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# **Research Paper**

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# Long-term organic and conventional management affects corn nutrient composition

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# Abstract

Corn (Zea mays L.) is an important crop that contributes to global food security, but understanding how farm management practices and soil health affect corn grain nutrient analysis and therefore human health is lacking. Leveraging Rodale Institute's Farming Systems Trial-a long-term field experiment established in 1981 in Kutztown, PA, USA-this study was conducted to assess the impact of different agricultural management systems on corn grain nutrient profiles in a long-term trial that has resulted in differences in soil health indicators between treatments as a result of long-term management. The main plot factor was two tillage practices (intensive and reduced) and the subplot factor was four cropping systems (non-diversified conventional [nCNV], diversified conventional [dCNV], legume-based organic [ORG-LEG], and manure-based organic [ORG-MNR]). Generally, the levels of amino acids, vitamins, and protein in corn grain were greatest in the ORG-MNR system, followed by the ORG-LEG and dCNV systems, and finally the nCNV system. It is important to consider that the observed difference between the organic and conventionally grown grain could be due to variations in corn hybrids that were used in those systems. However, nutrient composition of corn differed within cropping systems but between management practices (diversified crop rotation and cover cropping) which also contributed to differences in soil health indicators (soil compaction, soil protein, and organic C levels) that may also influence grain nutrient concentrations. With the exception of methionine, nutrient concentration in corn grain was not affected by different tillage regimes. These findings provide novel information on corn grain nutritional quality of organic and conventional cropping systems after long-term management and give insights into how system-specific components affect nutrient composition of corn grain.

# Introduction

Corn (*Zea mays* L.) is an important grain crop globally that is primarily used as feed for animals (livestock and poultry), food for humans, and ethanol for biofuel. Global corn production in 2019 was 1.14 billion tons, out of which 59, 19, 13, and 9% were used as animal feed, biofuel, human consumption, and others (seed, raw materials for industry, losses, etc.), respectively (FAOSTAT, 2022). The crop is consumed worldwide, with per capita food consumption of about 19 kg yr<sup>-1</sup>. In Africa and Latin America where corn serves as a dietary staple, its consumption level exceeds 40 kg capita<sup>-1</sup> yr<sup>-1</sup>. Given that billions of people in the world derive part of their daily calorie requirement from corn, the crop is considered a major contributor to global food and nutrition security (Palacios-Rojas et al., 2020).

Corn grain is nutrient-dense and provides macronutrients (carbohydrates including starch and non-starch polysaccharides [β-glucan and arabinoxylan], proteins, and lipids) and micronutrients (vitamins and minerals) for human and animal consumption. The relative fraction of the macronutrients in corn kernels, on a dry-weight basis, consists of 64–78% starch, 10% nonstarch polysaccharides, 6-11% protein, and 3-6% lipids (Watson, 2003; Ai and Jane, 2016). Due to its relatively high starch content and a high degree of unsaturated fat, corn is one of the most concentrated sources of energy among cereal grains, providing an energy density of about 365 kcal/100 g (Nuss and Tanumihardjo, 2010; Ranum, Peña-Rosas and Garcia-Casal, 2014). Relative to the requirement of humans and non-ruminant livestock, corn does not supply adequate quantities of all essential amino acids (Wang, Li and Malhi, 2008; Serna-Saldivar, 2019). For example, the crop has adequate and high levels of essential amino acids like leucine and proline but has low levels of methionine, lysine, and tryptophan (Blumenthal et al., 2008; Chaudhary, Kumar and Yadav, 2014; Serna-Saldivar, 2019). Due to an amino acid profile imbalance, most corn-based foods are supplemented with high-quality protein sources (legumes, eggs, dairy products, and meats) and most corn-based feeds are supplemented with proteins from legumes, notably soybean (Glycine max (L). Merrill) and oilseed, mainly canola (Brassica napus L.) (Nuss and Tanumihardjo, 2011; Serna-Saldivar, 2019). Minerals such as P, K, Mg, and S are abundant in corn relative to other cereal grains, while Ca, Mn, Cu, and Fe are limited (Suri and Tanumihardjo, 2016; Serna-Saldivar, 2019).

Corn contains some vitamins (vitamins A and E, and most B-vitamins) but lacks vitamins  $B_{12}$ , C, and D (Nuss and Tanumihardjo, 2010; Suri and Tanumihardjo, 2016).

Corn nutrient composition is controlled by genes interacting with environmental conditions (Scott et al., 2006; Győri, 2017). Genotypic variation exists in the nutrient composition of corn cultivars, with modern cultivars found to have lower nutrient concentrations compared to older, less improved cultivars (Ciampitti and Vyn, 2013; Győri, 2017). For example, Győri (2017) reported that grain protein concentration (GPC) (on dry-matter basis) of older corn cultivars exceeded 12%, while that of modern cultivars were below 10%. The reason for the lower nutrient concentrations in modern corn cultivar can be attributed to a focus on corn yield improvement by breeding programs, with little attention given to the concentrations of nutrients in the crop (Roberts and Mattoo, 2019). Even among modern cultivars, Scott et al. (2006) and Uribelarrea, Crafts-Brandner and Below (2009) found genotypic differences in amino acid and GPC levels among corn cultivars developed over an 80-106 yr period. Both studies found significant declines in GPC levels among corn cultivars over time while yield and starch levels increased. This inverse relationship between grain yield and protein concentrations that has resulted from improving cultivars for higher yields has been reported for other grains (Simmonds, 1995). Environmental factors including soil moisture and salinity, and ambient temperature affect the amount of nutrients removed from the soil by corn, which subsequently affects the nutrient composition of the crop (Győri, 2017; Roberts and Mattoo, 2019). For example, drought conditions elevated amino acid levels and GPC in corn (Bullock, Raymer and Savage, 1989; Lilburn et al., 1991).

In addition to genetic and environmental factors, management practices such as nitrogen (N) fertilization and crop diversification have been studied extensively and are known to influence the nutrient density of corn. For instance, an increase in N fertilizer rate can have a positive influence on GPC (Tsai et al., 1984; Oikeh, Kling and Okoruwa, 1998; Uribelarrea, Crafts-Brandner and Below, 2009) and amino acids like leucine, phenylalanine, glutamic acid, and proline (Rendig and Broadbent, 1979; Blumenthal et al., 2008), but a negative influence on amino acids like lysine, arginine, methionine, threonine, and tryptophan (Tsai et al., 1983; Mason and D'Croz-Mason, 2002). Diversified cropping systems, which can simply be defined as a set or multiple rotations of three or more crops, can enhance corn nutrient content by improving soil nutrient use efficiency, cycling, and availability (McDaniel and Grandy, 2016; Yang, Siddique and Liu, 2020; Shah et al., 2021). Considering that organic systems tend to employ more diverse crop rotations (Ponisio et al., 2015) but are also nitrogen limited (Clark et al., 1999; Alaru et al., 2014; Barbieri et al., 2023), it is difficult to predict how organic vs conventional management would affect corn grain nutrient content. The majority of these studies were short-term experiments or meta-analyses that did not take into account differences in soil quality indicators and nutrient cycling that can occur after long-term management. A comprehensive analysis of how long-term organic and conventional farm management may moderate corn nutritional factors is lacking.

