



Enhancing zinc and iron bioavailability through crop rotation and organic farming: Insights from a long-term study

Jing Hou^a, Xiaopeng Gao^{a,*}, Martin H. Entz^b

^a Department of Soil Science, University of Manitoba, Winnipeg, Manitoba, Canada

^b Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada

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ABSTRACT

Agronomic biofortification of Zinc (Zn) and iron (Fe) in cereal grains is a critical strategy to address human deficiencies in these micronutrients. Our study examined the effects of crop rotation and farming system on Zn and Fe bioavailability in wheat grains, using data from 2011 to 2020 from the Glenlea Long-term Crop Rotation Study, Canada's longest running study on organic farming. Two crop rotations, continuous annual (spring wheat, flax, oat, soybean) and annual-perennial (spring wheat, flax, alfalfa, alfalfa), managed both organically and conventionally, were fully phased. Compared to conventional farming, organic farming significantly increased grain Zn concentration in six out of ten years, with no significant effect on grain Fe. Additionally, grain Zn was also significantly higher in the annual-perennial rotation compared to the annual rotation in seven of the ten years. A significant interactive effect was observed for grain phytate, which was approximately 35 % lower in the annual-perennial rotation under organic farming than in other treatments. Consequently, the diversified rotation under organic farming significantly reduced the phytate/Zn and phytate/Fe molar ratios, indicating enhanced micronutrient bioavailability for human consumption. However, these effects were strongly associated with reduced phosphorus (P) availability in organic systems, particularly following alfalfa, which limited grain yields. Livestock manure-based compost application improved grain yield and nutrient uptake, but reduced Zn/Fe bioavailability due to increased phytate accumulation. This study demonstrates that diversified crop rotation and organic farming could improve micronutrient bioavailability, but at a cost of lower grain production tied to reduced P availability.

1. Introduction

Zinc (Zn) and iron (Fe) are essential micronutrients for the growth and development of plants, animals, and humans. In plants, Zn serves as a functional, structural or regulatory co-factor for numerous enzymes and proteins involved in critical biochemical processes. In animals and humans, approximately 3000 proteins, or 10 % of all proteins, require adequate Zn for their optimal function (Cakmak and Kutman, 2018). Zinc is a critical component in all six classes of enzymes including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases (Barak and Helmke, 1993). Similarly, Fe is an integral part of hemoglobin and myoglobin, where it is directly involved in oxygen transport, DNA synthesis, enzymatic functions, and energy production. Malnutrition resulting from Zn and Fe deficiencies, commonly referred to as “hidden hunger”, represents one of the most significant global health challenges. It is estimated that over 60 % of the global population is

affected by Fe deficiency, while more than 30 % are suffering from Zn deficiency (White and Broadley, 2009). According to Hotz and Brown (2004), about one-third of the global population lives in countries at high risk for Zn deficiency, leading to approximately 1.1 billion people are at risk, with 90 % residing in Asia and Africa (Kumssa et al., 2015).

The primary cause of Zn and Fe deficiencies is inadequate dietary intake, particularly in regions where people rely heavily on cereal-based foods that have lower Zn and Fe concentrations, and bioavailability compared to animal-based foods. Cereal crops naturally have low Zn and Fe concentration and cultivating them on nutrient-deficient soil further reduces their bioavailability in grains (Cakmak, 2008). Wheat (*Triticum aestivum* L.), the most extensively grown cereal crop in the world, provides 70 % of daily calorie intake and feeds around 40 % of the world's population (Giraldo et al., 2019). Despite being the most traded commodity globally, wheat is considered a poor source of minerals, particularly Zn, Fe, and vitamins. A meta-analysis of 109 studies reported that

* Correspondence to: Department of Soil Science, University of Manitoba, 311 Ellis Building, 13 Freedman Crescent, Winnipeg, MB R3T 2N2, Canada.

E-mail address: xiaopeng.gao@umanitoba.ca (X. Gao).

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Zn concentrations in wheat grain from major wheat-producing regions around the world vary from 20.4 to 30.5 mg kg⁻¹ with an average of 27.3 mg kg⁻¹ (Chen et al., 2017), far below the target level of 40–60 mg kg⁻¹ to meet needs of human body (Cakmak, 2008). Minerals in wheat grain are concentrated in the aleurone layer, which is usually removed during milling, resulting in refined flour with only small amounts of Zn and Fe. Bioavailability of micronutrients such as Zn and Fe is further impacted by presence of phytate, which is the dominant storage form of phosphorus (P) in cereal grains and binds micronutrients strongly to prevent their absorption by the human digestive system. The phytate to Zn or Fe molar ratio has been generally used to categorize their bioavailability (Bouis and Welch, 2010).

Several strategies are employed to tackle micronutrient deficiency, including supplementation, food fortification, dietary diversification, and biofortification. Supplementation provides nutrients directly in the form of chemical or pharmaceutical products. Food fortification involves adding nutrients to commonly consumed foods, beverages or condiments to increase their nutrient contents, such as iodized salt. Dietary diversification focuses on expanding the variety of nutrient-rich foods consumed to improve overall nutritional intake. These strategies, however, often face challenges related to accessibility and affordability, particularly in remote or impoverished regions where dietary options are limited. Biofortification, which increases nutrient levels in edible plant parts through agricultural practices, could offer a more sustainable solution to global nutritional deficiencies. For Zn biofortification in food crops, a combination of conventional breeding, genetic engineering, and agronomic practices like soil amendments and fertilization is typically used (Yadav et al., 2023; Younas et al., 2023). Compared to breeding or genetic approach, the agronomic approaches provide a timesaving, economical, and effective solution to improving Zn bioavailability in cereal grains, along with increased grain yield (Zou et al., 2012; Zulfiqar et al., 2020). Many studies have demonstrated that soil and foliar Zn fertilization effectively improved wheat grain Zn concentration and bioavailability (Rehman et al., 2018; Erdal et al., 2002; Cakmak, 2008; Wang et al., 2015). In contrast, the impact of crop rotation and farming system management on micronutrient biofortification in crop grain has not been extensively explored.

