



Effects of long-term organic amendment on the fertility of soil, nodulation, yield, and seed quality of soybean in a soybean-wheat rotation system

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Abstract

Purpose Organic amendment has long been recognized as an effective approach to improve soil fertility and plant nutrition. Most studies on long-term organic amendment in fields have focused on cereals. The effects of long-term organic amendment on legumes remain largely unknown. Here we studied the impact of organic amendments, including straw and manure on the soil fertility, nodulation, seed yield, and seed quality in a 27-year experiment.

Materials and methods The long-term fertilizer treatments were laid out using a randomized complete block design designed with three replications in the first year of experimentation (1990). Fertilization treatments were CK (no fertilization), NPK (inorganic nitrogen, phosphorus and potassium fertilizers), NPKS (Straw together with inorganic N, P, and K fertilizers), and NPKM (manure together with inorganic N, P, and K fertilizers). The layout was subsequently maintained in the following years to clearly understand the effect of long-term application of each treatment. Maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.) rotation was the main long-term experiment cropping system from 1990 to 2011; from 2012 it becomes wheat-soybean (*Glycine max* L.) rotation.

Results and discussion We found that NPKS, NPKM, and NPK increased organic matter by 34.7%, 36.24%, and 17.17%, respectively, and total nitrogen by 28.9%, 29.7%, and 25.2%, respectively, in comparison with CK. Organic amendment also increased enzymatic activity of phenol oxidases and β -1,4-glucosidases. The NPKS and NPKM increased soybean yields by 30.8% and 29.6%, respectively, in comparison with NPK alone and CK. The soybean yields significantly correlated with $\text{NO}_3\text{-N}$, available phosphorus, available potassium and soil organic matter. Both the inorganic fertilizers and organic amendments did not significantly alter soybean nodule number and size. NPKS and NPKM applications significantly reduced fat content by 1.17% and 1.26%, respectively, as opposed to NPK fertilized treatments alone (0.34%), compared with those under CK, but did not affect soybean protein.

Conclusion Overall, the combination of straw returning with inorganic fertilizers and manure with inorganic fertilizers improves soil fertility and increases soybean productivity. However, organic amendments together with inorganic fertilizers might have an undesirable effect on soybean fat content. Organic amendments combined with NPK application have the highest value of protein content and increased nitrogen to the soil. High nitrogen reduced fat and increased protein content. Therefore, rate of N inputs should be adjusted under the application of organic amendments together with inorganic fertilizers in fluvo-aquic soil.

Keywords Organic amendments · Straw returning · Soil fertility · Nodulation · Seed quality · Yield

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1 Introduction

Soil provides the base for planting and therefore affects the growth, development, and reproduction. Fundamentally, soil can also profoundly affect other aspects of the environment, for example, mitigating climate change via carbon sequestration (Zomer et al. 2017; Lal 2013; Akeley 2015). Organic carbon in

soil is central to nutrient cycling (Wei et al. 2019) and enhances the fertility status of soil (Palmer et al. 2017). Organic carbon could be changed in response to various factors such as the application of inorganic fertilizers, crop rotation, tillage, and organic amendment (Ye et al. 2019; Berhane et al. 2020; Alvarez 2005). Among them, organic amendment such as straw returning or the use of manure is probably the best choice to increase organic matter in soil. However, the effect of organic amendment on soil fertility and organic matter is usually chronic, and thus requires long-term field experimentation to assess it.

Straw input and the use of organic manure have been found to substantially increase crop productivity in cereal-based crop systems (Diacono and Montemurro 2010; Jin et al. 2020). Straw returning can increase organic carbon in soil and thus increase the productivity of cereal crops compared to inorganic fertilizers alone (Berhane et al. 2020; Jin et al. 2020). Use of manure can also lead to an increase in maize yield and improve organic carbon in soil (Li et al. 2017). Higher levels of organic carbon in soil lead to higher mineral nutrients and soil enzyme activity, and thus result in high cereal yields (Verma et al. 2017). Unlike cereals that completely rely on soil nutrient supply, legumes can fix nitrogen from atmospheric dinitrogen through symbiosis from rhizobia. Nitrogen fixation results in releasing proton, which mobilizes soil undissolved phosphorus. Thus, legumes possess the power to restore soil fertility through nitrogen fixation (Giller and Cadisch 1995; Rashid et al. 2016). However, our knowledge is still very limited regarding whether and how legume responds to long-term fertilization. Such knowledge is especially scarce in China, which puts emphasis on cereal production in past decades.