In the USA, agricultural specialization in the last half century has resulted in cropping systems with unique management characteristics including cultivar or hybrid used, fertility source, pest management, crop rotation, and tillage practices. As such, a typical conventional corn cropping system is different from an organic system. One such system-based difference is crop rotation with many conventional producers relying on either monocropping or simple rotations (O'Brien et al., 2020). Across the US Midwest, the standard practice is a simplified crop rotation of corn and soybeans or corn or soybeans alone for two or more consecutive years (Plourde, Pijanowski and Pekin, 2013; Wang and Ortiz-Bobea, 2019), while certified organic farms are required to have more diverse crop rotations to mitigate pests and manage soil fertility (USDA-AMS, 2000). This reduction in crop diversity has been partly driven by the increased adoption of genetically modified (GM) seeds (Wang and Ortiz-Bobea, 2019) which now accounts for over 90% of the corn planted in the USA (USDA-NASS, 2020, 2021a, 2021b). Traits conferred by GM seeds typically include tolerance to herbicides and resistance to insect pests. Based on USDA National Organic Program standards (USDA-AMS, 2000), GM seeds are prohibited in organic farming, and organic corn producers must use certified organic seeds when available or, if unavailable, opt for untreated, non-GM seeds. Conventional grain producers in the USA are slowly adopting the use of cover crops (Tully and McAskill, 2020). In contrast, organic producers tend to implement diverse and longer crop rotation, including the use of cover crops, to break pest and weed cycles and optimize nutrient cycling (Carr et al., 2012; Barbieri, Pellerin and Nesme, 2017; Tully and McAskill, 2020). Another system-based difference between organic and conventional corn producers is planting date. Organic corn production typically involves later planting dates compared to conventional corn production (especially in the mid-Atlantic region). This practice is adopted as a strategic measure to optimize the performance of organic systems by preventing stand losses caused by high prevalence of diseases and pests, commonly observed with early planting (Mirsky et al., 2012). Delaying planting in organic corn systems also allows for maximum biomass production and N content of the preceding leguminous cover crop, a major source of fertility for corn in organic systems (Ryan et al., 2009; Mirsky et al., 2012). Organic and conventional corn systems differ in their weed management, with conventional producers trending toward the use of herbicide-tolerant GM seeds and herbicides that results in reduced tillage (Benbrook, 2012; Dentzman and Burke, 2021), whereas organic corn producers rely heavily on primary tillage and cultivation for weed control (Mirsky et al., 2012; Hinson et al., 2022b). Fertility sources also vary between organic and conventional systems. While conventional producers typically use synthetic fertilizers, organic producers use carbon-based amendments (notably animal and green manures) to provide crop nutrients (Hinson et al., 2022a). Given these inherent differences between organic and conventional corn cropping systems, it is imperative to use a systems research approach to understand how each system functions as a whole and how each system is influenced by the relationships among its components. To better understand the ecological outcomes over time related to farming practices, long-term experiments can provide the best and foremost scientific information. Long-term experiments are particularly useful in evaluating the combined effects of different management practices and can provide valuable insights into system optimization (Jernigan et al., 2020).

The effect of long-term organic and conventional cropping systems on grain nutrient profile has been studied in oats (*Avena sativa* L.) (Omondi et al., 2022) and wheat (*Triticum aestivum*) (Campiglia et al., 2015), but few studies have been conducted to compare grain nutrient composition of organic and conventional corn grown in a long-term, side-by-side, replicated trial (Delate and Cambardella, 2004; Pearsons et al., 2022).

Therefore, the objective of this study was to assess the long-term impact of cropping systems management (two organic and two conventional systems) and tillage practices (intensive *vs* reduced) on corn grain nutrient content. This study reflects current, real-world cropping systems and is warranted for a broader understanding of the nutritional value of corn grown using conservation practices and in organic and conventional systems and how this might widely impact animal and human health.

# **Materials and methods**

## Experimental site, history, and design

Rodale Institute's Farming Systems Trial (FST) is a long-term field experiment established in 1981 to compare conventional and organic cropping systems. With FST still ongoing, the trial is the longest-running side-by-side comparison of organic and conventional cropping systems in North America. The FST is a 4.9-ha, rainfed experiment located in Kutztown, Berks County, Pennsylvania (40°33' N, 75°43' W) with a mean annual temperature of 10.3°C and mean annual rainfall of 1133 mm. Soil type at the experimental site is primarily ( $\sim$ 70%) a Clarksburg silt loam (fine-loamy, mixed, superactive, mesic Oxyaquic Fragiudalf), but a portion of the area (~30%) has a Berks channery loam (loamy-skeletal, mixed, active, mesic Typic Dystrudept) (USDA-NRCS, 2019). Before FST establishment, the field was managed under long-term tillage (>25 yr) with corn and winter wheat rotation. After wheat harvest in the summer of 1980, the field was fallow until the experiment began in 1981 (Liebhardt et al., 1989).

From 1981 to 2007, FST was a split-plot design experiment with eight replications arranged in a randomized complete block design. The main plots (92 m long by 18 m wide) were three cropping systems: (i) non-diversified conventional system (nCNV), (ii) legume-based organic system (ORG-LEG), and (iii) manure-based organic system (ORG-MNR). Main plots were separated by 1.5-m annual ryegrass (Lolium multiflorum Lam.) buffer strips to minimize movement of fertilizer, pesticides, and soil from the conventional plots to the organic plots. This reduces the risk of contamination in the organic plots. The subplots (92 m long by 6 m wide) were three rotation entry points, which allowed for the cultivation of three crop rotational phases and the comparison of three crops within a system in any given year. During this period, FST was managed under intensive tillage, in which the organic and conventional plots were tilled with a moldboard plow and chisel plow, respectively.

In 2008, four replicates of each treatment combination (cropping system by entry point) were converted to reduced tillage management, and the other four continued following intensive tillage management. With the addition of tillage as a factor, the experimental design of FST from 2008 to date can be described as a split-split plot with four replications arranged in a randomized complete block design. The main plots are tillage practices (intensive and reduced); the sub-plots are cropping systems (nCNV, ORG-LEG, ORG-MNR); and the sub-sub-plots are three rotation entry points making a total of 72 plots. In 2014, one of the three entry points of nCNV system was slightly modified by including wheat as a cash crop and cereal rye (Secale cereale L.) as a cover crop, referred to hereafter as diversified conventional (dCNV) system. This treatment was added due to the recognition of increased acreage across the USA that include cover crops within conventional grain cropping systems and that cereal rye is the most commonly used cover crop planted following harvest of corn and soybeans (*G. max* L. Merr.)

#### Field operations

#### Cropping systems

Management of the conventional system is synthetic input-based, relying on synthetic fertilizers and herbicides. In the FST, nitrogen fertilizer application rates are determined based on the yield goal, and P and K are based on soil test recommendations (Penn State Extension, 2019). Herbicides and their application rates are made based on Pennsylvania State University recommendations. The two organic systems follow the USDA National Organic Program guidelines (USDA-AMS, 2000) and are therefore operated with certifiable organic practices, though not certified organic or the grain marketed as organic because of the proximity of the organic plots to the conventional plots. Farm-scale equipment is used for all field management operations. Seeds planted in the conventional plots are either GM or chemically treated, while seeds planted in the organic plots are neither GM nor chemically treated.

Each cropping system (nCNV, dCNV, ORG-LEG, and ORG-MNR) has a specific rotation and includes various combinations of cash crops and/or cover crops. Across systems, major cash crops include corn, soybean, wheat, oats, and mixed-species hay crop; and major cover crops include hairy vetch (*Vicia villosa* Roth), red clover (*Trifolium pratense* L.) and cereal rye. A detailed description of each cropping system is presented below.