Organic farming can influence the concentration and uptake of micronutrients in crop grains through its practices of soil management, crop rotation, and the avoidance of synthetic fertilizers. Specifically, organic farming often promotes more diverse crop rotation and the use of natural sources like compost and manure, which can enhance soil organic matter and microbial activity. These factors may improve the availability of micronutrients in the soil, leading to higher uptake by plants. However, the effects of organic farming on grain Zn concentration are not always consistent, as they can vary depending on soil type, crop variety, and specific management practices. For example, a study by Ryan et al. (2004) found that organic grown wheat grain had higher Zn concentrations compared to conventionally grown wheat, which was attributed to increased mycorrhizal colonization in organic systems. Similarly, Nelson et al. (2011) reported higher Zn concentrations in wheat grown organically on the Canadian Prairies, with effects varying by wheat cultivar. Preliminary results from the same site as the current study showed that wheat produced organically in the perennial rotation had higher Zn than annual rotation, whereas there was no crop rotation effect when wheat was produced conventionally (Turmel et al., 2009). Conversely, Mäder et al. (2007) reported no significant differences in Zn concentration between organic and conventional wheat after a 21-year field experiment, despite 14 % lower yields in the organic system; this indicated that grain Zn differences between the two systems was not related to yield dilution. In addition to Zn, it has also been documented that Fe levels in organically produced crops are 21 % greater than in conventionally grown crops (Rembiałkowska, 2007). However, other studies have found no significant differences in the nutrient quality between organically and conventionally produced food (Dangour et al., 2009; Smith-Spangler et al., 2012). These inconsistent results indicates

that while organic farming has the potential to enhance grain Zn and Fe levels, the outcomes may vary depending on the specific rotation and environmental conditions. Moreover, none of these studies have investigated how these agricultural practices affect grain phytate levels and their impact on Zn and Fe bioavailability. Therefore, to fully understand the nutritional impact of different farming systems and crop rotations, future research should explore the relationship between Zn/Fe concentration and phytate levels in crop grains.

The Glenlea Long-term Crop Rotation Study Site, established in 1992, is Canada's longest-running organic farming systems field plots. The present study, conducted as part of this long-term research, aims to investigate how farming systems and crop rotations affect Zn and Fe bioavailability in wheat grain. The specific objectives are 1) to compare micronutrient bioavailability of wheat grain produced organically versus conventionally; 2) to evaluate the differences in micronutrient bioavailability between wheat grown under annual crop rotation and annual-perennial crop rotation; 3) to examine the relationships between grain Zn levels and other mineral nutrients, grain protein, and crop yield, as influenced by different farming systems and crop rotation strategies; and 4) to understand whether correcting P deficiencies in organic production would compromise Zn and Fe bioavailability due to increases in P availability.

2. Materials and methods

2.1. Experimental site and design

The Glenlea Long-term Crop Rotation Study Site (N 49.39.0, W 97.7.0) is located in the Red River Valley in Southern Manitoba, Canada. The site lies within a glaciolacustrine clay floodplain of near-level topography (0 to < 2 % slope) and has an extreme humid-continental climate (Köppen *Dfb*). The soils at this site are classified as Gleyed Humic Vertisols under the Canadian soil classification system and as Typic Humicryerts under the US system, with a clay texture (65 % clay, 26 % silt and 9 % sand).

The experimental design included two crop rotations: annual (oat-soybean-spring wheat-flax) and annual-perennial (spring wheat-flax-alfalfa-alfalfa), both of which were managed under organic and conventional systems. These rotations were fully phased each year across three replicate blocks from 2011 to 2020, during which data for the present study were collected. Under organic management, no synthetic fertilizer herbicide or pesticide were used, whereas under conventional management, fertilizer application followed soil tests and local recommendations for each crop rotation. The experiment employed a randomized complete block design in a split-plot arrangement, where crop rotation served as the main plot and the farming system as the subplot. The plot size for organic systems were 12 m by 4 m, while the conventional systems had a plot size of 28 m by 4 m. Seeding rates were typically 165 kg ha⁻¹ for the organic plots and 125 kg ha⁻¹ for the conventional plots, with occasional adjustments made depending on spring conditions. The spring wheat cultivar used in this experiment was Cardale, a semi-dwarf hard red cultivar released in 2011 by Agriculture and Agri-Food Canada (Fox et al., 2013). Additionally, a comparison of compost and non-compost treatments was implemented within the annual-perennial crop rotation under the organic farming to evaluate their effects on crop productivity and micronutrient bioavailability. The compost treatment involved the annual application of composted beef cattle manure to achieve a target rate of 80 kg ha⁻¹ of P in the soil from 2011 to 2020. Throughout the study years, same source of beef solid manure was used for the compost. The total N content ranged from 0.54 % to 0.84 %, P₂O₅ ranged from 0.65 % to 0.91 %, K₂O ranged from 1.1 % to 3.9 %, total Zn ranged from 65 mg kg⁻¹ to 219 mg kg⁻¹, and total Fe ranged from 6176 mg kg⁻¹ to 9100 mg kg⁻¹. Total N was reported on an as-received basis for solid manure, while other nutrients were reported on a dry-mass basis. All analyses were conducted by Agvise Laboratories (Northwood, ND, USA). Herbicide and pesticide

were applied at the recommended rates in the conventional plots only.

2.2. Analysis of grain samples for nutrient concentration

Wheat grain was harvested using a custom plot combine in late August or early September, depending on crop maturity and weather conditions. Harvested grain samples were dried on specialized drying beds, cleaned using a Carter Day dockage tester, and then determined for dry yield. Subsequently, a representative sample was finely ground using a zirconium ball mill, and then digested using a mixture of sulfuric acid and hydrogen peroxide (Westman, 1990). Briefly, 0.4 g of ground wheat grain was added to 4.4 mL of acid solution, left at room temperature for 1 hour, then digested for 1 hour at 100°C followed by 2 hours at 350 °C. Nitrogen (N) in the digest solution was determined using a Technicon Autoanalyzer II (Technicon Industrial Systems, Tarrytown, NY, USA). Other nutrients, including Zn, Fe, P, Mg, Mn and Cu were analyzed using an ICP-MS (Agilent 7850, Agilent, Santa Clara, USA). Grain protein concentration was calculated assuming a protein to N ratio of 6.25.

