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Contrasting Impacts of Long-Term Application of Biofertilizers and Organic Manure on Grain Yield of Winter Wheat in North China Plain

Amara Cisse ^{1,2,*}, Adnan Arshad ³ , Xiaofen Wang ¹, Fanta Yattara ² and Yuegao Hu ^{1,*}

¹ College of Agronomy and Biotechnology, China Agricultural University, Haidian district, Beijing 100193, China; wxiaofen@cau.edu.cn

² Department of Agronomy, Higher Institute of Agronomy and Veterinary of Faranah, Faranah 131, Guinea; yattarafanta22@gmail.com

³ College of Resources and Environmental Sciences, China Agricultural University, Haidian district, Beijing 100193, China; ad@cau.edu.cn

* Correspondence: amaracisse577@gmail.com (A.C.); huyuegao@cau.edu.cn (Y.H.)

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Abstract: The effects of long-term incorporation of organic manure and biofertilizers have been investigated on winter wheat in the North China Plain (NCP). The five-year field experiment (2013–2018) has illustrated the responses of grain yield and yield components. Seven fertilization approaches, included pig farm-yard-manure and biofertilizers amendments combined with five NPK% drop levels of chemical fertilizer ratio + organic fertilizer + biofertilizer (0, C+O+B) 25%, CL4; 50%, CL3; 75%, CL1; and 100%, CL0), without fertilizer as control (CK), in NCP during the years 2013–2018. Results showed that the grain yields of CL1 and CL2 were equivalent to CL0 in all growing seasons except 2014/2015. The grain yields of CL4 were 29.9% to 46.6% lower than that of CL0 during 2014/2015, 2016/2017, and 2017/2018. The valuable spike-number, grain number per-spike, and 1000-grain weight showed significant variations among different growing periods. Regression analysis of grain yield and yield components indicated that number grains per-spike showed significant increase in seed yield formation. The 1000-grain weight was the major parameter that influenced yield of moderate and low yielding periods, respectively. The results revealed that application of 30 m³ ha⁻¹ pig farm-yard-manure and 20 kg ha⁻¹ biofertilizers has reduced at least 50% of the NPK fertilization without dropping grain yields in the North China Plain.

Keywords: biofertilizers; organic manuring; grain yield; winter wheat; sustainable agriculture

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in the world and provides approximately 20% of the entire caloric intake of the world population [1–3]. About one third of human being nutritional protein is derived from grains worldwide. China is a major wheat producing country and contributed to 11.1% (24.3 million hectares) and 17.6% (131.7 million tons) of the world total wheat harvest area and yield production in 2016, respectively [4–6]. The North China Plain (NCP) is the most important dryland grain production region in China. The plain is known for its intensively managed double-cropping systems with rotations of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). Winter wheat and summer maize rotation is the primary cropping system in the North China Plain [3,7,8] and this region produced 2/3 of the nation's total production. Consequently, wheat production in the NCP plays a very important role to fulfill the increased food demands in China to ensure food security. As such, potential soil regions around the world, could play an important role to combat future climate change impacts on soil health and sustainable agriculture [9–11].

Fertilization is one of the most key driving forces in agriculture and play important role in crop grain yield formation [12–14] and farmers in general apply a high dose of chemical fertilizers during wheat production to harvest high grain yields [7,15,16]. However, the long-term unnecessary use of compound fertilizers in agricultural farming has unanticipated environmental impacts, including degradation of soil fertility, organic matter absorption and decreased water holding capacity (WHC), nutrient mobilization, and uptake by root zone [3,17]. Moreover, the excess input of chemical fertilizers resulted in NO_3 leaching, volatilization of NH_3 , and NO_x emission from agricultural fields and damaged both environment and human health [3,5,6,18,19]. Efforts have been devoted to reduce chemical fertilizers application in wheat production, and organic manure and biofertilizers amendments are considered to be feasible ways to realize this goal without decreasing grain yields [11,20–22]. Application of organic manure can increase soil carbon, improve soil fertility, and obtain similar yield compared with inorganic fertilizer addition [18,23,24]. Research [25,26] reported that the wheat grain yields were comparable in the treatments that received $80 \text{ t ha}^{-1} \text{ yr}^{-1}$ of organic manure or $204\text{--}252 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ chemical N-fertilizers. As for biofertilizers, [27–29] reported that application of one biofertilizers obtained from sewage sludge at 7.2 L ha^{-1} increased maize grain yield by 17% compared with the untreated control and [10,30,31] revealed that biofertilizers amendments reduced inorganic N-fertilizers by 25% without decreasing rice yield in India [32].

Application of organic manure and biofertilizers showed great advantages in dry matter, grain yield, and nutrient content in grain [11,29,33], and had the potential to reduce chemical fertilizers without decreasing grains yield. Nevertheless, limited literature is available on how organic manure or farm-yard-manure (FYM) and biofertilizers amendments combined with reduced chemical fertilizers can improve grain yield and yield components in the NCP winter wheat [18,33,34]. Furthermore, the national level food production depended upon the crop rotation system which is applied to 30 million hectare of wheat and maize crops in the North China Plain [31], accounting for 48% and 39%, respectively.

In the present study, we investigated the effects of organic manure and biofertilizer amendments pooled with different reduction rates of compound fertilizers on wheat grain yield and yield components during the 2013–2018 growing seasons. The objectives were: (1) to estimate what percentage of chemical fertilizers can be substituted by organic manure and biofertilizers amendments without decline in grain yield and (2) to explore how effective spike number, grain number per spike, and 1000-grain weight contribute to grain yields and economic production under different grain yield levels.

2. Materials and Methods

2.1. Status of Agrometeorology and Soil Profile

The long-term field experiment was conducted during the 2013–2018 (2013/2014, 2014/2015, 2015/2016, 2016/2017, and 2017/2018) winter wheat growing seasons at the Wuqiao Experimental Station of China Agricultural University ($37^{\circ}37' \text{ N}$, $116^{\circ}26' \text{ E}$) in Cangzhou, Hebei, China. This site has a warm temperate semi-humid continental monsoon climate with a mean frost-free period of 201 days. The long-term average annual precipitation, temperature, and sunshine time were 562 mm, 12.6° , and 2724 h, respectively. Daily precipitation and mean air temperature during the 2013–2018 growing seasons are shown in Figure 1.