The nCNV system follows a 3-yr rotation (corn-corn-soybean). This simple rotation is practiced by most conventional producers in the mid-Atlantic region (Cavigelli et al., 2009; Wallace et al., 2017). This system can be described as a high-input, chemically intensive farming system. The dCNV system, which is an entry point of the nCNV system, follows a 3-yr rotation (corn/ rye-soybean/wheat-wheat/rye) with cereal rye planted as a cover crop following corn and wheat planted after soybean harvest in the fall of each year.

The ORG-LEG system follows a mid-length rotation (4 yr), and the rotational sequence is corn/rye-oats + clover/rye-soybean/wheat-wheat/vetch. This system is classified as a low-input system, relying on leguminous cover crops (hairy vetch and red clover) only to supply crops with N fertility.

The ORG-MNR system is a 9-yr rotation consisting of annual grain crops, perennial hay crops (mixed species), and cover crops. The crop rotational sequence is oats/rye-soybean/wheat-wheat/hay-hay-hay-hay-corn silage/wheat-wheat/vetch-corn/rye. The mixed-species hay crop planted in this system included alfalfa (*Medicago sativa* L.) and perennial orchardgrass (*Dactylis glomerata* L.). Fertility sources in this system include leguminous cover crop and periodic applications of composted dairy manure. Composted manure is applied twice during the 9-yr rotation (before planting corn silage or following oat harvest) (Alfahham et al., 2021). Compost application rates are determined based on a target N input of 90 kg N ha<sup>-1</sup>, with the assumption of 40% N availability in the first year (Hepperly et al., 2009; Alfahham et al., 2021).

### Tillage practices

Two tillage practices are employed in FST: intensive and reduced. Intensive tillage practices in the conventional systems (nCNV and dCNV systems) and the organic systems differ. In the two conventional systems, intensive tillage involves the use of a chisel plow (a primary tillage tool) at crop transitions for seedbed preparation and weed control. Following chisel plowing, disking, and packing are used to break large clods before planting. Weed control after planting is accomplished by using herbicides. In contrast, intensive tillage in the two organic systems involves using a primary tillage tool—moldboard plow—at crop transitions for seedbed preparation and weed control. Disking and packing follow moldboard plowing before planting. After planting, weeds are managed mechanically by cultivation using implements such as rotary hoe, tine weeder, and between row cultivators.

Reduced tillage in conventional and organic cropping systems differ-continuous no-till in the conventional systems and rotational no-till in the organic systems. Continuous no-till is achieved in the conventional system by using broad-spectrum herbicides and herbicide-resistant corn and soybeans seeds. Thus, tillage is not used in this system and the conventional reduced tillage (no-till) plots have not been tilled since 2007. In contrast, the organic reduced tillage system utilizes rotational, cover crop-based no-till, which involves growing high-biomass cover crops, terminating them with a roller-crimper (I&J Manufacturing, Gap, PA, USA), and no-till planting cash crops into the weed-suppressive mulch (Moyer, 2020). In rotational no-till, tillage is used prior to the seeding of cover crops in the fall but not used prior to seeding cash crops in late spring. Rotational no-till at FST entails no-till planting soybean and corn into cereal rye and hairy vetch, respectively. Rotational no-till in FST has been thoroughly described by Mirsky et al. (2012), Seidel et al. (2017), Mover (2020), and Littrell et al. (2021). For this study, hairy vetch was planted following wheat harvest in late-August of 2019 (Table 1). Cover crop termination and corn planting occurred simultaneously in the organic reduced tillage system by rolling-crimping hairy vetch when plants had reached 75% inflorescence using an I&J Manufacturing rollercrimper front-mounted on the tractor and corn planted with a rear-mounted Monosem NGPlus vacuum precision no-till planter (Monosem Inc., Edwardsville, KS, USA).

## Study procedures

#### 2020 corn management

In the summer of 2020, 32 plots out of the 72 FST plots, were planted to corn. This provided an ideal opportunity to evaluate long-term effects of cropping systems and tillage on the nutrient composition of corn. Cropping sequence (2017–2020) for the entry points used in this study is shown in Table 1. Details regarding crop management are presented in Table 2 including tillage implements used, dates of field activities, and herbicide application rates, with additional information provided below.

Corn seed planted in all conventional plots was a GM hybrid (LC0297 SSXRIB, Local Seed Company, Memphis, TN, USA) that confers resistance to above and below ground insect pests, as well as herbicide tolerance, while the seeds planted in the organic plots were certified organic (Blue River 51T59, Albert Lea Seed, Albert Lea, MN, USA). These hybrids were used because they are adapted to regional growing conditions. Seeds in all plots were direct seeded using a Monosem NGPlus vacuum precision planter (Monosem Inc., Edwardsville, KS, USA). Corn was planted on May 15, 2020 in the conventional plots, and June 9, 2020 in the organic plots. It is not unusual for organic corn producers, particularly in the mid-Atlantic region, to plant late relative to conventional corn producers. This approach is employed as a strategy to avoid stand losses due to high incidence of diseases and pests, which are typically associated with early planting (Ryan et al., 2009; Mirsky et al., 2012). In the present study, corn was planted late in the organic systems to allow for maximum biomass production and N content of the preceding cover crop (hairy vetch), which supplies N to corn. The timing also allowed hairy vetch to reach the flowering stage (75% or more) which is the required phenological stage to terminate hairy vetch in organic rotations when using a roller crimper to maximize weed suppression and minimize hairy vetch regrowth and competition with corn (Cook et al., 2010; Mirsky et al., 2012; Moyer, 2020). In the organic reduced tillage system, further weed management included a single pass of a high-residue cultivator (Kelley Manufacturing Company, Tifton, GA) on July 17th just prior to canopy closure. The high residue cultivator undercuts weed roots and leaves cover crop residue on the soil surface. Corn planting for both the intensive tillage and reduced tillage treatments occurred on the same day in the conventional (May 15th) and organic (June 9th) systems. In all plots, corn was seeded at a rate of 79,090 seeds ha<sup>-1</sup> with a row spacing of 76 cm.

In the conventional plots, 45 kg N ha<sup>-1</sup> was applied at planting. At the six-collared leaf (V6) stage of development (on June 24, 2020), N fertilizer (urea ammonium nitrate) was side-dressed at a rate of 135 kg N ha<sup>-1</sup>. Thus, each conventional plot received a total N application of 180 kg ha<sup>-1</sup>, and this decision was made based on a yield goal of 7.5–9.0 Mg ha<sup>-1</sup>, a typical corn yield goal for Berks County, PA (Beegle and Durst, 2003; USDA-NASS, 2021a, 2021b). All plots were harvested on November 6, 2020.

#### Data collection

At physiological maturity (R6 stage), which was detected using Biologische Bundesanstalt, Bundessortenamt und CHemisch Industrie (BBCH) growth scale, corn ears were hand harvested from a 4.05 m<sup>2</sup> area in the center of each plot. The ears were dried at 65°C for 96 h in a forced-air oven. All corn ears were

Table 1. Details of crop sequence from winter 2017/2018 to summer 2020 at the FST, Rodale Institute, Kutztown, PA, USA

	2017-	-2018	2018-	-2019	2019-	2020
Cropping system	Fall-winter	Spring-summer	Fall-winter	Spring-summer	Fall-winter	Spring-summer
nCNV	-	Soybean	-	Corn	-	Corn
dCNV	Rye*	Soybean	$Wheat^\dagger$		Rye*	Corn
ORG-LEG	Rye*	Soybean	$Wheat^\dagger$		Vetch*	Corn
ORG-MNR	Нау	Corn Silage	$Wheat^\dagger$		Vetch*	Corn

nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR, manure-based organic system. \*Cover crop.