2.3. Analysis of grain samples for phytate concentration and Zn/Fe bioavailability

Grain phytate was determined according to Haug and Lantzsch (1983), with slight modifications. Finely ground wheat grain samples (0.15 g each) were extracted in 25 mL of 0.2 N HCl. The solutions were vortexed, and 0.5 mL of each solution was transferred to 2 mL centrifuge tube, followed by the addition of 1 mL ferric solution. The mixtures were heated in a water bath at 100°C for 30 minutes, then immediately cooled in an ice water bath for 15 minutes before returning to room temperature. After centrifugation at 5700 rpm for 30 minutes, 1 mL of supernatant was mixed with 1.5 mL of 2,2'-bipyridine solution (prepared by dissolving 5 g of 2,2'-bipyridine and 5 mL of thioglycolic acid in deionized water, then diluted to 500 mL). Phytate concentration was measured by the decrease in absorbance of the pink color developed by unreacted Fe with 2,2'-bipyridine at 519 nm using a UV/visible spectrophotometer (Ultrospec 2100 pro, GE Healthcare, UK). All grain samples for nutrient and phytate determinations were prepared and analyzed in triplicates. Grain nutrient and phytate uptake were calculated by multiplying grain yield with their respective concentrations. Molar ratios of phytate to Zn or Fe were calculated to estimate the bioavailability of Zn and Fe in wheat grain, with lower ratios indicating higher bioavailability.

$$\text{Phytate : Zn molar ratio} = \frac{(\text{phytate concentration in mg kg}^{-1})/660.04}{(\text{Zn concentration in mg kg}^{-1})/65.38}$$

$$\text{Phytate : Fe molar ratio} = \frac{(\text{phytate concentration in mg kg}^{-1})/660.04}{(\text{Fe concentration in mg kg}^{-1})/55.85}$$

2.4. Statistical analysis

Data analysis was conducted using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA). An initial three-way analysis of variance (ANOVA) was performed with the Mixed procedure, treating farming system and crop rotation as fixed effects and year as a random effect. For each individual year, a two-way ANOVA was applied to evaluate the main and interactive effects of farming system and crop rotation. When significant main or interactive effects were detected, means were compared using Tukey's Honestly Significant Differences test. Differences between compost and non-compost treatments within the annual-perennial crop rotation under the organic farming system were assessed using a paired *t*-test. Linear regression was used to examine relationships between measured parameters. In all analyses, statistical significance was set at a 5 % probability level.

3. Results

3.1. Impact of farming system and crop rotation on grain nutrients, protein and yield

Wheat grain yield and nutrient concentrations of Zn, Mg and P were significantly ($p < 0.05$) affected by farming system, crop rotation, year and their interactions (Table 1). Grain protein concentration was significantly ($p < 0.05$) affected by crop rotation and year. Additionally, grain Cu concentration varied across years, and was significantly ($p < 0.05$) impacted by the interactions between farming system and crop rotation. In contrast, grain Fe concentration differed between years but did not show significant response to farming systems, crop rotation, or their interactions. Grain Mn concentration was only affected by the three-way interaction among farming system, crop rotation and year, but was not significantly affected by each factor individually.

Compared to conventional farming, organic farming exhibited significantly ($p < 0.05$) higher Zn concentrations in wheat grain in six out of ten years (Table 2). Across the study period, grain Zn concentrations ranged between 25.6 and 36.9 mg kg⁻¹ for the organic system, and between 19.7 and 31.7 mg kg⁻¹ for the conventional system. The 10-year average Zn concentration in the organic system was 16.9 % higher than that in the conventional system. Wheat in the annual-perennial rotation had significantly ($p < 0.05$) higher grain Zn concentrations than that in the continuous annual rotation in seven out of ten years. As a result, the 10-year average grain Zn in the annual-perennial rotation was 19.6 % higher than that in the annual rotation. In six out of ten years, grain Zn was significantly ($p < 0.05$) affected by an interaction between farming system and crop rotation, where the combination of organic system and annual-perennial rotation consistently had the highest grain Zn concentrations.

There was a large variation in grain Fe concentration among different years, ranging from 25.5 to 71.1 mg kg⁻¹ over the 10-year study period, with relatively higher values observed in 2017 and 2018 compared to other years (Table 3). Farming system, crop rotation and their interaction did not influence grain Fe concentration in any individual year ($p > 0.05$), except for 2016, where the annual-perennial rotation exhibiting significantly ($p < 0.05$) higher Fe concentration compared to the annual rotation.

In each of the ten years, wheat grain yield was significantly ($p < 0.05$) lower in the organic system compared to the conventional system (Table 4). The 10-year average yield in the organic system was only approximately half of that in the conventional system. In five out of ten years, yields were significantly ($p < 0.05$) lower in the annual-perennial rotation compared to the annual rotation, leading to a 35 % lower 10-year average for the annual-perennial rotation. Additionally, the 10-year average yield was significantly ($p < 0.05$) affected by the interaction between farming system and crop rotation, which was contributed to that wheat yields were significantly lower in the annual-perennial rotation than the annual rotation for the organic system but yields between the two crop rotations were comparable for the conventional system.

3.2. Impact of farming systems and crop rotation on phytate, and molar ratios of phytate to Zn/Fe

Over the 10-year study period, the organic annual-perennial rotation had a phytate concentration of 7.9 mg g⁻¹, which was 34.2 % lower ($p < 0.05$) than in the other treatments (Fig. 1A). Additionally, the [Phytate]:[Zn] ratio ranged from 24.6 to 50.7, and the [Phytate]:[Fe] ratio ranged from 18.4 to 32.3. Both ratios were significantly lower ($p < 0.05$) in the organic annual-perennial rotation system compared to other treatments (Fig. 1B, C), suggesting improved bioavailability of Zn and Fe.