Nodule is an organ for nitrogen fixation during symbiosis in legume. The formation of nodules involves a complex process of signal perception and transduction between legume roots and rhizobia (Dolgikh et al. 2008; Popp and Ott 2011). After nodule formation, legumes can regulate the supply of resources to nodule through a dual sanction mechanism based on nitrogen needs, and thus control nodule size (Ferguson et al. 2019). Hence, nodulation and nitrogen fixation are vulnerable to soil properties outside roots and nodules that influence legume's performances. It is reported that number, biomass, and nitrogen fixation ability of nodule are negatively correlated with nitrogen supply (Adams et al. 2018). Nodulation and nitrogen fixation are also significantly impacted by soil phosphorus availability (Yusuf and Silas 2016). Long-term organic amendment has been found to significantly influence soil properties, such as organic carbon, other organic matter, pH, inorganic nitrogen, and available phosphorus in soil (Šimanský and Kováčik 2015; Ge et al. 2018; Nagwanshi et al. 2018). However, how different soil amendment approaches over the long term and their long-term fertilization impact on soybean nodulation has not been fully studied.

Soybean is a major source of protein and fatty acids for human nutrition (Bellaloui et al. 2011). Higher yields and higher proportion of proteins and fatty acids are constantly

sought from soybean by scientists. Soil conditions may not affect the yield and quality of soybean seeds profoundly. However, research results are inconclusive regarding to how and to what extent different long-term fertilization strategies impact yield and quality of soybean seeds (Bellaloui et al. 2009; White and Brown 2010; Krueger et al. 2015). Some studies suggest that there is no association between soil chemical elements and soybean seed composition (Jaureguy 2012), whereas other reports suggest a significant increase in proteins and oleic acid, and a concomitant decrease in oil and linolenic acid in soybean seeds when soybean plants are fertilized (Bellaloui et al. 2010). Krueger et al. (2015) has found that the levels of phosphorus and potassium are negatively correlated with content of proteins in soybean seeds.

China is the largest soybean consumer in the world with a total import of 83 million metric tons (MMT) in 2019 and a domestic production of 18.1 million tons for 1.95 million ha planted, or 7.56% of the soybean planted area in the world (USDA/FAS 2020). The low production has been attributed to many factors among which include soil properties, fertility status, and enzyme activities (Zhang and Xue 2007; Yang et al. 2017; Adetunji et al. 2017). However, researches on the management of soil fertility for increasing soybean yield and quality are much less compared with cereal crops. Few long-term field studies on soil management have been carried out for soybean production in China despite its importance in yield improvement. Looking at the numerous advantages of soil management, we therefore hypothesized that the combined application of inorganic fertilizer and organic amendment will be a more effective fertilization practice to increase the organic carbon and enzyme activities in soils as well as improve the nodulation, yield, and seed quality of soybean than the sole application of inorganic fertilizer under a soybean-wheat cropping system.

2 Materials and methods

2.1 Experimental site, design, and sampling

A field experiment was carried out at the National soil fertility and fertilizer efficiency long-term monitoring station of China, located in Yuanyang County, Xinxiang City, Henan Province (E 113° 40' 042" and N 34° 47' 725") in fluvo-aquic soil (central China). This location has a warm temperate, sub-humid continental monsoon climate. The site annual average temperature and precipitation are 14.4 °C and 645 mm, respectively (Zha et al. 2015).