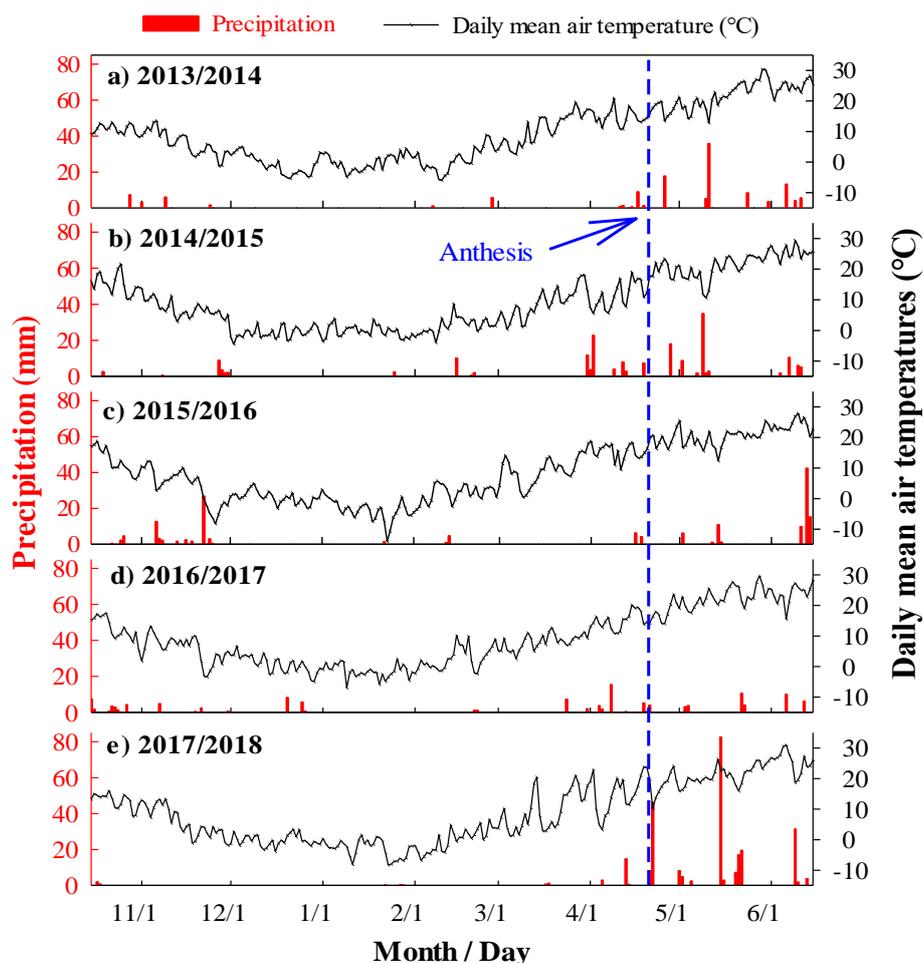


Figure 1. Agrometeorological status of daily precipitation, daily mean air temperature of Wujiao Experimental Station during the 2013–2018 winter wheat growing period in Cangzhou, Hebei, China.

Precipitation during the 2017 to 2018 growing season was 49% higher than the long-term average, but nearly 70% of the rainfall was concentrated after flowering stage of the winter wheat crop [3]. The soil type of the research site is sandy to clay loam (Table 1). Basic properties of the topsoil (0–20 cm) layer were: Soil pH 8.2 (1: 2.5, soil to water), soil organic matter 16.09 g kg⁻¹, total nitrogen 1.02 g kg⁻¹, Olsen phosphorus 20.31 mg kg⁻¹, and available potassium 87.49 mg kg⁻¹, respectively, before the experiment started in 2013.

Table 1. Basic soil properties of the experimental site before the start of the experiment in 2013 winter wheat growing period in Cangzhou, Hebei, China North Plain.

Sand ^a (%)	Silt ^a (%)	Clay ^a (%)	pH	SOM (g kg ⁻¹)	Total N (g kg ⁻¹)	Olsen P (mg kg ⁻¹)	Available K (mg kg ⁻¹)
88.39	11.46	0.76	8.05	2.29	1.02	20.31	87.49

^a The method of soil texture classification was defined as the following: sand, 0.05–2.0 mm; silt, 0.002–0.005 mm, clay, <0.002; SOM: soil organic matter.

2.2. Long-Term Fertilization Strategies

The field fertilization experiment started from the 2013/2014 winter wheat growing season in winter wheat-summer maize rotation system and then maintained the same fertilization approaches. The experiment contained seven treatments with three replicates in a randomized complete-block design (RCBD): (i) without fertilization as control (CK); (ii) 100% chemical fertilizers NPK (CL0);

(iii) 75% chemical fertilizers NPK + organic manure + biofertilizers (CL1); (iv) 50% chemical fertilizers NPK + organic manure + biofertilizers (CL2); (v) 25% chemical fertilizers NPK + organic manure + biofertilizers (CL3); (vi) organic manure+biofertilizers (CL4); and (vii) 100% chemical fertilizers NPK+organic manure + biofertilizers (C+O+B). The plot size was 20 m² (5 m by 4 m).