3.3 Impact of compost application on grain Zn and Fe bioavailability under annual-perennial crop rotation in the organic farming system

Table 1

p-values of three-way ANOVA evaluating the effect of farming system (FS), crop rotation (CR) and year (Y) on wheat grain nutrient concentrations, protein and yield from the Glenlea Long-term Crop Rotation study over 2011–2020.

Source	DF	Mg	P	Mn	Fe	Cu	Zn	Protein	Yield
FS	1	< 0.0001	< 0.0001	0.0696	0.5236	0.5720	< 0.0001	0.9208	< 0.0001
CR	1	< 0.0001	< 0.0001	0.1720	0.1899	0.3155	< 0.0001	0.0046	< 0.0001
Y	9	< 0.0001	< 0.0001	0.0962	< 0.0001	0.0043	< 0.0001	< 0.0001	< 0.0001
FS × CR	1	< 0.0001	< 0.0001	0.3304	0.7398	0.0131	< 0.0001	0.9353	0.0003
FS × Y	9	< 0.0001	0.0011	0.1023	0.4329	0.0370	0.0347	0.7687	0.0396
CR × Y	9	0.2601	0.1433	0.1985	0.7794	0.0692	0.2956	0.1626	0.0024
FS × CR × Y	9	0.0189	0.0210	0.0475	0.7284	0.1984	0.0197	0.7208	0.0394

Table 2

Effect of farming system (FS) and crop rotation (CR) on wheat grain Zn concentration at the Glenlea Long-term Crop Rotation study site over 2011–2020.

Treatment	Grain Zn concentration (mg kg ⁻¹)										
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2011–2020
FS											
Org.	27.9 ± 1.6 a	28.5 ± 2.4 a	25.6 ± 1.5 a	25.6 ± 2.1 a	26.9 ± 1.7 a	27.2 ± 1.1 a	29.1 ± 2.9 a	31.5 ± 3.4 a	30.8 ± 2.6 a	36.9 ± 1.9 a	29.0 ± 0.8 a
Conv.	22.5 ± 2.2 a	22.2 ± 0.6 b	23.3 ± 0.7 b	21.9 ± 0.4 b	25.3 ± 0.5 a	25.0 ± 0.4 b	19.7 ± 0.6 b	29.4 ± 1.3 b	27.1 ± 1.1 a	31.7 ± 0.9 b	24.8 ± 0.5 b
CR											
A.	22.9 ± 2.3 a	22.4 ± 0.8 b	22.4 ± 0.6 b	21.5 ± 0.5 b	24.3 ± 0.6 b	24.7 ± 0.5 b	21.8 ± 1.5 b	26.4 ± 1.1 b	26.1 ± 2.2 a	32.6 ± 1.9 a	24.5 ± 0.6 b
A.P.	27.6 ± 1.6 a	28.3 ± 2.4 a	26.4 ± 1.2 a	26.0 ± 1.9 a	27.9 ± 1.3 a	27.5 ± 0.9 a	27.0 ± 3.6 a	34.5 ± 2.0 a	31.8 ± 1.2 a	36.0 ± 1.5 a	29.3 ± 0.7 a
FS × CR											
Org.-A.	26.4 ± 2.8 a	23.6 ± 1.3 b	22.5 ± 1.2 b	21.1 ± 1.1 b	23.5 ± 0.1 b	25.1 ± 1.0 b	23.3 ± 2.8 b	25.2 ± 0.8 c	27.2 ± 4.6 a	35.1 ± 3.6 a	25.3 ± 0.9 b
Conv.-A.	19.3 ± 2.5 a	21.2 ± 0.4 b	22.3 ± 0.5 b	22.0 ± 0.2 b	25.1 ± 0.9 b	24.3 ± 0.2 b	20.3 ± 1.1 b	27.7 ± 1.9 bc	25.0 ± 1.1 a	30.2 ± 0.6 a	23.7 ± 0.7 b
Org.-A.P.	29.4 ± 1.6 a	33.3 ± 1.7 a	28.7 ± 0.9 a	30.1 ± 1.0 a	30.3 ± 1.7 a	29.3 ± 0.5 a	34.9 ± 0.5 a	37.9 ± 0.4 a	34.4 ± 0.9 a	38.8 ± 1.6 a	32.6 ± 0.7 a
Conv.-A.	25.7 ± 2.7 a	23.2 ± 0.6 b	24.2 ± 1.2 b	21.9 ± 0.8 b	25.5 ± 0.7 b	25.7 ± 0.6 b	19.0 ± 0.5 b	31.1 ± 0.9 b	29.3 ± 0.6 a	33.2 ± 1.3 a	25.9 ± 0.8 b
Analysis of Variance											
Source	Pr ≥ F										
FS	0.0613	0.0005	0.0215	0.0018	0.1460	0.0087	0.0002	0.0363	0.1704	0.0365	< 0.0001
CR	0.0922	0.0007	0.0018	0.0007	0.0082	0.0025	0.0042	0.0001	0.0501	0.1443	< 0.0001
FS × CR	0.5140	0.0086	0.0296	0.0006	0.0146	0.0647	0.0014	0.0017	0.5504	0.8710	0.0015

Abbreviations: Org. = Organic farming, Conv. = Conventional farming, A. = Annual crop rotation, A.P. = Annual-perennial crop rotation, FS = Farming system, CR = Crop rotation.

Data presented are means ± standard error (n = 3).

Per column, within the farming system, or crop rotation, or their interaction, means followed by different letters are significantly different at *p* < 0.05.

Table 3

Effect of farming system (FS) and crop rotation (CR) on wheat grain Fe concentration at the Glenlea Long-term Crop Rotation study site over 2011–2020.