<sup>†</sup>Wheat is harvested in the summer.

Table 2. Summary of crop mana	agement practices for 2020 corr	grown at the FST, Rodale	Institute, Kutztown, PA, USA
		<u> </u>	, , ,

Cropping system	Primary tillage implement and date	Secondary tillage implement and date	Preemergence herbicide applied, rate, and date	Postemergence herbicide applied, rate, and date
Intensive tillage				
nCNV	CP on 5/7	D&P on 5/15	Warrant 3CS (6.7 L ha <sup><math>-1</math></sup> ), infantry (2.3 L ha <sup><math>-1</math></sup> ), and balance flexx (0.2 L ha <sup><math>-1</math></sup> ) on 5/28/2020	Bullzeye (1.8 L ha <sup>-1</sup> ) on 6/25/ 2020
dCNV	CP on 5/7	D&P on 5/15	Warrant 3CS (6.7 L $ha^{-1}$ ), infantry (2.3 L $ha^{-1}$ ), and balance flexx (0.2 L $ha^{-1}$ ) on 5/28/2020	Bullzeye (1.8 L ha <sup>-1</sup> ) on 6/25/ 2020
ORG-LEG	MBP on 5/12	D&P on 6/10, TW on 6/ 13, and ST on 7/01	None	None
ORG-MNR	MBP on 5/13	D&P on 6/10, TW on 6/ 13 and ST on 7/01	None	None
Reduced tillage				
nCNV	None	None	Warrant 3CS (6.7 L ha <sup><math>-1</math></sup> ), infantry (2.3 L ha <sup><math>-1</math></sup> ), and balance flexx (0.2 L ha <sup><math>-1</math></sup> ) on 5/28/2020	Bullzeye (1.8 L ha <sup>-1</sup> ) on 6/25/ 2020
dCNV	None	None	Warrant 3CS (6.7 L $ha^{-1}$ ), infantry (2.3 L $ha^{-1}$ ), and balance flexx (0.2 L $ha^{-1}$ ) on 5/28/2020	Bullzeye (1.8 L ha <sup>-1</sup> ) on 6/25/ 2020
ORG-LEG	None	HRC on 7/17	None	None
ORG-MNR	None	HRC on 7/17	None	None

nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR, manure-based organic system; MBP, moldboard plow; CP, chisel plow; D&P, disking and packing; TW, tine weeding; ST, S-tine cultivation; HRC, high residue cultivation.

shelled by hand, and grains were weighed to determine yield. Grain yields (in Mg  $ha^{-1}$ ) were adjusted to 15.5% moisture.

For grain nutrient analyses, corn grain was subsampled for each plot, and ground into fine powder using a coffee grinder (Cuisinart Model DCG-20N, East Windsor, NJ, USA). Grain nutrient analyses were performed by commercial laboratories. Complete amino acid profile, and GPC analyses were conducted by the University of Missouri Agricultural Experiment Station Chemical Laboratory (Columbia, MO). Grain mineral analysis was carried out by the Cornell University Nutrient Analysis Laboratory (Ithaca, NY). Grain nutrient analyses for carbohydrate, calories, crude fat, and vitamin B-complex (B<sub>3</sub>, B<sub>6</sub>, and B<sub>9</sub>) were performed by Eurofins Nutrition Analysis Center (Des Moines, IA). A ground sample of approximately 200 g from each plot was sent to each commercial lab for analysis.

Complete amino acid profile was determined through proximate analysis using AOAC Official Method 982.30 (AOAC International, 2019). This method provides concentrations for all 20 primary amino acids; but it estimates the sum of aspartate and asparagine as aspartic acid, and sum of glutamine and glutamate as glutamic acid. Therefore, the amino acid profile included essential amino acids (threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, and tryptophan) and non-essential amino acids (proline, glycine, serine, glutamic acid, alanine, aspartic acid, cysteine, tyrosine, and arginine). The GPC was determined indirectly through grain N concentration, which was determined by combustion analysis using a LECO gas analyzer. The GPC was calculated by multiplying grain N by 6.25 according to AOAC Official Method 990.03 (AOAC International, 2019). Grain minerals including P, K, Ca, Mg, S, Fe, Zn, Cu, and Mn were analyzed using hot plate digestion plus inductively coupled plasma atomic emission spectroscopy method. Carbohydrate and calories were determined via CFR 21 calculation and CFR Atwater calculation, respectively (AOAC International, 2019). Crude fat was determined by acid hydrolysis according to AOAC Official Method 954.02 (AOAC International,

2019). Vitamins B<sub>3</sub>, B<sub>6</sub>, and B<sub>9</sub> were determined according to AOAC Official Method 961.14, 985.32, and 952.20, respectively (AOAC International, 2019).

Soil samples were collected from all 32 plots after corn harvest (November 20th, 2020). Samples were collected using a hand-held auger (2 cm diameter), and from 0 to 20 cm depth. Soils were sampled from 10 representative locations across each plot following the USDA GRACEnet protocol (Liebig, Varvel and Honeycutt, 2010), then composited and homogenized into a single sample. Each composited soil sample was air-dried and ground to pass through a 2-mm sieve for nutrient analysis, which was done by commercial laboratories. The nutrient analyses included total N, available N (NO3-N+NH4-N), soil protein, P, K, Ca, Mg, S, Zn, and Cu. Soil protein analysis was performed by the Ohio State University's Soil Fertility Laboratory (Wooster, OH) using autoclaved-citrate extraction method. Total soil N, NO<sub>3</sub>-N, NH<sub>4</sub>-N, and the other nutrients were analyzed by the Pennsylvania State University's Agricultural Analytical Services Laboratory (University Park, PA). Total N was measured using the combustion method (Bremner, 1996; Nelson and Sommers, 1996), and soil NO<sub>3</sub>-N and NH<sub>4</sub>-N were determined using KCl-cadmium reduction method (Dahnke and Johnson, 1990). Soil P, K, Ca, Mg, and S were determined using Mehlich-3 (ICP) extractant (Wolf and Beegle, 1995), and Cu and Zn were determined by using EPA Method 3050B/3051 + 6010 (USEPA, 1986).

In addition to soil nutrient analyses, soil penetration resistance measurements were taken before corn planting (April 4th, 2020) using a Dickey-John Soil Compaction Tester (Dickey-John Corporation, Auburn, IL, USA). This soil compaction tester is a portable cone penetrometer with a 1.8-m long probe and has a pressure gauge meter ranging from 0 to 4 MPa. The penetrometer was gradually pushed into the soil until a gauge reading of 2 MPa was obtained. The penetrometer was then removed, and the depth to the compaction layer was measured. For each plot, 10 soil penetration resistance measurements were taken randomly. Weather data were obtained from an on-site weather station. Growing degree-days (GDDs), a measure of heat units, was computed using 30°C as the upper temperature threshold and 10°C as the base temperature.

# Statistical analysis

Statistical analysis was conducted using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). All data were analyzed by analysis of variance (ANOVA) ( $P \le 0.05$ ) using Proc MIXED. In the statistical model, system, tillage, and the system × tillage interaction were considered fixed effects, and block was considered a random effect. Prior to ANOVA, all data were tested for assumptions of normality (Shapiro–Wilk's test) and equality of variance (Levene's test). Tukey's honest significant difference test was used for pairwise mean comparisons at a probability level of 5%.