Fertilization strategies were the same for all the five years winter wheat growing periods. The 100% chemical fertilizers were 205.5 kg N ha⁻¹, 172.5 kg P₂O₅ ha⁻¹, and 112.5 kg K₂O ha⁻¹ in form of urea, diammonium phosphate, and potassium sulfate, respectively. The organic manure was applied with pig manure at a rate of 30 m³ ha⁻¹ which contained 226.2 kg N ha⁻¹, 903.8 kg P₂O₅ ha⁻¹, and 125.1 kg K₂O ha⁻¹, respectively. Microbial biofertilizer with the effective viable count of ≥2 million g⁻¹ mixed with microbial fertilizer with lignite as the base material used in this study which was supplied by the Beijing Liuhe Shenzhou Biotechnology Co. Ltd. Biofertilizers were applied with a rate of 20 kg ha⁻¹ (Table 2). All the phosphorus (P), potassium (K), organic manure, and biofertilizers were applied as base fertilizer and chemical N-fertilizer was applied with 50% as base fertilizer and 50% as topdressing at the jointing stage of the crop, correspondingly.

Table 2. Application combinations with amount of biofertilizer, synthetic fertilizers (kg ha⁻¹) applied to different concentrations rates during winter wheat growing seasons 2013–2018.

Treatment (~)	Synthetic Fertilizers			Organic Manure			Biofertilizer
	N	P ₂ O ₅	K ₂ O	N	P ₂ O ₅	K ₂ O	
CK	-	-	-	-	-	-	-
CL0	205.5	172.5	112.5	-	-	-	-
CL1	154.1	129.4	84.4	226.2	903.8	125.1	20
CL2	102.8	86.3	56.3	226.2	903.8	125.1	20
CL3	51.4	43.1	28.1	226.2	903.8	125.1	20
CL4	-	-	-	226.2	903.8	125.1	20
C+O+B	205.5	172.5	112.5	226.2	903.8	125.1	20

~(CK, without fertilization control; CL0, 100% synthetic fertilizers NPK; CL1, 75% synthetic fertilizers NPK + organic manure+biofertilizer; CL2, 50% synthetic fertilizers NPK + organic manure + biofertilizer; CL3, 25% synthetic fertilizers NPK + organic manure + biofertilizer; CL4, organic manure + biofertilizer; and C+O+B, 100% synthetic fertilizers NPK + organic manure + biofertilizer).

2.3. Crop Management and Sampling Analysis

Winter wheat was cultivated from 8 to 15 October and harvested from 10 to 13 June of the next year during the entire experiment (2013–2018) of wheat growing seasons, respectively. Before winter wheat sowing, the field was irrigated with 50m³ ha⁻¹ (75 mm) and received one more irrigation during the jointing and grain filling stages with 50 m³ ha⁻¹, in that order. Other agronomic management practices i.e., weeding, pest control, and disease control, were done as recommended and needed according to tangible conditions during the 2013 to 2018 growing period. At the crop maturity stage, an area of 1 m² (1 m by 1 m) winter wheat plants were selected for sampling, effective spike number and grain yield measurements, and 20 winter wheat plants were collected to determine the grain number per spike in each replication of different treatments. The 1000-grain weight was measured three times for each plot and treatment.

One-way analysis of variance (ANOVA) was performed to compare the differences in winter wheat yield and yield components in each growing season using the least significant difference test (LSD, $P = 0.05$) and two-way ANOVA was carried out to show the significance of year, fertilization, and their interaction on winter wheat yield and yield components using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Regression analysis was performed to present the correlations between yield and yield components under high, moderate, and low yield conditions. All figures were made by using SigmaPlot 14 (Systat Software Inc., San José, CA, USA).

3. Results

3.1. Soil Profile and Grain Yield

Significant variations were observed in grains yield among different fertilization treatments as reported in Figure 2. The two-way ANOVA showed that year ($F = 119.311$, $P < 0.001$), fertilization ($F = 34.329$, $P < 0.001$), and their interaction ($F = 5.236$, $P < 0.001$) all greatly influenced the grain yields at the maturity stage (Table 3). The grain yields in all the fertilized treatments were significantly high (0.28–0.54, 0.20–1.36, 0.24–0.91, 0.51–2.06, and 0.41–1.32 times more in the 2013/2014, 2014/2015, 2015/2016, 2016/2017, and 2017/2018 growing seasons, respectively) than that in CK with the exception of CL4 in 2015/2016. Treatments that received organic manure and biofertilizers showed enormous variations in grains yield compared with CL0 in different planting seasons. Furthermore, the grain yields drastically decreased with the decrease in chemical fertilizers application rates and the grain yield in CL4 was the lowest among the organic manure and biofertilizers amendment treatments in all the five growing seasons. In the 2014/2015 growing season, the grain yields in all the organic manure and biofertilizers amendments treatments were 53.4–93.0% as much as that in CL0 and the grain yields arranged in order by $CL0 > CL1$, $C+O+B > CL1 > CL3 > CL4$ in the fertilized treatments ($P < 0.001$).