Treatment	Grain Fe concentration (mg kg ⁻¹)										
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2011–2020
FS											
Org.	41.8 ± 7.2 a	30.7 ± 1.7 a	28.3 ± 2.5 a	25.5 ± 1.0 a	29.4 ± 2.6 a	42.8 ± 6.6 a	68.1 ± 7.2 a	53.0 ± 4.9 a	32.8 ± 4.6 a	46.2 ± 12.4 a	39.4 ± 2.4 a
Conv.	32.6 ± 2.2 a	29.4 ± 0.7 a	27.0 ± 0.3 a	29.1 ± 1.4 a	33.0 ± 4.1 a	35.7 ± 3.6 a	70.3 ± 14.3 a	71.1 ± 11.1 a	53.7 ± 10.3 a	39.5 ± 2.5 a	42.1 ± 2.9 a
CR											
A.	33.2 ± 2.7 a	31.2 ± 1.6 a	26.3 ± 1.0 a	28.0 ± 1.7 a	27.4 ± 0.9 a	29.6 ± 1.1 b	67.8 ± 14.0 a	56.0 ± 7.3 a	39.9 ± 5.4 a	46.2 ± 12.4 a	38.6 ± 2.6 a
A.P.	41.3 ± 7.2 a	28.9 ± 0.6 a	29.0 ± 2.2 a	26.6 ± 1.0 a	35.0 ± 4.3 a	48.9 ± 4.6 a	70.6 ± 7.7 a	68.1 ± 12.3 a	46.6 ± 11.7 a	39.6 ± 2.4 a	42.9 ± 2.7 a
Analysis of Variance											
Source	Pr ≥ F										
FS	0.2905	0.5071	0.6217	0.0534	0.4688	0.0688	0.9009	0.2398	0.1296	0.6154	0.4651
CR	0.3477	0.2450	0.3337	0.4227	0.1440	0.0006	0.8736	0.4229	0.6036	0.6178	0.2519
FS × CR	0.8205	0.8675	0.5055	0.1388	0.7846	0.0596	0.3941	0.8885	0.8357	0.2613	0.8471

Abbreviations: Org. = Organic farming, Conv. = Conventional farming, A. = Annual crop rotation, A.P. = Annual-perennial crop rotation, FS = Farming system, CR = Crop rotation.

Data presented are means ± standard error (n = 3).

Per column, within the farming system or crop rotation, means followed by different letters are significantly different at *p* < 0.05.

Table 4

Effect of farming system (FS) and crop rotation (CR) on wheat grain yield at the Glenlea Long-term Crop Rotation study site over 2011–2020.

Treatment	Wheat grain yield (kg ha ⁻¹)										
	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2011–2020
FS											
Org.	586 ± 120 b	1521 ± 281 b	1691 ± 452 b	1789 ± 359 b	1140 ± 248 b	1675 ± 108 b	1904 ± 540 b	1479 ± 256 b	1014 ± 345 b	1256 ± 226 b	1405 ± 107 b
Conv.	1952 ± 107 a	3606 ± 554 a	3266 ± 240 a	2885 ± 299 a	2508 ± 149 a	3183 ± 210 a	3775 ± 193 a	2038 ± 271 a	2038 ± 324 a	3146 ± 83 a	2840 ± 115 a
CR											
A.	1325 ± 294 a	2705 ± 311 a	3142 ± 338 a	2688 ± 308 a	2142 ± 221 a	2416 ± 294 a	3317 ± 253 a	2341 ± 137 a	2185 ± 330 a	2104 ± 477 a	2436 ± 114 a
A.P.	1214 ± 353 a	2422 ± 846 a	1814 ± 469 b	1987 ± 441 a	1506 ± 426 b	2443 ± 443 a	2362 ± 723 b	1175 ± 144 b	867 ± 214 b	2298 ± 429 a	1809 ± 162 b
FS × CR											
Org.-A.	713 ± 168 b	2091 ± 259 ab	2582 ± 437 a	2506 ± 222 ab	1677 ± 61 b	1823 ± 107 b	3043 ± 391 a	2052 ± 37 b	1615 ± 468 b	1167 ± 497 b	1927 ± 146 b
Conv.-A.	1937 ± 169 a	3319 ± 197 ab	3703 ± 256 a	2869 ± 627 a	2608 ± 151 a	3008 ± 266 a	3591 ± 304 a	2631 ± 94 a	2754 ± 47 a	3040 ± 111 a	2946 ± 116 a
Org.-A.P.	460 ± 167 b	950 ± 37 b	800 ± 196 b	1073 ± 288 b	602 ± 120c	1527 ± 156 b	765 ± 85 b	906 ± 157 d	414 ± 130c	1344 ± 20 b	885 ± 77c
Conv.-A.	1967 ± 170 a	3893 ± 1190 a	2829 ± 174 a	2901 ± 230 a	2409 ± 280 ab	3358 ± 346 a	3959 ± 243 a	1444 ± 82 c	1321 ± 85 bc	3252 ± 179 a	2733 ± 199 a
Analysis of Variance											
Source	Pr ≥ F										
FS	< 0.0001	0.0089	0.0011	0.0204	< 0.0001	0.0002	0.0002	0.0001	0.0033	< 0.0001	< 0.0001
CR	0.2819	0.6241	0.0026	0.1026	0.0062	0.9131	0.0053	< 0.0001	0.0007	0.477	< 0.0001
FS × CR	0.1844	0.1689	0.1427	0.0903	0.035	0.2112	0.001	0.8080	0.6512	0.948	0.0040

Abbreviations: Org. = Organic farming, Conv. = Conventional farming, A. = Annual crop rotation, A.P. = Annual-perennial crop rotation, FS = Farming system, CR = Crop rotation.

Data presented are means ± standard error (n = 3).

Per column, within the farming system, or crop rotation, or their interaction, means followed by different letters are significantly different at $p < 0.05$.

Compost application had no effect on grain Fe concentration but significantly ($p < 0.05$) reduced Zn concentration to 26.4 mg kg⁻¹, compared to 32.6 mg kg⁻¹ in the non-compost control (Table 5). As expected, compost application significantly ($p < 0.05$) increased grain P concentration from 1.3 g kg⁻¹ to 1.8 g kg⁻¹, and phytate from 7.9 mg g⁻¹ to 10.6 mg g⁻¹. Consequently, the [Phytate]:[Zn] and [Phytate]:[Fe] ratios were significantly ($p < 0.05$) higher with compost application compared to the non-compost control. Additionally, compost nearly doubled grain yield but significantly ($p < 0.05$) reduced grain protein concentration from 15.3 % to 13.9 % (Table 5). As a result, compost application significantly increased grain Zn uptake by 56 %, and nearly doubled Fe uptake. More notably, compost led to a 1.6-fold increase in grain P and phytate uptake (Table 6).