# Results

# Weather data

Cumulative GDDs, daily precipitation, and minimum and maximum temperatures for the 2020 growing season are presented in Figure 1. The cumulative GDDs were 1741 and 1523 for the conventional and organic systems, respectively. Precipitation was evenly distributed throughout the 2020 corn growing season. The total precipitation from planting to harvest in the conventional and organic systems was 790 and 732 mm, respectively. In the conventional systems, season-long mean minimum and maximum air temperatures were 12.2 and 25.9°C, respectively. Season-long mean minimum and maximum air temperatures for the organic systems were 12.3 and 26.1°C, respectively.

#### Grain yield

Significant differences in corn yield were observed among the different cropping systems. Corn grain yield in the ORG-LEG system  $(6.3 \text{ Mg ha}^{-1})$  was significantly lower than the other three systems—ORG-MNR ( $(8.6 \text{ Mg ha}^{-1})$ , dCNV ( $8.4 \text{ Mg ha}^{-1}$ ), and nCNV ( $7.9 \text{ Mg ha}^{-1}$ ). Neither tillage treatments nor the

interaction between cropping systems and tillage had a significant effect on corn yield (Fig. 2A).

# Grain nutrient analyses

#### Essential amino acids

Total essential amino acid (TEAA) was affected by cropping systems but not tillage or system × tillage interaction (Table 3). The concentration of TEAA was similar among dCNV, ORG-LEG, and ORG-MNR systems. Corn produced in the nCNV system, however, had the lowest levels of TEAA. Although the two conventional systems had the same corn hybrid, TEAA was 15% greater in the dCNV system than in the nCNV system. Notably, TEAA level was 20% lower in the nCNV system than in the ORG-MNR system. The concentrations of six essential amino acids (i.e. threonine, valine, isoleucine, leucine, phenylalanine, and histidine) were influenced by cropping systems, while the other three (methionine, lysine, and tryptophan) were not affected by cropping systems. Overall, the concentrations of the six essential amino acids that were influenced by cropping systems were greater in the ORG-MNR system than in the nCNV system but did not differ between the ORG-LEG and dCNV systems. There were no differences among the essential amino acids between the two organic systems, while the ORG-MNR system had higher isoleucine, leucine, and phenylalanine levels than the dCNV system. The concentrations of valine, isoleucine, leucine, and phenylalanine in corn grain harvested from the dCNV system were 14, 16, 20, and 18% greater than that of the nCNV system, respectively. Tillage affected only methionine concentration, where the corn produced under the reduced tillage had a greater methionine concentration than that produced under intensive tillage. There was no system × tillage interaction effect on any of the nine essential amino acids.

#### Non-essential amino acids

Total non-essential amino acid (TNAA) was affected by cropping systems but not by tillage or the interaction between system and tillage (Table 4). The highest and lowest levels of TNAA were observed in the ORG-MNR and nCNV systems, respectively. The TNAA in the nCNV system was 11% lower than that of



Figure 1. Weather data for 2020 corn growing season. FST, Rodale Institute, Kutztown, PA. The dashed vertical line represents planting date in the organic systems.



Figure 2. Mean (±SEM) and ANOVA results for corn grain yield (A) and protein concentration (B) in different cropping systems (non-diversified conventional [nCNV], diversified conventional [dCNV], legume-based organic [ORG-LEG], and manure-based organic (ORG-MNR) systems) and under intensive and reduced tillage practices.

the dCNV system. The concentration of TNAA in corn grain harvested from the ORG-MNR system was 19 and 6% greater than that of the nCNV and ORG-LEG systems, respectively. The concentrations of aspartic acid, serine, glutamic acid, proline, alanine,

and tyrosine differed among cropping systems. The concentrations of aspartic acid, serine, proline, alanine, and tyrosine in corn grain were 11, 28, 12, 13, and 16% greater in the dCNV than the nCNV system, respectively. The concentrations of

Table 3. ANOVA and means separation results for essential amino acids concentration (g 100 g<sup>-1</sup>) of 2020 corn grown in the FST, Rodale Institute, Kutztown, PA, USA

Variable										
ANOVA	Thr	Val	Met	Ile	Leu	Phe	Lys	His	Trp	TEAA
Cropping system (CS)	0.007	<0.001	0.110	<0.001	<0.001	<0.001	0.171	0.015	0.062	<0.001
Tillage (T)	0.852	0.516	0.039	0.269	0.054	0.184	0.807	0.742	0.446	0.409
CS × T	0.602	0.739	0.469	0.625	0.480	0.508	0.958	0.660	0.409	0.662
Mean separation										
Cropping system										
nCNV	0.226b	0.282b	0.149	0.210c	0.630c	0.265c	0.240	0.178b	0.053	2.20b
dCNV	0.240ab	0.320a	0.153	0.243b	0.755b	0.314b	0.246	0.189ab	0.056	2.52a
ORG-LEG	0.250ab	0.328a	0.139	0.255ab	0.806ab	0.340ab	0.245	0.199a	0.058	2.62a
ORG-MNR	0.261a	0.346a	0.141	0.270a	0.864a	0.363a	0.256	0.204a	0.059	2.76a
Tillage										
Intensive	0.245	0.322	0.141b	0.249	0.793	0.328	0.248	0.193	0.056	2.51
Reduced	0.244	0.316	0.150a	0.240	0.758	0.313	0.246	0.191	0.057	2.58

Values within each column followed by the same letter are not statistically different (P = 0.05).

nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR, manure-based organic system; Thr, threonine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Lys, lysine; His, histidine; Trp, tryptophan; TEAA, total essential amino acid.

Tabl

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**Table 4.** ANOVA and means separation results for non-essential amino acids concentration (g  $100 \text{ g}^{-1}$ ) of corn grain grown in the FST, Rodale Institute, Kutztown, PA, USA

Variable	Asp	Ser	Glu	Pro	Gly	Ala	Cys	Tyr	Arg	TNAA
ANOVA										
Cropping system (CS)	<0.001	0.006	0.013	0.007	0.090	0.011	0.135	<0.001	0.221	0.003
Tillage (T)	0.085	0.738	0.578	0.422	0.585	0.607	0.170	0.411	0.555	0.543
S × T	0.278	0.399	0.529	0.519	0.889	0.496	0.851	0.157	0.907	0.475
Mean separation										
Cropping system										
nCNV	0.421d	0.248c	1.076c	0.510b	0.270	0.448c	0.156	0.146b	0.299	3.61c
dCNV	0.467c	0.318b	1.166bc	0.573a	0.281	0.505b	0.163	0.170a	0.316	4.06b
ORG-LEG	0.514b	0.318b	1.243ab	0.583a	0.284	0.499b	0.168	0.175a	0.303	4.08b
ORG-MNR	0.555a	0.336a	1.309a	0.599a	0.291	0.531a	0.166	0.181a	0.316	4.29a
Tillage										
Intensive	0.500	0.316	1.212	0.573	0.280	0.500	0.161	0.170	0.306	3.98
Reduced	0.470	0.312	1.185	0.559	0.283	0.490	0.166	0.166	0.311	4.04

Values within each column followed by the same letter are not statistically different (P = 0.05).

nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR, manure-based organic system; Asp, aspartic acid; Ser, serine; Glu, glutamic acid; Pro, proline; Gly, glycine; Ala, alanine; Cys, cysteine; Tyr, tyrosine; Arg, arginine; TNAA, total non-essential amino acid.

aspartic acid, serine, and alanine were all greatest in the ORG-MNR system while glutamate was greater in the ORG-MNR than both conventional systems but not the ORG-LEG system.