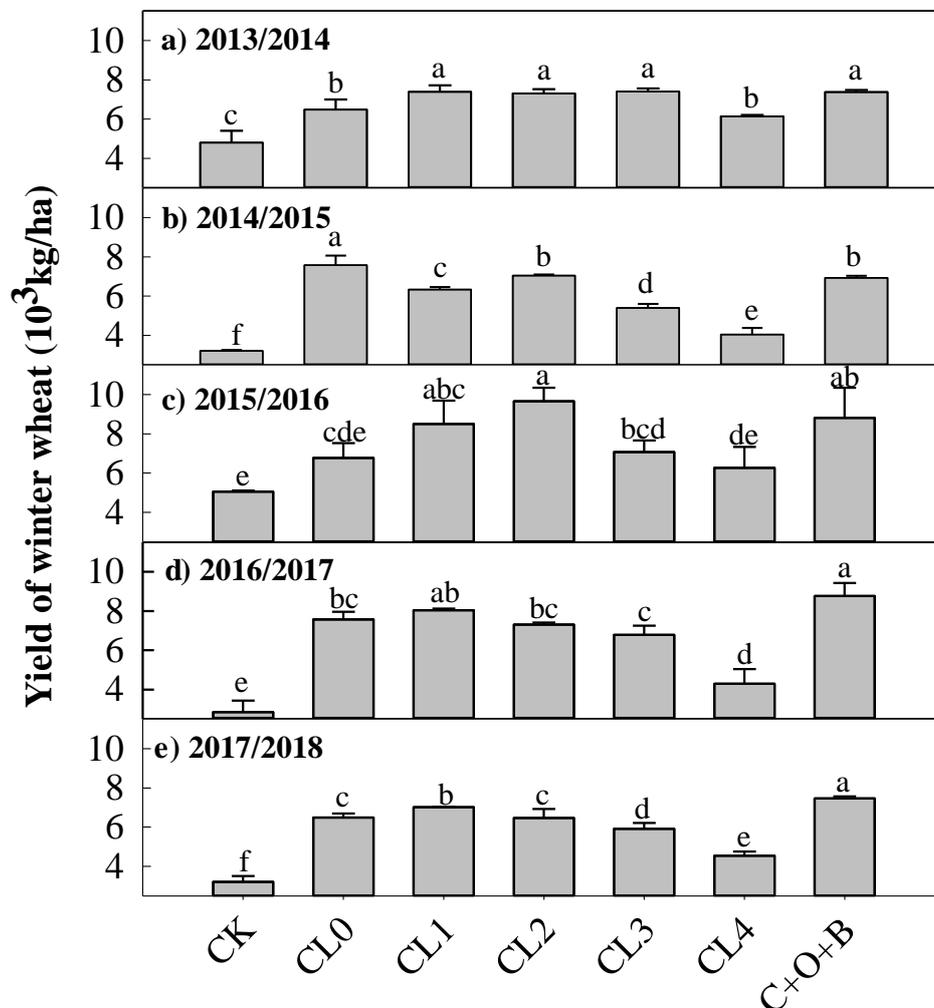


Figure 2. Grain yield of fertilization treatments (CK, CL0, CL1, CL2, CL3, CL4, and C+O+B) during the winter wheat production years 2013–2018 at the China North Plain.

Table 3. Yield components of winter wheat yield parameters in different fertilization treatments during the 2013–2018 wheat growing seasons of China North Plain.

Treatment	Effective Spike (10 thousands ha ⁻¹)	Grain Number (per Spike)	1000-Grain Weight (g)	Grain Yield (10 ³ kg ha ⁻¹)
2013/2014				
CK	574.67 ± 1.53 f	24.00 ± 3.60 d	46.77 ± 0.25 a	4.80 ± 0.60 c
CL0	623.67 ± 1.15c	36.00 ± 1.73 a	44.93 ± 2.53 a	6.48 ± 0.51 b
CL1	724.67 ± 1.15 b	35.33 ± 2.08 a	45.90 ± 1.22 a	7.39 ± 0.33 a
CL2	614.67 ± 0.58 d	33.67 ± 1.15 ab	45.33 ± 1.53 a	7.31 ± 0.22 a
CL3	581.33 ± 0.58 e	30.33 ± 3.05 bc	44.53 ± 2.63 a	7.40 ± 0.16 a
CL4	565.67 ± 1.53 g	27.00 ± 0.00 cd	43.70 ± 0.65 a	6.14 ± 0.06 b
C+O+B	732.67 ± 2.08 a	36.00 ± 1.00 a	43.40 ± 2.26 a	7.37 ± 0.11 a
2014/2015				
CK	479.67 ± 3.05 g	22.67 ± 0.6 f	41.47 ± 0.47 a	3.21 ± 0.04 f
CL0	634.67 ± 1.53 d	40.63 ± 0.58 a	43.97 ± 2.00 a	7.57 ± 0.48 a
CL1	695.33 ± 1.53 b	37.00 ± 1.73 b	38.50 ± 1.32 b	6.33 ± 0.13 c
CL2	707.67 ± 0.58 a	35.00 ± 1.00 c	41.17 ± 0.29 a	7.05 ± 0.05 b
CL3	578.67 ± 1.53 e	32.00 ± 1.00 d	42.50 ± 1.32 a	5.40 ± 0.21 d
CL4	533.67 ± 1.53f	27.00 ± 0.00 e	41.7 ± 2.31 a	4.04 ± 0.34e
C+O+B	647.00 ± 1.00 c	41.37 ± 1.53 a	38.33 ± 1.53 b	6.93 ± 0.09 b
2015/2016				
CK	485.00 ± 1.00 f	27.63 ± 1.53 d	36.70 ± 2.52 c	5.05 ± 0.58 e
CL0	609.70 ± 2.00 c	36.67 ± 1.15 b	39.46 ± 0.23 bc	6.77 ± 0.38 cde
CL1	660.00 ± 3.00 b	37.37 ± 1.15 ab	38.57 ± 0.81 c	8.49 ± 0.08 abc
CL2	661.03 ± 2.52 b	39.67 ± 0.58 a	39.00 ± 0.00 bc	9.65 ± 0.10 a
CL3	499.07 ± 1.53 e	36.37 ± 0.58 b	42.60 ± 0.87 a	7.07 ± 0.47 bcd
CL4	533.37 ± 3.78 d	32.30 ± 1.00 c	41.62 ± 3.61 ab	6.26 ± 0.74 de
C+O+B	763.70 ± 2.64 a	38.67 ± 4.04 ab	39.30 ± 0.26 bc	8.80 ± 0.66 ab
2016/2017				
CK	460.00 ± 1.00 g	23.33 ± 1.52 d	36.20 ± 2.52 c	2.86 ± 0.58 e
CL0	637.00 ± 2.00 d	33.67 ± 1.15 b	43.87 ± 0.23 ab	7.57 ± 0.39 bc
CL1	726.00 ± 3.00 b	37.67 ± 1.15 a	45.87 ± 0.81 a	8.04 ± 0.08 ab
CL2	694.33 ± 2.52 c	37.67 ± 0.58 a	44.00 ± 0.00 ab	7.30 ± 0.10 bc
CL3	580.67 ± 1.53 e	32.67 ± 0.58 b	43.50 ± 0.87 ab	6.78 ± 0.47c
CL4	537.67 ± 3.78 f	27.00 ± 1.00 c	41.20 ± 3.60 b	4.30 ± 0.74 d
C+O+B	762.00 ± 5.03 a	38.67 ± 4.04 a	46.20 ± 0.26 a	8.76 ± 0.67 a
2017/2018				
CK	502.33 ± 3.21 g	23.67 ± 2.52 d	35.00 ± 0.00 d	3.22 ± 0.29 f
CL0	616.33 ± 2.52 d	32.33 ± 2.52 b	39.77 ± 0.64 c	6.48 ± 0.21 c
CL1	723.67 ± 1.15 b	38.67 ± 0.58 a	45.70 ± 2.04 a	7.00 ± 0.03 b
CL2	625.67 ± 1.53 c	35.00 ± 0.00 b	42.83 ± 1.75 b	6.46 ± 0.45 c
CL3	588.33 ± 2.52 e	33.67 ± 1.53 b	42.17 ± 1.04 b	5.91 ± 0.30 d
CL4	539.67 ± 1.15 f	28.33 ± 0.58 c	38.43 ± 1.29 c	4.54 ± 0.21 e
C+O+B	789.00 ± 8.71 a	40.00 ± 3.00 a	45.87 ± 0.15 a	7.47 ± 0.10 a
Significance of				
Year (Y)	***	***	***	**
Fertilization (F)	***	***	***	***
Y × F	***	***	***	***