The long-term fertilization experiment was initiated in 1990. In the current study, we selected the treatments that are having a combination of the macronutrients (N, P, and K), as well as a combination of NPK fertilizers with straw returning (NPKS) or use of manure (NPKM) and no fertilization as controls which resulted in four treatments. The long-

term fertilizer treatments were laid out using a randomized complete block design designed with three replications in the first year of experimentation (1990). The layout was subsequently maintained in the following years to clearly understand the effect of long-term application of each treatment. Maize (*Zea mays* L.)-wheat (*Triticum aestivum* L.) rotation was the main long-term experiment cropping system from 1990 to 2011 (Zhang et al. 2017); from 2012, it becomes wheat-soybean (*Glycine max* L.) rotation. Each treatment plot size was 54 m² (6 m × 9 m) and had three replications, which were separated from adjacent plot by 100-cm cemented barriers. For the NPK treatment, fertilizer N, P, and K were applied in the form of urea for N, calcium superphosphate for P, and potassium chloride for K, respectively (Table 1). Manure was cattle manure or horse dung on dry weight containing 6.1–21.4 g N kg⁻¹, 2.3–10.6 g P₂O₅ kg⁻¹, and 2.3–15.9 g K₂O kg⁻¹ (Ai et al. 2018). The rate of organic fertilizer addition in the NPKM and NPKS treatments to fields is mostly calculated based on N input rates. Thus, in 1 year, the total N input applied (inorganic plus organic) was equal for the NPK, NPKS, and NPKM treatments. The ratio of organic N fertilizer to inorganic N fertilizer was 7:3. All organic manure and inorganic fertilizers P and K were applied as a basal application, while two-thirds of N was applied as basal application and one-third as topdressing.

Soil samples were collected from each experimental plot to a depth of 0–20 cm from the surface on 1 September 2017, before soybean maturity. An auger with 5-cm internal diameter was used to take soil samples at five soil cores at randomly selected locations, and soil was mixed into a single composite at the start of the experiment. To collect rhizosphere soil, plants were uprooted. Then roots were shaken carefully to remove the coarse soil that adhered to roots. The remaining soil adhere to root surface was defined as rhizosphere soil and collected by thoroughly shaking. The fresh samples were mixed thoroughly and divided into two groups. Some samples were stored in a refrigerator at -4 °C to analyze soil enzyme activities, and the others were air-dried, sieved through a 2-mm sieve, and stored in individual plastic bags for soil properties analysis. The initial soil parameters (1990) were soil pH of 8.3 (H₂O), total nitrogen

(TN) of 0.67 g kg⁻¹, total P (TP) of 0.65 g kg⁻¹, total potassium (TK) of 16.9 g kg⁻¹, available phosphorus (AP) of 6.5 mg kg⁻¹, and organic carbon (OC) of 6.7 g kg⁻¹ (Ai et al. 2018).

2.2 Laboratory analysis

Soil samples were air-dried and passed through a 2-mm and 1-mm sieves. Soil organic matter was determined by a Walkley-Black chromic acid wet oxidation method (Gelman et al. 2011). Total nitrogen was determined by Kjeldahl distillation. Available potassium was determined by flame photometric method, while available phosphorus was determined by blue molybdenum with 0.5 M NaHCO₃ solution at pH 8.5, a method developed by Olsen (Molina 2011). Soil pH was measured in an air-dried soil: water ratio (1:2.5) with a digital Mettler Toledo meters (pH meter) (Ge et al. 2010). Soil water content was determined gravimetrically by weighing and drying at 105 °C for 24 h, reweighing and calculating the mass of water lost as a percentage of the mass of the dried soil. Fresh soil samples were used for the determination of ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N). NH₄⁺-N and NO₃⁻-N absorbance values were measured calorimetrically at a wavelength of 625 nm and 275 nm against a reference solution, respectively. Bulk and rhizosphere soil samples were also collected and stored at -20 °C and 4 °C for further molecular and enzyme assays. Soybeans nodule number and biomass were sampled in September at soybean reproductive growth stages (during the R1-R2 stages) (Zhang et al. 2020). Twenty-seven plants (9 per plot) were harvested from the field; the roots were washed and the nodules were separated by hand and the normal root nodules were counted.

