropping systems and cover crops. In *Efficient Crop Water Use in Kansas*; Kansas State University: Manhattan, KS, 2012.

(3) Batey, T.; Reynish, D. J. The influence of nitrogen fertiliser on grain quality in winter wheat. *J. Sci. Food Agric.* **1976**, *27*, 983–990.

(4) Moss, H. J.; Wrigley, C. W.; MacRitchie, F.; Randall, P. J. Sulfur and nitrogen fertilizer effects on wheat. II. Influence on grain quality. *Aust. J. Agric. Res.* **1981**, *32*, 213–226.

(5) Wang, Z.-H.; Li, S.-X.; Malhi, S. Effects of fertilization and other agronomic measures on nutritional quality of crops. *J. Sci. Food Agric.* **2008**, *88*, 7–23.

(6) Lestingi, A.; Bovera, F.; De Giorgio, D.; Ventrella, D.; Tateo, A. Effects of tillage and nitrogen fertilisation on triticale grain yield, chemical composition and nutritive value. *J. Sci. Food Agric.* **2010**, *90*, 2440–2446.

(7) Galantini, J. A.; Landriscini, M. R.; Inglesias, J. O.; Miglierina, A. M.; Rosell, R. A. The effects of crop rotation and fertilization on wheat productivity in the Pampean semiarid region of Argentina 2. Nutrient balance, yield and grain quality. *Soil Tillage Res.* **2000**, *53*, 137–144.

(8) Wilkes, M. A.; Seung, D.; Levavasseur, G.; Trethowan, R. M.; Copeland, L. Effects of soil type and tillage on protein and starch quality in three related wheat genotypes. *Cereal Chem.* **2010**, *87*, 95–99.

(9) Kaye, N. M.; Mason, S. C.; Jackson, D. S.; Galusha, T. D. Crop rotation and soil amendment alters sorghum grain quality. *Crop Sci.* **2007**, *47*, 722–729.

(10) Blanco-Canqui, H.; Mikha, M. M.; Presley, D. R.; Claassen, M. M. Addition of cover crops enhances no-till potential for improving soil physical properties. *Soil Sci. Soc. Am. J.* **2011**, *75*, 1471–1482.

(11) Blanco-Canqui, H.; Claassen, M. M.; Presley, D. R. Summer cover crops fix nitrogen, increase crop yield, and improve soil-crop relationships. *Agron. J.* **2012**, *104*, 137–147.

(12) Nelson, D. W.; Sommers, L. E. Total carbon, organic carbon, and organic matter: Laboratory methods. In *Methods of Soil Analysis, Part 3*; Sparks, D. L., Ed.; SSSA Book Series 5; Soil Science Society of America: Madison, WI, 1996; pp 961–1010.

(13) Bean, S. R.; Chung, O. K.; Tuinstra, M. R.; Pedersen, J. F.; Erpelding, J. Evaluation of the single kernel characterization system (SKCS) for measurement of sorghum grain attributes. *Cereal Chem.* **2006**, *83*, 108–113.

(14) AACC International. Method 46-30.01. Crude protein—combustion method. *Approved Methods of Analysis*, 11th ed.; AACC International: St. Paul, MN, 2012a.

(15) Mertz, E. T.; Hassen, M. M.; Cairns-Whittem, C.; Kirleis, A. W.; Tu, L.; Axtell, J. D. Pepsin digestibility of proteins in sorghum and other major cereals. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1–2.

(16) Bean, S. R.; Ioerger, B. P.; Blackwell, D. L. Separation of kafirins on surface porous reversed-phase high-performance liquid chromatography columns. *J. Agric. Food Chem.* **2011**, *59*, 85–91.

(17) AACC International. Method 76-13.01. Total starch assay procedure (Megazyme amyloglucosidase/ $\alpha$ -amylase method). *Approved Methods of Analysis*, 11th ed.; AACC International: St. Paul, MN, 2012b.

(18) Park, S. H.; Bean, S. R.; Wilson, J. D.; Schober, T. J. Rapid isolation of sorghum and other cereal starches using sonication. *Cereal Chem.* **2006**, *83*, 611–616.

(19) Magdoff, F.; van Es, H. *Building Soils for Better Crops*, 2nd ed.; Sustainable Agriculture Publications: Burlington, VT, 2000.

(20) Zebarth, B. J.; Warren, C. J.; Sheard, R. W. Influence of the rate of nitrogen fertilization on the mineral content of winter wheat in Ontario. *J. Agric. Food Chem.* **1992**, *40*, 1528–1530.