Values are mean ± SD ($n = 3$). CK, without fertilization control; CL0, 100% synthetic fertilizers NPK; CL1, 75% synthetic fertilizers NPK + organic manure + biofertilizer; CL2, 50% synthetic fertilizers NPK + organic manure + biofertilizer; CL3, 25% synthetic fertilizers NPK + organic manure + biofertilizer; CL4, organic manure + biofertilizer; and C+O+B, 100% synthetic fertilizers NPK + organic manure + biofertilizer. (**: $P < 0.01$; ***: $P < 0.001$) All SD values means are different superscript as mentioned in the table.

The grain yields in CL1 and C+O+B were 1.06–1.25 and 1.14–1.30 times more and that in CL4 were 0.57–0.95 times more as much as those in CL0 in the rest four growing seasons ($P < 0.001$), respectively. The grain yields in CL2 and CL3 were 1.13–1.43 and 1.05–1.14 times more in the 2013/2014 and 2015/2016,

and 0.96–1.00 and 0.90–0.91 times as much as that in CL0 in the 2016/2017 and 2017/2018 growing seasons ($P < 0.001$) (Figure 2, Table 3), respectively. During the five growing seasons, the highest mean annual grain yield was observed in C+O+B, followed by CL2, CL1, CL0, CL4, and CK. The mean grain yields of the 2013–2018 growing seasons in the fertilized treatments were 0.32–1.05 times greater than that in CK and those in combined chemical fertilizers (NPK) and organic manure treatments were 0.29–0.56 times more than that in organic manure and biofertilizers amendments treatment (CL4), respectively.

3.2. Assessment of Biofertilizer and Manure Effects on Growth and Seed Yield

The effectual spikes, number of grains per-spike, and 1000-grain weight varied greatly among different fertilization in all the five growing spells except for 1000-grain weight in the 2013/2014 growing season (Table 3). The two-way ANOVA showed that year ($F = 506.477$, $P < 0.001$; $F = 13.354$, $P < 0.001$, and $F = 34.941$, $P < 0.001$), fertilization ($F = 17307.338$, $P < 0.001$; $F = 140.882$, $P < 0.001$, and $F = 10.999$, $P < 0.001$), and their interaction ($F = 599.855$, $P < 0.001$; $F = 3.937$, $P < 0.001$, and $F = 8.403$, $P < 0.001$) all profoundly affected the effective spike number, grain number per spike, and 1000-grain weight (Table 3), in that order. The highest effective spike numbers were obtained in C+O+B in the 2013/2014, 2015/2016, 2016/2017, and 2017/2018 growing seasons and in CL2 in the 2014/2015 growing season, which were 1.1–29.5%, 15.5–57.5%, 5.0–65.7%, 9.0–57.1%, and 1.8–47.5% higher than those in the rest treatments (Table 3), respectively.

3.3. Effects of Biofertilizer on Yield Components

As for number of grain per spike, the highest values were obtained in C+O+B and CL0 in the first two growing seasons and in C+O+B, CL1, and CL2 in later three growing seasons, and the lowest number was found in CK in all the five growing seasons with 24.7–41.0% lower in average than those in the rest of the treatments in the five growing seasons, respectively. Pertaining to 1000-grain weight, significant differences were found in the later four growing seasons. In the 2014/2015 growing season, significant lower 1000-grain weight was detected in CL1 and C+O+B and they were 7.0–12.3% and 3.0–12.8% less than those in the rest of treatments. The highest 1000-grain weight was found in CL3 in the 2015/2016 growing season and in CL1 and C+O+B in the 2016/2017 and 2017/2018 growing seasons, which were 2.4–16.1%, 4.1–27.6%, 6.8–30.9%, and then those in the rest of the treatments in the three growing seasons (Table 3), equally.

During the 2013–2018 growing seasons, the mean effective spike number and grain number per spike both ranked in a decreasing order with C+O+B>CL1>CL2>CL0>CL3>CL4>CK while the mean 1000-grain weight ranked in a decreasing order with CL1>C+O+B>CL3>CL2>CL0>CL4>CK. The mean effective spike number, grain number per spike, and 1000-grain weight in the fertilized treatments were 8.3–47.75%, 16.9–60.6%, and 2.2–9.3% higher than that in CK and those in combined chemical fertilizers (NPK) and organic manure treatments were 4.4–36.3%, 16.4–37.4%, and 1.7–6.9% greater than that in the organic manure and biofertilizers amendments alone treatment (CL4). Our five-year field experiment results are varied with the two year field experiment of [34] who reported the effects of full-straw incorporation on soil fertility richness, and crop yield in a rice-wheat (*Oryza sativa* L.–*Triticum aestivum* L.) under rotation system on sandy, loamy soil.