2.3 Enzyme activity

The activity of enzymes including acid phosphatase, β-1,4-N-acetyl-glucosaminidase, phenol oxidase, and β-1,4-glucosidase was measured in both bulk and rhizosphere soils. Activities of extracellular enzymes acid phosphatase, β-1,4-N-acetyl-glucosaminidase, and β-1,4-glucosidase were analyzed using MUF-linked model substrates yielding the highly

Table 1 Annual inorganic fertilizers, straw returning and manure application input rates used in this study

Treatments				N from manure	P from manure	K from manure	Wheat straw
	N kg ha ⁻¹	P ₂ O ₅ kg ha ⁻¹	K ₂ O kg ha ⁻¹	N g kg ⁻¹	P g kg ⁻¹	K g kg ⁻¹	kg ha ⁻¹
CK	0	0	0	0	0	0	0
NPK	352.5	176.25	176.25	0	0	0	0
NPKS	352.5	176.25	176.25	0	0	0	115.5
NPKM	352.5	176.25	176.25	6.1–21.4	2.3–10.6	2.3–15.9	0

No fertilizer (CK); nitrogen, phosphate, and potassium (NPK); organic manure plus NPK (NPKM); straw plus NPK (NPKS)

fluorescent cleavage products 4-methylumbelliferone (4-MUB) (4-MUB phosphate, 4-MUB-N-acetyl- β -D-glucosaminide, and 4-MUB- β -D-glucoside; respectively) in soil solutions (Saiya-cork et al. 2002; Deforest 2009; Sinsabaugh et al. 2000; Marx et al. 2001). The non-fluorometric enzyme, phenol oxidase, was measured spectrophotometrically in the clear 96-well microplate using the substrate of L-3, 4-dihydroxyphenylalanine (L-DOPA) (Ai et al. 2018).

One gram of fresh soil sample was mixed with 50 mL of double-distilled water (ddH₂O) in a centrifuge tube and was shaken to homogenize. The mixture was incubated at 25 °C with a shaking speed of 180 rpm for 30 min, and was then mixed and poured into a larger beaker. Fifty-microliter substrate solution was added in each microplate well, which contained 200- μ L sample suspension. The microplate was covered and incubated in the dark at 25 °C for 4 h for fluorometric enzymes (acid phosphatase, β -1,4-N-acetyl-glucosaminidase, and β -1,4-glucosidase) and 18 h for the spectrophotometric enzyme (phenol oxidase) (Deforest 2009; Sinsabaugh et al. 2000). Fluorescence was quantified using a microplate fluorometer at excitation 365 nm and emission 450 nm for black plates or at 450 nm for white plates (Ai et al. 2018; Fan et al. 2012; Xu et al. 2014).

Activities were expressed in units of nanomole per gram of soil per hour (nmol g⁻¹ h⁻¹).

2.4 Soybean seed quality

Soybean seed protein, fat, water content and water-soluble protein (Wprotein) contents were collected using a Perten DA7200 (Perten Instruments North America, Springfield, IL, USA), a near-infrared spectroscopy (NIRS) with wavelength 950–1650 nm designed for seed analyses at 5-nm intervals (He et al. 2019).

2.5 Statistical analysis

Data were evaluated with one-way analysis of variance using ANOVA to compare the effects of different treatments on the measured variables. Differences between treatments were compared using Fisher's LSD method at the 0.05 probability level. All of the statistical analyses were processed with SPSS software of the International Business Machines Corporation Company (IBM SPSS Statistics v22 x64). Pearson linear regression was conducted between parameters to determine their relationship.