#### Minerals and vitamins

## Grain protein concentration

The GPC differed among cropping systems but was not influenced by tillage or system by tillage interaction (Fig. 2B). No significant difference was found among ORG-MNR, ORG-LEG, and dCNV systems, while the lowest GPC was observed in the nCNV system. Notably, corn GPC was 21% greater in the dCNV system than the nCNV system. Corn grain mineral concentrations were not affected by tillage. Cropping system did not affect the concentrations of P, K, Mg, Fe, and Cu in corn grain, but significantly affected the concentrations of Ca, S, Zn, and Mn (Table 5). The concentrations of Ca, S, and Zn were greater in the conventional systems (nCNV and dCNV) compared to the two organic systems (ORG-MNR and ORG-LEG). The concentration of Ca, S, and Zn in corn grain harvested from the nCNV system were 28, 17, and 42% greater than the ORG-MNR system, respectively. The greatest differences in concentrations of Ca, S, and Zn occurred between dCNV and ORG-LEG, being 35, 21, and 44% greater in the dCNV system,

Table 5. ANOVA and means separation results for grain mineral concentrations (mg kg<sup>-1</sup>) of 2020 corn grown in the FST, Rodale Institute, Kutztown, PA, USA

Variable	Р	К	Са	Mg	S	Fe	Zn	Cu	Mn
ANOVA									
Cropping system (CS)	0.147	0.274	0.012	0.078	0.001	0.188	<0.001	0.134	0.004
Tillage (T)	0.463	0.752	0.060	0.455	0.388	0.855	0.952	0.592	0.879
S × T	0.567	0.598	0.815	0.525	0.760	0.887	0.887	0.720	0.796
Mean separation									
Cropping system									
nCNV	3034	3096	31.2a	936	761a	11.9	19.3a	1.17	3.19b
dCNV	3540	3593	32.7a	1142	796a	14.7	22.9a	1.28	4.39a
ORG-LEG	2763	3008	21.4b	842	628b	14.6	12.7b	0.73	2.77b
ORG-MNR	2918	3106	24.4b	914	650b	16.6	13.6b	1.01	3.09b
Tillage									
Intensive	3154	3237	29.9	989	693	14.3	17.1	1.09	3.39
Reduced	2974	3164	24.9	928	724	14.6	17.2	1.00	3.33

Values within each column followed by the same letter are not statistically different (P = 0.05).

nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR, manure-based organic system.

respectively. The greatest concentration of corn grain Mn was observed in the dCNV system.

Vitamins  $B_3$  (niacin),  $B_6$  (pyridoxine), and  $B_9$  (folic acid) were all affected by cropping systems, but not by tillage or the interaction of system by tillage (Table 6). The two organic systems had similar levels of vitamins  $B_3$ ,  $B_6$ , and  $B_9$ , and these levels were all significantly greater than the levels observed in the conventional systems. The concentration of vitamin  $B_3$  in ORG-LEG corn was twice as high as that of the nCNV system, and the concentration of Vitamin  $B_9$  in the ORG-MNR corn was 45% greater than that of both conventional systems.

# Calories, carbohydrates, and crude fat

Calories and carbohydrates were not affected by cropping systems or tillage or their interaction (Table 6). Crude fat differed among systems but did not differ between the two tillage practices. Cropping system × tillage interaction did not affect crude fat concentration in corn grain. Crude fat was similar between the two organic systems and similar between the two conventional systems. Crude fat was 5–7% greater in the conventional systems than in the organic systems.

## Soil

Among the measured soil properties, cropping systems affected soil protein, total N, available N, Mg, S, and compaction (Table 7). Soil protein was significantly greater in the organic systems compared to the conventional systems. Soil protein in the ORG-MNR system was 11, 35, and 39% greater than the ORG-LEG, dCNV, and nCNV systems, respectively. Total soil N and available S concentrations were both similar between the ORG-MNR and nCNV systems, and greater than the ORG-LEG and dCNV systems. Soil available N was significantly greater in the nCNV system compared to the ORG-MNR system. Similar to soil total N, the ORG-LEG and dCNV systems had the lowest concentrations of available soil N. Soil Mg was significantly lower Tillage showed a significant effect only on soil available N and K (Table 7). Across the four cropping systems, soil available N and K concentrations were greater under reduced tillage than under intensive tillage. Neither cropping system nor tillage showed significant effects on soil P, Ca, Zn, and Cu. Cropping system  $\times$  tillage interaction was not observed in any of the measured soil properties.

# Discussion

Corn nutrient composition was most influenced by the cropping system with little impact resulting from tillage management. This reflects previous research on nutrient concentrations of grains grown over a 12 yr period in the FST (Pearsons et al., 2022). That study compared a more limited set of nutrients of corn, soybean, wheat, and oats over a 12 yr period starting when reduced tillage treatments were added to the FST. The most relevant findings to this study include-similar corn yields between the nCNV and MNR systems; greater corn crude protein levels in both organic systems compared to nCNV; greater corn Mg levels but lower percent starch in the MNR system compared to the nCNV system. However, due to crop rotation lengths, rarely were there direct comparisons of crops on an annual basis between all cropping systems and tillage treatments. This study allowed us to dig deeper into how management practices affect one of the most highly grown and consumed crops in the world by having a direct annual comparison, conducting a complete amino acid profile, expanding the minerals tested, and including B vitamins. In this study, significant differences between corn grain amino acid and GPC levels were found between the four cropping systems that did not indicate a clear distinction between

Table 6. ANOVA and means separation results for calories, carbohydrate, crude fat, and vitamins (B<sub>3</sub>, B<sub>6</sub>, and B<sub>9</sub>) concentrations of 2020 corn grown in the FST, Rodale Institute, Kutztown, PA, USA

Variable	Calories (kcal/100 g)	Carbohydrate (g kg <sup>-1</sup> )	Crude fat (g kg <sup>-1</sup> )	Vit. $B_3$ (mg 100 g <sup>-1</sup> )	Vit. $B_6$ (mg 100 g <sup>-1</sup> )	Vit. B <sub>9</sub> (mg 100 g <sup>-1</sup> )
ANOVA						
Cropping system (CS)	0.162	0.373	<0.001	<0.001	<0.001	0.003
Tillage (T)	0.108	0.077	0.235	0.509	0.818	0.372
S×T	0.476	0.332	0.885	0.426	0.656	0.534
Mean separation						
Cropping system						
nCNV	390.5	812	45.1a	1.57b	0.23b	0.022b
dCNV	395.9	820	45.7a	1.45b	0.24b	0.022b
ORG-LEG	391.1	812	42.8b	3.36a	0.27a	0.030a
ORG-MNR	390.8	809	42.8b	3.16a	0.27a	0.032a
Tillage						
Intensive	393.6	818	43.5	2.40	0.25	0.026
Reduced	390.5	809	44.3	2.36	0.25	0.028

Values within each column followed by the same letter are not statistically different (P = 0.05).

nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR, manure-based organic system.