3.4. Correlations among Grain Yield and Other Components

Among the five growing seasons, the grain yield can be divided into three groups: (a) high yield growing season ($>7 \text{ t ha}^{-1}$, 2015/2016), (b) moderate yield growing seasons ($6\sim7 \text{ t ha}^{-1}$, 2013/2014 and 2016/2017), and (c) low yield growing seasons ($5\sim6 \text{ t ha}^{-1}$, 2014/2015 and 2017/2018), based on the mean grain yield of all the treatments. Regression analysis was performed to show the correlations between grain yield and yield components at the three grain yield levels (Figure 3). For the high yield growing season, grain yield correlated significantly with effective spike number and grain number per spike with determination coefficient (R^2) values of 0.52711 and 0.4294, respectively. The grain

yield correlated significantly with effective spike number, grain number per spike, and 1000-grain weight with R^2 values of 0.68277, 0.63335, and 0.40524 and 0.76309, 0.88114, and 0.2577 in the moderate yield growing season, while R^2 values of 0.76309, 0.88114, and 0.2577 were respectively obtained in the low yield growing season. The slope between grain yield and grain number per spike in the high yield growing season (0.27125) was higher than those in the moderate and low yield growing seasons (0.24009 and 0.22913), while the slope between grain yield and effective spike number showed an opposite result. The 1000-grain weight and slope between grain yield and 1000-grain weight in the moderate yield growing seasons were much higher than those in the low yield growing seasons. These results demonstrated that the effective spike number dominated in yield formation in the high yield growing season while the 1000-grain weight was a very important yield development factor in the moderate and low yield growing seasons, respectively.

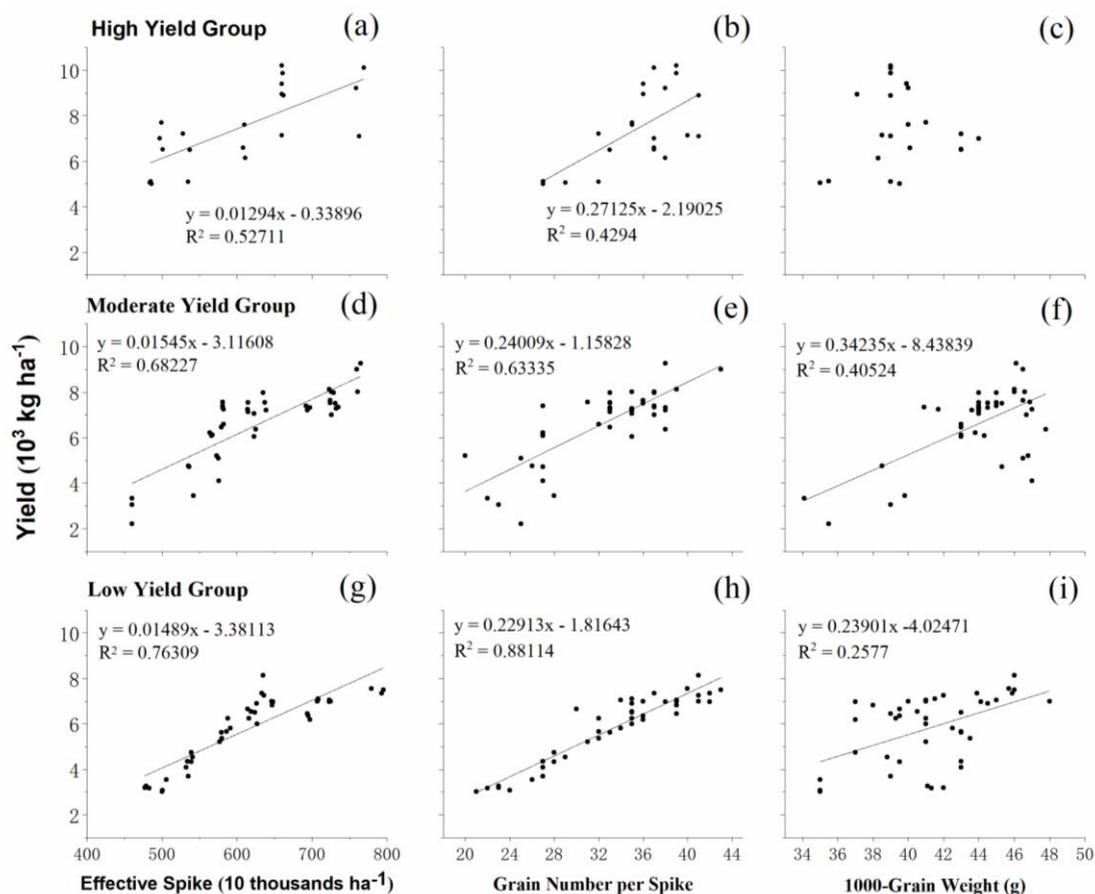


Figure 3. Regression correlations of yield (10^3 kg ha^{-1}) grain yield, effective spikes, 1000-seed weight at the high (a–c), moderate (d–f), and low (g–i) grain yield groups during the cropping years of 2013 to 2018 in China North Plain experiment sites.

The cumulative data analysis of yield parameters of winter wheat in China North Plain study sites showed significant effects of long-term incorporation of biofertilizers and organic manure in soil which enhances the soil properties; enrich nutrients and soil fertility levels (Figure 4, Appendix A). The contrasting impact of biofertilizers into soil also promote the plant growth and booted the vegetative growth, which ensure effective number of spikes, significant increase in grain weight and grain yield 103 kg ha^{-1} . The applications of $30 \text{ m}^3 \text{ ha}^{-1}$ pig FYM and 20 kg ha^{-1} biofertilizers might reduce at least 50% of the NPK application without reducing wheat grain yields in the North China Plain. Therefore, the applications of biofertilizers and FYM are advantageous for soil $\text{NO}_3\text{-N}$ content balance in the soil (Appendix A). These outcomes (Figure 3, Figure 4, and Appendix A) showed a greater potential for

utilization of biofertilizers and farm-yard-manure to improve the grain yield while decrease the input cost of farm practices.