3.3. Relationships of grain Zn concentration with grain P concentration, yield and protein concentration

Over the 10-year study, grain Zn concentration showed a significantly ($p < 0.001$) negative correlation with both grain P concentration ($r = -0.423$) and grain yield ($r = -0.555$) (Fig. 2A, B). In contrast, grain Zn was positively correlated with protein concentration ($r = 0.585$) (Fig. 2C).

4. Discussion

4.1. Annual-perennial crop rotation with organic farming enhanced grain Zn and Fe bioavailability

This study revealed key findings on the impact of farming system and crop rotation on micronutrient concentration and bioavailability in wheat grain, focusing on Zn and Fe. Organic farming significantly enhanced Zn concentration compared to conventional systems, while the annual-perennial crop rotation was more effective than continuous annual rotation. Further, the combination of organic farming and annual-perennial rotation led to significantly higher Zn concentration, lower phytate level, and consequently a reduced [phytate]:[Zn] ratio,

indicating improved bioavailability for human consumption. These findings are consistent with those of Ryan et al. (2004) and Turmel et al. (2011), who also reported higher Zn and lower P concentration in organic systems with annual-perennial rotations. Therefore, our study confirms that organic farming, particularly when combined with diverse crop rotations, is a promising strategy for enhancing Zn bioavailability in wheat grain. Unlike conventional systems, organic systems' reliance on natural inputs and the absence of synthetic fertilizers could promote the long-term sustainability of micronutrient biofortification in crops.

However, despite these improvement, Zn and Fe bioavailability in wheat grain in the current study remains suboptimal to meet human consumption needs. A [phytate]:[Zn] ratio exceeding 15:1 and [phytate]:[Fe] ratio over 1:1 are associated with poor absorption by humans (Gibson et al., 1997; Hurrell and Egli, 2010). In our study, the organic annual-perennial system had ratios of 24.4 for Zn and 18.2 for Fe, exceeding critical thresholds. Similarly, previous studies have also reported low Zn and Fe bioavailability in wheat varieties. For example, Hussain et al. (2011) reported [phytate]:[Zn] ratios ranging from 23.9 to 41.1 in 65 bread wheat varieties in Pakistan, indicating low Zn bioavailability. Magallanes-López et al. (2017) and Tran et al. (2020) analyzed 46 and 101 wheat genotypes, respectively, and found that all genotypes fell into the low bioavailability category for both Zn and Fe. This outcome is not surprising, given that wheat grain inherently contains low levels of Zn and is rich in compounds such as polyphenols and phytate that inhibit micronutrient absorption (Cakmak, 2008).

Furthermore, we observed a considerable trade-off between improved micronutrient bioavailability and reduced yield productivity, highlighting the challenges for organic farming systems. At the Glenlea study site, organic systems produced only about half the yield of the conventional systems. Organic farming often produces lower yields due to reliance on natural inputs, slower nutrient availability, higher weed pressure, and potential soil nutrient depletion over time, particularly P deficiency (Welsh et al., 2009; Nelson et al., 2011). A survey of 14 organic farms in the Canadian prairie provinces and North Dakota revealed that while these farms generally maintain sufficient levels of N, K, and S, they often lack adequate P (Entz et al., 2001). Similarly, a

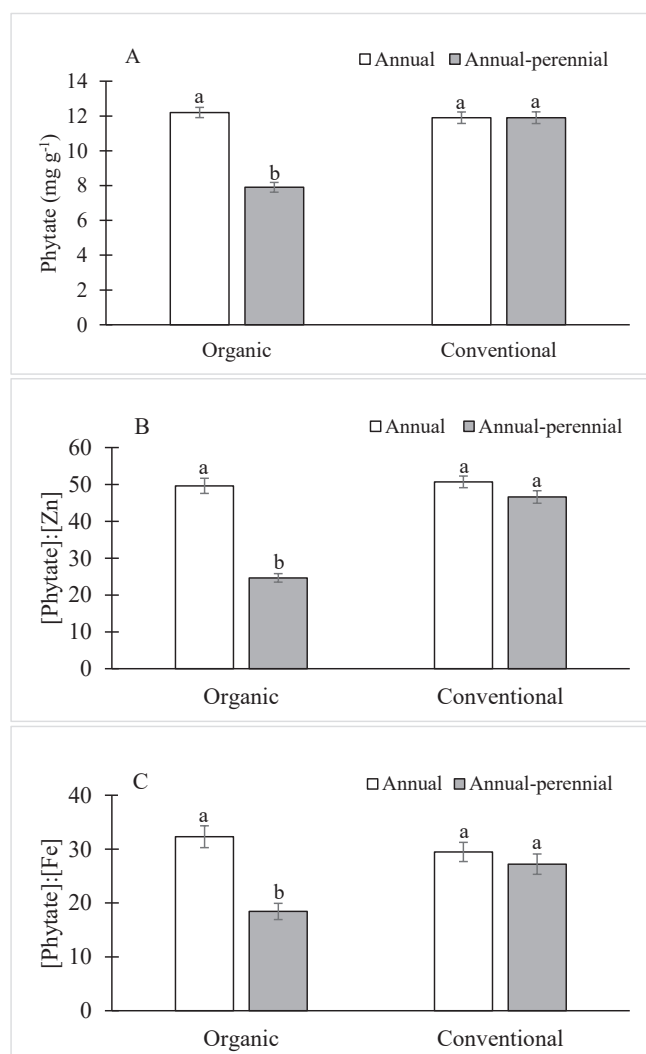


Fig. 1. Effect of farming system (organic vs. conventional) and crop rotation (continuous annual vs. annual-perennial) on grain phytate content (A), and molar ratio of phytate to grain Zn (B) and Fe (C). Data presented are the 2011–2020 means $\pm$ standard error ($n = 30$). Bars with different letters indicate statistically significant differences ($p < 0.05$).

long-term crop rotation study conducted in Saskatchewan of Canada also reported significant soil P depletion, particularly when legume hay crops are part of the rotation (Campbell et al., 1993). In the current study, wheat was grown after two years of alfalfa in the rotation. High nutrient removal of harvesting alfalfa appeared to be responsible for depleted soil P reserves (Welsh et al., 2009). Therefore, the reduced P availability and its effects on subsequent wheat grain yield and nutrient composition are partially attributed to the two years of alfalfa in the rotation.