Variable	Protein (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Available N (mg kg <sup>-1</sup> )	P (mg kg <sup><math>-1</math></sup> )	K (mg kg <sup><math>-1</math></sup> )	Ca ( $mg kg^{-1}$ )	Mg (mg $kg^{-1}$ )	S (mg kg <sup><math>-1</math></sup> )	Zn (mg kg <sup><math>-1</math></sup> )	Cu (mg $kg^{-1}$ )	Depth to compaction layer (cm)
ANOVA											
Cropping system (CS)	<0.001	<0.001	<0.001	0.839	0.104	0.114	<0.001	<0.001	0.300	0.659	<0.001
Tillage (T)	0.904	0.586	0.023	0.945	0.036	0.256	0.419	0.084	0.744	0.882	0.587
$CS \times T$	0.668	0.363	0.128	0.909	0.802	0.774	0.340	0.097	0.445	0.438	0.510
Mean separation											
Cropping system											
nCNV	4.78c	3.12a	27.7a	94	77.4	1138	107a	8.77a	2.63	3.65	21.7b
dCNV	4.92c	2.75b	14.4c	109	77.5	1005	94.0a	6.39b	2.39	3.37	21.5b
ORG-LEG	5.97b	2.74b	15.8c	16	73.5	1043	98.4a	6.53b	2.29	3.05	28.5a
ORG-MNR	6.64a	3.18a	20.8b	112	91.3	963	62.8b	8.50a	2.64	3.37	30.4a
Tillage											
Intensive	5.56	3.01	17.8b	102	74.2b	1066	92.1	7.19	2.46	3.30	26.0
Reduced	5.59	2.91	21.5a	101	85.6a	1008	88.9	7.90	2.51	3.36	25.2
Values within each column fo	Ilowed by the si	ame letter are not	statistically differe	nt $(P = 0.05)$ .							

manure-based organic system. nCNV, non-diversified conventional system; dCNV, diversified conventional system; ORG-LEG, legume-based organic system; ORG-MNR,

management, as well as genetics, plays a significant role in shaping corn grain nutritional content. TEAA, TNAA, and GPC were lowest in nCNV but did not differ between the other cropping systems. In general, those amino acids that differed were greatest in the ORG-MNR, and lowest in the nCNV. Differences between some amino acids were observed within systems, with ORG-MNR having greater levels than ORG-LEG in the organic systems, and dCNV having greater levels than nCNV in the conventional system. Minerals, B vitamins, and crude fat showed more clear distinctions between organic and conventional grain. The minerals Ca, S, and Zn and percentage of crude fat were all greater in the conventionally grown corn grain, while levels of all B vitamins were greater in the organically grown corn grain. These differences may have been influenced by differences in soil health indicators that have changed over time. However, they may have also been influenced by different corn genetics used in the organic and conventional cropping systems. While this trial reflects real-world farming scenarios that is typical of the majority of the farmland across the USA, this research was not setup or intended to specifically determine differences between GM and non-GM corn hybrids. However, differences between corn grain nutrient concentrations were observed between the two organic (ORG-LEG, ORG-MNR) and two conventional (nCNV, dCNV) systems, implicating management practices as key indicators driving these changes. While there is little difference between soil health indicators between the conventional systems, the ORG-MNR had improved soil health outcomes compared to the ORG-LEG and both conventional systems. Therefore, improved soil health may be a factor contributing to the observed differences in corn grain nutrient content. However, the variation in nutrient composition within and across organic and conventional systems cannot be attributed to single factors, but are derivative of interconnected factors of genetics, management practices, and soil factors. Additionally, as a system-level experiment with multiple factors within and between cropping systems, this trial does not allow testing of single factors that may be leading to changes in crop nutritional quality. Thus, this trial reflects a real-world scenario in which crop nutrient quality is a function of interrelated factors of genetics, management practices, and environmental factors. Yet, these findings do suggest corn grain nutrition is not fixed by genetics and a greater exploration of the factors related to corn grain nutrition that could impact livestock and human health is warranted.

organic and conventional management, suggesting that crop

Corn nutrient composition has genetic bases, and can vary between corn cultivars (Bullock, Raymer and Savage, 1989; Menkir, 2008; Hinson et al., 2022a). Scott et al. (2006) and Uribelarrea, Crafts-Brandner and Below (2009) found genotypic differences in amino acid and protein concentrations in corn germplasm/cultivars. The differences in nutrient composition of corn grain observed in this study could be a result of the differences in hybrids between the conventional and organic systems. The corn hybrid, Blue River 51T59 (certified organic seed, which are non-GM and not chemically treated) was planted in the two organic systems, whereas the corn hybrid, LC0297 SSXRIB (GM seed) was planted in the two conventional systems. Both hybrids were chosen based on their adaptation to regional growing conditions and previous performance in the mid-Atlantic region. In addition, the conventional corn hybrid was selected to be compatible with the recommended herbicide regimen that was instituted to manage herbicide resistant weeds. Thus, these hybrids are standard varieties used by commercial

Table 7. ANOVA and means separation results for soil protein, total N, available N, minerals, and compaction

conventional and organic corn producers in the region. Several studies have compared the nutritional profiles of standard, non-GM corn cultivars to GM corn cultivars that have been engineered to confer herbicide tolerance to glyphosate (Ridley et al., 2002), or provide protection against European corn borer (Ostrinia nubilalis (Hubner)) (Aeschbacher et al., 2005), and corn rootworm (Diabrotica spp.) (George et al., 2004). These studies found little to no difference in most nutrients tested. In some cases, specific amino acids differed between transgenic and non-transgenic corn cultivar, but this was not replicated across years and locations (George et al., 2004; Aeschbacher et al., 2005). However, these studies are limited in that they only tested one or two transgenic corn lines to a single standard non-transgenic control and were few in number. To properly test the genetic factors leading to nutritional differences found in this study would require a separate, multi-year trial and we are therefore limited in inferring that the differences found between the four cropping systems in this study are mainly due to management and soil health as opposed to genetics.

However, crop management practices did contribute to the variation in the nutrient composition of corn grain. The two conventional systems (nCNV and dCNV)-which had the same corn hybrid (LC0297 SSXRIB)-showed significant differences in grain nutrient composition. The conventional systems significantly differed from each other in regard to TEAA, TNAA, and GPC levels. Specifically, the concentrations of TEAA, TNAA, and GPC in corn grain harvested from the dCNV system were 15, 13, and 21% greater than that of the nCNV system, respectively. Essential amino acids (valine, isoleucine, leucine, and phenylalanine) and non-essential amino acids (aspartic acid, serine, proline, alanine, and tyrosine) were 11-28% greater in the dCNV system than in the nCNV system. The greater levels of amino acids and protein in the dCNV system relative to the nCNV system could be due to a more diverse crop rotation, including cover cropping, in that system. Diversified crop rotations can improve nutrient composition of grain crops (Galantini et al., 2000; Kaye et al., 2007) likely through an increased soil microbial abundance, activity, and diversity (Tiemann et al., 2015; McDaniel and Grandy, 2016), enhanced mycorrhizal associations (Johnson, Tilman and Wedin, 1992; Guzman et al., 2021), increased soil organic matter levels (Grandy and Robertson, 2007; Renwick et al., 2021), and improved nutrient availability and nutrient uptake (Benitez et al., 2021). Riedell et al. (2009) found that a 4-yr rotation under a conventional setting that included corn, wheat, and alfalfa resulted in greater N in corn grain than a continuous corn or 2-yr corn-soybean rotation due to improved soil N availability and uptake. Between the two organic systemswhich had the same corn hybrid (Blue River 51T59), the ORG-MNR system, which has the longest and most diverse crop rotation among the four cropping systems, had 6-8% greater levels of TNAA, alanine, arginine, and serine than the ORG-LEG system. This also shows the critical role of management and crop diversification on the nutrient composition of corn.