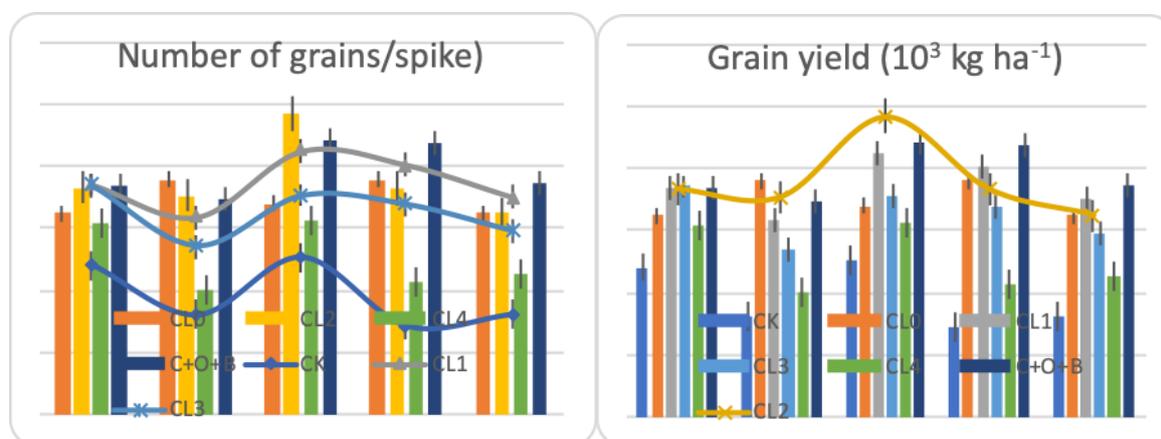


Figure 4. Cumulative evaluation analysis of number of grains/spike and total grain yield (10^3 kg ha^{-1}) of winter wheat in the North China Plain during the long-term research intervention 2013–2018 (data attached).

4. Discussion

Contrasting impacts of long-term application of organic manure and crop residual straw nitrate-N along with the soil profile in the NCP, amendments significantly increased the plant's growth and development leads to economic yields as The finding was supported by [14,34]. Five years of research work investigated response of grain yield and yield components of wheat to organic manure and biofertilizers amendments combined with different application rates of chemical fertilizers. Treatment C+O+B generated the highest grain yields apart from the 2014/2015 growing season and the highest and lowest mean grain yields were observed in C+O+B and CK, respectively, during the whole growing seasons. In accordance with findings, (Abbasi and Yousra, 2012 [35]) found high wheat grain yield in the treatment that received organic manure, biofertilizers, and inorganic fertilizers and [14,24,36] observed higher maize grain yield in the organics, biofertilizers, and recommended dose of NPK treatment during the experiment period.

The grain yields in the treatments that received up to 50% reduction of chemical fertilizers (CL0, CL1, and CL2) were 6.8–12.4% higher, and those in treatments with 25% and 0% recommending dose of chemical fertilizers (CL3 and CL4) in the study area, even the same dose of organic manure and biofertilizers, were much less than that in the chemical fertilizers application alone treatment during the growing seasons, respectively. Our results indicate that organic manure and biofertilizers amendments could substitute about half of the recommended chemical fertilizers in winter wheat production without decreasing grain yield, and this was supported with other findings [24,27]. These results (Figure 4) were in accordance with previous studies about the effects of organic manure and biofertilizers application on cotton and wheat yields [25,37,38]. The increases in grain yield of low chemical fertilizers substitute rates treatments might be due to the positive effects of organic manure and biofertilizers on wheat plants. As previously reported, organic manure amendment had the advantages of slowly releasing nutrients and preventing losses due to leaching [39,40] while biofertilizers could produce vitamins and substances that were beneficial to root development [32,41], which can promote wheat plants to absorb more nutrients.

The effects of organic manure and biofertilizers amendments on grain yield and 1000-grain weight varied in different studies. Research [31,42] reported that the grain yield and 1000-grain weight of maize received a half dose of N ($102.75 \text{ Kg ha}^{-1}$) fertilizer and biofertilizers were 13% and 10% greater than that in treatment with a full dose of N fertilizer (175 kg N ha^{-1}), respectively. On the contrary, two studies revealed that biofertilizers and half dose of N fertilizer produced 25% and 17% less in

grain yield and 1000-grain weight compared with that received a full dose of N fertilizer [26,38]. In this study, half dose chemical fertilizers ($102.5 \text{ kg N ha}^{-1}$, $86.25 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$, and $56.25 \text{ kg K}_2\text{O ha}^{-1}$) combined with organic manure and biofertilizers amendments produced 7.0% to 42.5% excessive in grain yield compared with full dose chemical fertilizers and less difference was detected in 1000-grain weight between these treatments among the five growing seasons except for 2017/2018, respectively. The treatment CL0 which was composed of only the mineral fertilizer (NPK) provided more grain per spike with an average of 36.0 compared to CL2 of 33.6 which is constituted by a proportional mixture of the mineral fertilizer (NPK) 50% + manure organic + biofertilizer. This was explained by the slow and gradual release of the elements contained in the dose CL2 compared to CL0 the findings supported by the results of [20,26].

Solar radiation, accumulated temperature, and precipitation were the major important meteorological factors that caused wheat grain yield changes from year to year [14,30,43–45]. Hence, it could be explained by the meteorological conditions and potential grain yields in different growing seasons. On the other hand, number of spikes, grain numbers per spike, and 1000-grain weight were three yield component factors resulted in yield formation and, generally, grain number per spike was strongly source-limited and 1000-grain weight was limited by its particular sink-strength, respectively [2,46,47]. The long-term observations resulted that, CL1-CL4 contains more nutrients (N, P_2O_5 , and K_2O) than CL0 under the same weather conditions. CL0 has the ability to provide instant nutrients essential for plant growth more quickly as compared with organic manure, FYM, and biofertilizers supported by the studies of [33,40,48]. In this study, the grain yield showed great variations in different growing seasons, and it can be divided into three yield levels based on the mean grain yield of all the treatments. Garcia et al. [49] reported that grain yield under warmer conditions was mostly determined by grain number per spike, whereas 1000-grain weight dominantly affected grain production in the colder environments. Contrary to these findings, long-term results indicated that grain number per spike was the main factor that affected grain yield in lower mean temperature growing season while 1000-grain weight was the main factor that influenced grain yield in growing seasons with higher mean temperature, respectively. The reason might be that water was the first limiting factor determining the grain yield formation and could affect the contributions of yield components to yield [50–52].