Additionally, higher Zn concentration in organic than conventional systems could be attributed to enhanced root arbuscular mycorrhizal

fungi (AMF) colonization, which facilitate nutrient absorption. For instance, on low-P soils in Australia, Ryan et al. (2004) reported higher Zn concentration in organically grown wheat grains, which correlated positively with the percentage of root length colonised by AMF. Previous studies at the Glenlea study site also reported greater colonization and spore populations of AMF in organic than conventional systems (Welsh, 2006) but with less AMF in the manure amended organic system compared with the unmanured organic system (Mukungu et al., 2024). Therefore, the enhanced Zn and Fe bioavailability in organic farming with diversified crop rotation can be attributed to several factors including a concentration effect due to yield reduction, reduced phytate accumulation, and increased Zn uptake facilitated by mycorrhizal fungi. While the organic systems show promise for improving micronutrient bioavailability, the associated yield reductions highlight the need for strategies that enhance productivity while at the same time reducing P accumulation in the crop (Carkner et al., 2023).

4.2. Compost application decreased Zn and Fe bioavailability for organic annual-perennial crop rotation

Though livestock manure-based compost is a good source of micro-nutrients, we observed that compost application significantly increased grain [Phytate]:[Zn] and [Phytate]:[Fe] ratios, indicating reduction of their bioavailability. The decreased Zn bioavailability was a combination of a yield dilution impact and an increase in phytate level resulted from compost application. The doubling of wheat yields following compost application reflects its effectiveness in replenishing soil P reserves, a common limitation in organic systems that rely on natural inputs (Ryan et al., 2004). Studies have shown that the benefits of compost, such as improved soil water retention and nutrient availability, are critical for maintaining yields in organic systems, which often suffer from lower productivity compared to conventional systems (Edmeades, 2003). As evidenced in the current study, enhanced yield productivity by compost application accompanied with significant increases in grain uptake of micronutrients including Zn and Fe. However, compost application also resulted in significantly higher grain phytate concentration and uptake, leading to decreased Zn and Fe bioavailability. This highlights the complexity of nutrient management in organic systems, where interventions aimed at improving macronutrient availability can inadvertently hinder micronutrient biofortification. Effective compost management, therefore, must consider enhancing the bioavailability of essential micronutrients like Zn and Fe, while promoting yield in organic farming systems. For instance, selecting manure sources with low P

Table 6

Effect of compost application on uptake of Zn, Fe, P and phytate in wheat grains under annual-perennial crop rotation in organic farming system.

Treatment	Zn (g ha ⁻¹)	Fe (g ha ⁻¹)	P (kg ha ⁻¹)	Phytate (kg ha ⁻¹)
Non-compost	29.0 $\pm$ 2.7 b	35.8 $\pm$ 4.4 b	1.2 $\pm$ 0.1 b	7.1 $\pm$ 0.7 b
Compost	45.1 $\pm$ 3.3 a	75.2 $\pm$ 9.8 a	3.1 $\pm$ 0.2 a	18.6 $\pm$ 1.3 a

Means within each column followed by different letters are significantly different at $p < 0.05$.

Data presented are the 2011–2020 means $\pm$ standard error ($n = 30$).

Table 5

Effect of compost application on grain phytate content, molar ratio of phytate to Zn and Fe, protein concentration and yield of wheat under annual-perennial crop rotation in organic farming system.

Treatment	Zn (mg kg ⁻¹)	Fe (mg kg ⁻¹)	P (g kg ⁻¹)	Phytate (mg g ⁻¹)	[Phytate]:[Zn]	[Phytate]:[Fe]	Protein (%)	Yield (kg ha ⁻¹)
Non-compost	32.6 $\pm$ 0.7 a	41.9 $\pm$ 3.6 a	1.3 $\pm$ 0.03 b	7.9 $\pm$ 0.3 b	24.6 $\pm$ 1.2 b	18.2 $\pm$ 1.5 b	15.3 $\pm$ 0.4 a	884.7 $\pm$ 77.1 b
Compost	26.4 $\pm$ 0.9 b	46.0 $\pm$ 6.3 a	1.8 $\pm$ 0.04 a	10.6 $\pm$ 0.2 a	41.1 $\pm$ 1.5 a	26.4 $\pm$ 2.4 a	13.9 $\pm$ 0.3 b	1764.1 $\pm$ 127.8 a

Means within each column followed by different letters are significantly different at $p < 0.05$.

Data presented are the 2011–2020 means $\pm$ standard error ($n = 30$).