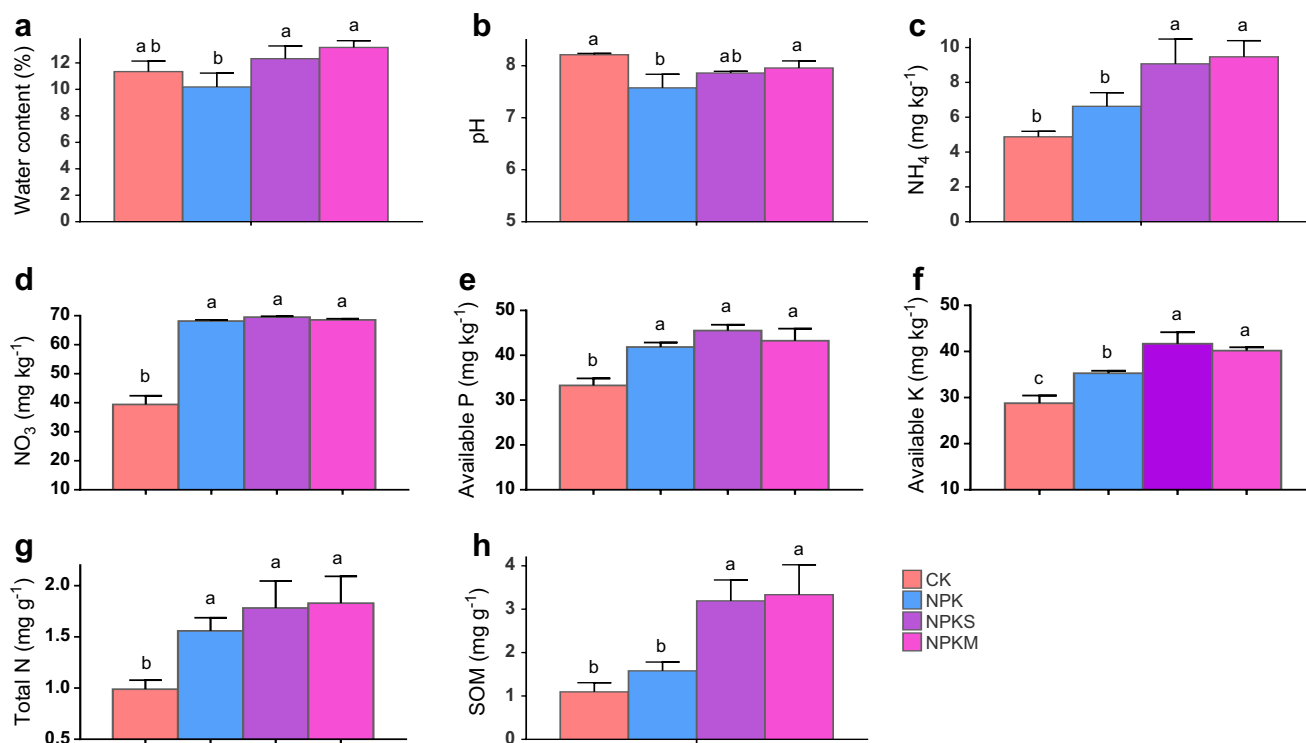
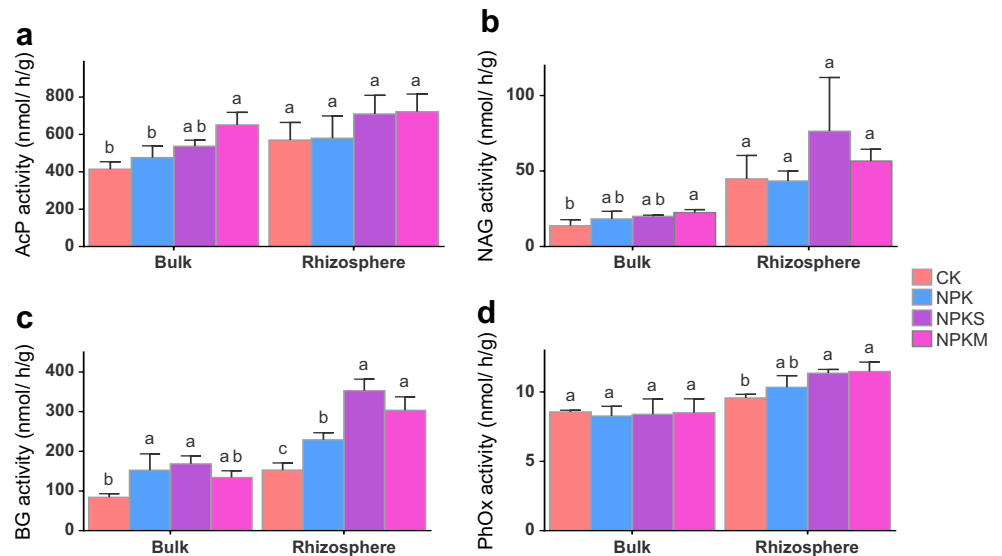