5. Conclusions

In conclusion, these results indicated that the grain yields in C+O+B, CL1, and CL2 were comparable to CL0 during all the growing seasons except for 2014/2015. The results revealed that application of $30 \text{ m}^3 \text{ ha}^{-1}$ pig FYM and 20 kg ha^{-1} biofertilizers possibly reduce at least 50% of the NPK application without dropping wheat grain yields in the North China Plain. Therefore, we concluded that biofertilizers and FYM applications are advantageous for soil $\text{NO}_3\text{-N}$ content balance. These results (Figure 3, Figure 4, and Appendix A) indicated a more significant potential for utilization of biofertilizers and FYM to improve the grain yield while decrease total input cost to ensure sustainable agriculture farming. The number of wheat grains per spike played very important role in total yield formation at the high yield growing season and 1000-grains weight contributed greatly to yield formation at the moderate and low yield growing seasons, respectively. The five-year contrasting field experiment demonstrated that $30 \text{ m}^3 \text{ ha}^{-1}$ organic manure and 20 kg ha^{-1} biofertilizers amendments could reduce at least 50% of the recommended dose of chemical fertilizers that is typically applied for winter wheat production without affecting the grain yields in the North China Plain. This long-term research results recommend that, the application of $30 \text{ m}^3 \text{ ha}^{-1}$ pig farm-yard-manure and 20 kg ha^{-1} biofertilizers will reduce at least 50% of the NPK application without dropping grain yields in North China Plain soils. While this study recommends that $30 \text{ m}^3 \text{ ha}^{-1}$ pig farm-yard-manure and 20 kg ha^{-1} biofertilizers will reduce at least 50% of the NPK application without dropping grain yields in North China Plain soils, it was the only rate of pig farm-yard-manure and biofertilizer tested in this study. These rates provided additional amounts of nutrients (N, P_2O_5 , and K_2O) which are actually higher

than those in the CL0 treatment (Table 2). Therefore, future research should test other FYM (probably lower rates) and biofertilizer application rates.

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Appendix A

Table A1. Analysis of soil chemical properties after harvesting of winter wheat in North China Plain (NCP) experimental site during years 2017/2018, soil pH, Soil total nitrogen, NH_4^+ -N, and NO_3^- -N at different soil depths under different fertilizer treatments.

Treatment	Soil Total N (g kg ⁻¹)			NH_4^+ -N (mg kg ⁻¹)			NO_3^- -N (mg kg ⁻¹)		Soil pH		
	10 cm	20 cm	40 cm	10 cm	20 cm	40 cm	10cm	(1:2.5 H ₂ O)	(1:2.5 KCl)	(1:5CaCl ₂)	
CK	0.47 ± 0.01d	0.73 ± 0.01e	1.20 ± 0.01e	0.23 ± 0.00g	0.32 ± 0.00f	0.19 ± 0.01f	0.32 ± 0.00g	7.4 ± 0.14a	6.8 ± 0.17a	6.9 ± 0.22a	
CL0	0.84 ± 0.03ab	1.35 ± 0.00bc	2.48 ± 0.02ab	1.01 ± 0.00e	1.12 ± 0.00d	0.67 ± 0.01a	6.12 ± 0.08e	6.9 ± 0.07a	6.3 ± 0.12b	6.4 ± 0.23b	
CL1	0.87 ± 0.06ab	1.39 ± 0.02ab	2.65 ± 0.02a	1.53 ± 0.01b	1.56 ± 0.03b	0.50 ± 0.01b	9.02 ± 0.03b	6.8 ± 0.08a	6.4 ± 0.13b	6.3 ± 0.22b	
CL2	0.85 ± 0.01ab	1.37 ± 0.02abc	2.35 ± 0.01bc	1.44 ± 0.01c	1.31 ± 0.02c	0.45 ± 0.00c	7.76 ± 0.12c	7.1 ± 0.15a	6.8 ± 0.23a	6.9 ± 0.12a	
CL3	0.77 ± 0.01bc	1.33 ± 0.02c	2.25 ± 0.01c	1.34 ± 0.01d	1.10 ± 0.02d	0.39 ± 0.00d	7.18 ± 0.06d	7.5 ± 0.14a	6.9 ± 0.23a	7.1 ± 0.11a	
CL4	0.70 ± 0.00c	1.23 ± 0.00d	1.70 ± 0.14d	0.67 ± 0.01f	0.76 ± 0.02e	0.29 ± 0.01e	5.09 ± 0.05f	7.7 ± 0.18a	7.1 ± 0.12a	7 ± 0.12a	
C+O+B	0.88 ± 0.03a	1.41 ± 0.01a	2.64 ± 0.03a	1.82 ± 0.02a	2.02 ± 0.02a	0.68 ± 0.00a	9.99 ± 0.02a	7.4 ± 0.07a	7 ± 0.12a	7.2 ± 0.12a	

In each column lower case lettering is used to show the significant differences between different types of treatments at $P < 0.05$ level. Values show Standard errors (SE) ± mean of four replicates. CK, without fertilization control; CL0, 100% synthetic fertilizers NPK; CL1, 75% synthetic fertilizers NPK + organic manure + biofertilizer; CL2, 50% synthetic fertilizers NPK + organic manure + biofertilizer; CL3, 25% synthetic fertilizers NPK + organic manure + biofertilizer; CL4, organic manure + biofertilizer; and C+O+B, 100% synthetic fertilizers NPK + organic manure + biofertilizer.

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