Soil health indicators (such as soil compaction, soil protein, and soil organic carbon) that are influenced by management practices may have contributed to the differences in corn grain quality observed among systems. Soil compaction impedes root growth and exploration for nutrients (Unger and Kaspar, 1994; Ishaq et al., 2001), which can reduce nutrient uptake and crop grain quality (Ishaq, Ibrahim and Lal, 2003; Miransari et al., 2009; Wasaya et al., 2018). Ahmad, Hassan and Belford (2009) reported that shallow penetration depth reduced wheat GPC by 12–25%. In the present study, shallower penetration depth (indicating more soil compaction) was observed in the conventional systems than the organic systems, and this may explain the lower concentration of some nutrients (amino acids, protein, and vitamins B<sub>3</sub>, B<sub>6</sub>, and B<sub>9</sub>) in conventional systems. Soil protein represents a readily mineralizable and bioavailable soil N pool that supplies N for plant uptake (Hurisso et al., 2018). The high grain protein and vitamin concentrations observed in the organic systems could be due to the high soil protein in these systems, which supplied a more evenly distributed and available source of N throughout the growing season through mineralization processes (Sprunger et al., 2019). However, the nCNV system had the highest levels of available N based on soil sampling after crop harvest but this did not translate into greater grain protein, amino acid, or B vitamin levels compared to the other systems. It is possible that soil measurements and available N pools were out of synchrony with crop needs and highlights the necessity to sample multiple times over a growing season to be able to truly decipher the role of N or other fertility sources in driving grain crop nutritional quality. Yet this and other studies help pinpoint areas of study that would aid in our understanding of the soil and grain quality connection. Soil organic carbon fractions, though not measured in this study, have recently been measured by Littrell et al. (2021) in the FST. They found that total and biologically active soil organic carbon (SOC), in the 0-30 cm soil depth, were 16-132% greater in the ORG-LEG and ORG-MNR systems than the nCNV system, respectively. Elevated levels of biologically active SOC fractions (mineralizable C, microbial biomass C, permanganate oxidizable C, water extractable organic C) have been associated with increased nutrient cycling efficiency, nutrient availability, and nutrient uptake (Manzoni and Porporato, 2009; Liu et al., 2009; Kallenbach and Grandy, 2011; Bhowmik et al., 2017), which can lead to increased nutrient concentrations in crops.

In the present study, the GPC observed across systems (60-70  $g kg^{-1}$ ) was lower than the typical average reported in most US studies. For instance, in a 3-yr study evaluating US commercial corn germplasm under a conventional farming setting, Butts-Wilmsmeyer, Mumm and Bohn (2017) found GPC of corn hybrid across years averaged 91.2 g kg<sup>-1</sup>. Delate and Cambardella (2004), in a 4-yr cropping system study comparing two organic corn systems to a conventional system in Iowa, found greater GPC in the conventional system than the organic systems in 2 out of the 4 yr, and GPC across years averaged 82 and 78 g kg<sup>-1</sup> for the conventional and the organic systems, respectively. Corn crude fat and carbohydrate contents were greater in the current study relative to other studies (Bullock, Raymer and Savage, 1989; Seebauer et al., 2010; Butts-Wilmsmeyer et al., 2019), and these high crude fat and carbohydrate levels may have contributed to the low corn GPC. Corn GPC and carbohydrate, which primarily consists of starch, are typically inversely related (Uribelarrea, Below and Moose, 2004; Seebauer et al., 2010). Mixed results have been reported on the relationship between crude fat and GPC in corn, with a negative relationship found in some studies (Jaradat and Goldstein, 2014; Ray et al., 2019) and a positive relationship observed in others (Doehlert and Lambert, 1991; Nankar et al., 2017).

Corn grain is primarily used as feedstuff for domestic monogastric (poultry and swine) and ruminant animals in the USA and many other developed countries (Serna-Saldivar, 2019). Corn grain is a good source of energy in animal feeds, but it has limited nutritional quality due to its deficiencies in amino acids such as methionine, cystine, and lysine (Nankar et al., 2017; Blair, 2018). Methionine is an essential amino acid, and it is commonly considered the most limiting amino acid for poultry in terms of their dietary needs (Chalova et al., 2016; Fanatico et al., 2018). The deficiency of methionine in the poultry diet can result in growth inhibition, cannibalism, and increased susceptibility to disease (Bunchasak, 2009; Burley, Patterson and Anderson, 2015). Corn grain with a high methionine concentration is typically high in protein (Jaradat and Goldstein, 2014). In the current study, corn grain had low levels of methionine, cystine, lysine, and tryptophan regardless of the management system, and this is consistent with many other studies (Nankar et al., 2017; Blair, 2018). The low methionine levels across systems indicate that methionine is inherently constrained by genetics, and therefore there is the need to develop adapted corn hybrids with greater methionine levels.

With the exception of methionine, tillage practices did not influence the nutrient composition of corn grain. Methionine levels were greater in the reduced tillage systems (Table 3). The lack of tillage effect on corn nutrient concentrations has been reported previously (Al-Kaisi and Kwaw-Mensah, 2007; Pearsons et al., 2022). In a long-term ( $\geq 20$  yr) tillage study comparing chisel plow and no-till practices in Missouri, Houx, Wiebold and Fritschi (2016) found no difference in corn grain protein, macronutrients (P, K, Ca, and Mg), and micronutrient (Fe, Mn, Zn, and B) concentrations. In a 3-yr study in Iowa, Singer et al. (2007) compared tillage practices (moldboard plow, chisel plow, and no-till) and soil amendments (compost and no-compost), and they found no effect of tillage and tillage x compost interaction on corn grain N, P, and K concentrations. Soil nutrient concentrations generally did not differ by tillage, and this likely contributed to the lack of tillage effect on corn grain nutrient concentrations observed in this study.

Previous studies comparing nutrient concentrations of grains have found a 'dilution effect' or negative correlation between yield and certain nutrient components (Simmonds, 1995; Scott et al., 2006; Davis, 2009). Simmonds (1995) found a negative relationship between yield and protein for all the major global grain crops, including corn. Scott et al. (2006) evaluated 45 corn cultivars released between 1920 and 2001 in side-by-side studies at multiple locations and found a similar result. These findings were mostly attributed to breeding over time targeted at increasing yields. In some cases however, increased soil fertility contributed to increased yields and reductions (dilution) in crop nutrient levels (Davis, 2009). In the present study, differences existed in total and available soil N pools and fertilizer regimes. However, a 'dilution effect' did not appear to be a factor in determining nutrient levels, as the ORG-MNR system had both the highest yields and highest GPC and the ORG-LEG system, which had the lowest yields, had GPC similar to ORG-MNR and dCNV.

#### Conclusions

Conventional and organic corn cropping systems in the USA vary in multiple management components and these system-based differences may contribute to differences in nutrient composition of corn grain. In this study, grain nutrient profile of corn was influenced by cropping systems, with the two organic systems generally showing greater concentrations of amino acids and protein compared to the conventional systems but differences between the ORG-MNR and ORG-LEG and between the dCNV and nCNV were also observed, suggesting that management may be playing a role in shaping nutrient concentrations while differences due to hybrid genetics between the organic and conventional systems cannot be ruled out. Management practices (diversified crop rotation and cover cropping) may have influenced corn nutrient composition, as evidenced by the ORG-MNR system having higher levels of some amino acids compared to the ORG-LEG system and the dCNV system exhibiting significantly greater levels of most amino acids and GPC than the nCNV system, despite both systems using the same hybrid. Some minerals and crude fat were higher in both conventional systems while B vitamins were higher in both organic systems, suggesting these nutrients may be more influenced by corn hybrid genetics and less crop management or soil factors. Soil factors (such as deeper compaction layer, greater soil protein, and organic C levels) may also have contributed to the greater concentrations of corn grain amino acids, protein, and B vitamin levels in the organic systems than the conventional systems. The study showed that 12 yr of tillage treatment did not affect the nutrient composition of corn grain. The results of this study provide novel information on the legacy impacts of organic and conventional cropping systems under different tillage practices on the nutrient composition of corn grain.

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