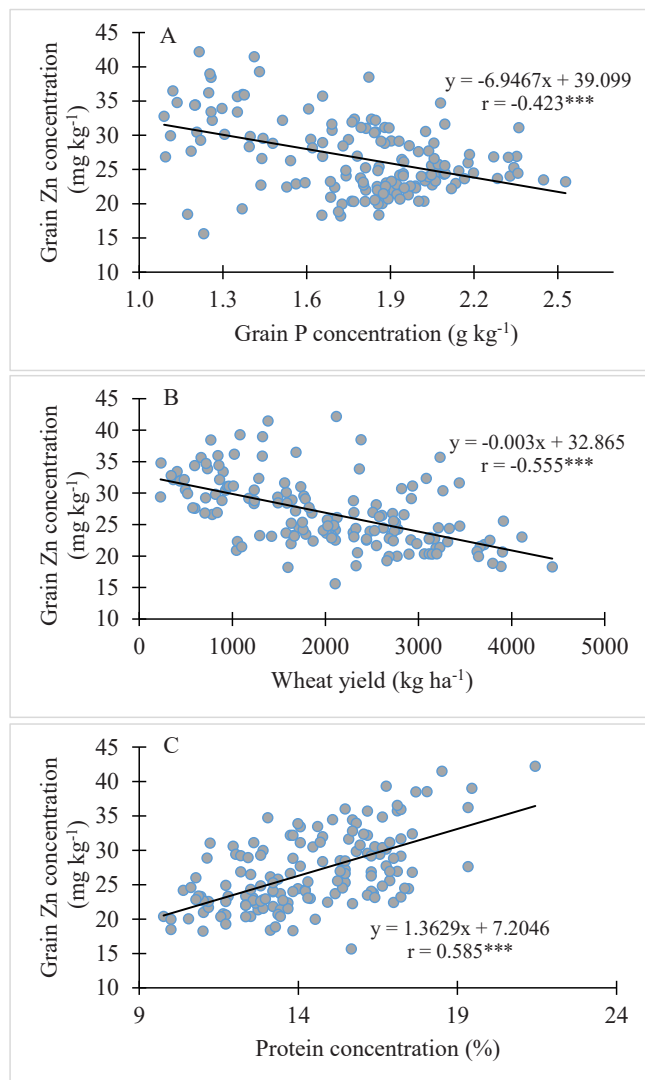


Fig. 2. Linear relationships of grain Zn with grain P (A), wheat yield (B), and protein concentration (C). *** indicated significance at $p < 0.001$.

content could possibly maintain a balanced nutrient profile in the soil, and minimize phytate accumulation in crop grains. This approach can thus help achieve a more sustainable nutrient management in organic farming to improve crop productivity and nutritional quality.

4.3. Grain Zn concentration correlates negatively with grain P concentration, but positively with protein concentration

We found that grain Zn concentration was negatively correlated with grain P concentration, confirming the well-recognized P-Zn antagonistic relationship in plant nutrition (Bindraban et al., 2020). Ryan et al. (2008) reported that P fertilizer application increased grain P and phytate level and wheat yield, but reduced grain Zn concentration by 33 %–39 % due to decreased root AMF colonization. This aligns with our previous findings at the same study site, where high soil P availability in conventional systems led to reduced root AMF colonization than organic systems (Entz et al., 2004), but manure still had higher AMF than conventional production (Mukungu et al., 2024). Additionally, our most recent study demonstrated that AMF inoculation significantly increased Zn and Fe uptake in spring wheat under conditions of low soil P availability (Gao and Zhang, 2024). The positive correlation between grain Zn and protein agrees with previous studies, supporting that protein acts as a sink for Zn in the grain (Dapkekar et al., 2018; Cakmak et al., 2010;

Kutman et al., 2011; Helfenstein et al., 2016). It implies that strategies aimed at enhancing grain Zn concentration are likely to also increase grain protein concentration, providing dual nutritional benefits for human health. These findings suggest that managing the macronutrient-micronutrient balance is critical to ensuring their availability for plant growth, and the maintenance of micronutrient bioavailability, particularly in organic farming systems.

4.4. Implications for organic farming and micronutrient bioavailability

Our study highlights a key challenge in organic farming system: the trade-off between wheat grain yield and micronutrient bioavailability, particularly for Zn and Fe. Organic farming with diverse crop rotation is an effective strategy to improve micronutrient biofortification, whereas it remains a challenge to maintain crop productivity through an optimal nutrient management. The reduced yields observed in the organic annual-perennial crop rotation were likely due to P limitations. Therefore, the reduced phytate and increased Zn levels in the organic system are more likely a consequence of reduced P availability rather than an intrinsic effect of the organic management system. Compost application presents a promising solution to this dilemma by addressing P deficiencies and substantially increasing yields. However, it also led to a marked reduction in grain Zn and Fe bioavailability. This trade-off could be mitigated by using compost sources rich in micronutrient or enriching manure sources with organic micronutrient additives to balance yield and nutrient quality. Such a strategy is particularly valuable for organic farming systems in regions like the Canadian prairies, where P deficiency is prevalent and difficult to manage (Entz et al., 2001). This study thus highlights how P management strategies influence grain nutrient composition is closely tied to soil and climate conditions, and the findings from the current study may not be generalized to all organic systems worldwide. Furthermore, selecting and breeding wheat cultivars with enhanced P uptake capacity while minimizing P accumulation in grain could help offset the negative effects of P addition on wheat grain Zn concentration (Carkner et al., 2023; Yang et al., 2023). Integrating these approaches could optimize both yield and micronutrient bioavailability in organic farming systems, promoting sustainable agriculture and better nutritional outcomes.

5. Conclusion

Our long-term field study reveals that crop management can affect the bioavailability of micronutrients in wheat grain. Organic farming, particularly using annual-perennial crop rotations, significantly enhanced grain Zn and Fe bioavailability in wheat. However, the trade-off between achieving high yields and maintaining micronutrient availability presents a significant challenge for organic farming, primarily due to P limitations. While compost application effectively addressed P deficiency and substantially increased grain yields and micronutrient uptake, it also resulted in higher grain phytate levels, thereby reducing Zn and Fe bioavailability. Therefore, our attempt to produce wheat grains with high bioavailability of Zn and Fe was successful only in scenarios where organic yields were unsustainably low. Addition research is required to find more effective mechanisms to raise organic wheat yields at lower levels of available P. We observed that grain Zn correlated negatively with grain P and positively with protein, suggesting the importance of managing the macronutrient-micronutrient balance. To optimize both yield and micronutrient bioavailability, future approaches should emphasize using compost sources rich in micronutrients and low in P, and breeding wheat cultivars with high P uptake efficiency but low accumulation in grain. These integrated strategies could promote sustainable agriculture and improve nutritional outcomes in organic farming systems.

CRedit authorship contribution statement

Jing Hou: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Xiaopeng Gao:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Martin Entz:** Writing – review & editing, Supervision, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Xiaopeng Gao reports financial support was provided by Natural Science and Engineering Research Council of Canada. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

Data will be made available on request.

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