Fig. 1 Impact of inorganic fertilizers containing nitrogen, phosphorus, and potassium (NPK), NPK together with straw returning (NPKS) or manure application (NPKM) on soil properties. CK—No fertilizer control. Soil properties measured include: (a) water content (%); (b) pH; (c) nitrate-nitrogen (NO₃⁻-N) concentration (mg kg⁻¹); (d) ammonium

(NH₄⁺-N) concentration (mg kg⁻¹); (e) available phosphorus (mg kg⁻¹); (f) available potassium (mg kg⁻¹), (g) total nitrogen (mg g⁻¹), and (h) soil organic matter. Same letters on top of the bars indicate no significant differences between the treatments at $P < 0.05$

Fig. 2 Impact of inorganic fertilizers containing nitrogen, phosphorus, and potassium (NPK), NPK together with straw returning (NPKS) and manure application (NPKM) on soil enzyme activities in the rhizosphere. CK—No fertilizer control. Other abbreviations include (a) AcP, acid phosphatase; (b) NAG, β -1,4-N-acetyl-glucosaminidase; (c) BG, β -1,4-glucosidase; (d) PhOx, phenol oxidase. Units for all enzyme activities are $\text{nmol activity h}^{-1} \text{g}^{-1} \text{soil}$. Same letters on bars indicate no significant differences between the treatments at $P < 0.05$



3 Results

3.1 Soil properties

The impact of different long-term soil fertilization strategies on soil property is shown in Fig. 1. Significant ($P < 0.05$) differences were observed between different strategies. Both NPKS and NPKM resulted in increased water content in comparison with fertilizers alone (NPK). The NPK treatment recorded the lowest pH of the soil. Both NPKS and NPKM resulted in higher levels of $\text{NH}_4\text{-N}$, AK, and SOM in comparison with NPK and CK. NPKS, NPKM, and NPK resulted in increased levels of nitrate ($\text{NO}_3\text{-N}$), available phosphorus (AP), and total nitrogen in comparison with CK. No significant difference ($P > 0.05$) was observed between NPKM and NPKS.

3.2 Enzyme activity

Enzymatic activities in both bulk and rhizosphere soils are shown in Fig. 2. Almost all samples showed higher enzymatic activities in comparison with CK. Enzyme

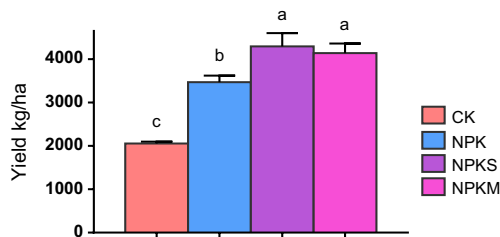


Fig. 3 Impact of inorganic fertilizers containing nitrogen, phosphate, and potassium (NPK) and NPK together with straw returning (NPKS) and manure addition (NPKM) on soybean yields. CK—No fertilizer control. Same letters on top of the bars indicate no significant differences between the treatments at $P < 0.05$

activities were higher in the rhizosphere than in bulk soil among different treatments. Significant ($P < 0.05$) differences were observed between samples in the activity of the extracellular enzymes. NPKM resulted in significant increase in the activities of acid phosphatase in the bulk soil by 11.39%, while NPK and NPKS had no detectable effect compared with CK. In the bulk soil samples of the β -1,4-N-acetyl-glucosaminidase, the higher enzyme activity was detected in the NPKM samples (11.65%) compared with CK. NPKS and NPK resulted in the highest activity of β -1,4-glucosidase in bulk soil with 12.64 % and 15.63 %, respectively, compared to CK. No significant difference was observed between the treatments in the rhizosphere in terms of acid phosphatase and the activities of β -1,4-N-acetyl-glucosaminidase and phenol oxidase.

3.3 Crop yields

The NPK resulted in a significant increase in soybean yield in comparison with no fertilizer control. NPKS and NPKM resulted in additional increase in soybean yields (Fig. 3).

3.4 Nodule number and biomass

Long-term fertilizer treatments had a significant impact on nodule number and total nodule biomass, but no significant ($P > 0.05$) difference was observed in individual nodule biomass (Fig. 4). Specifically, NPK and NPKM resulted in reduced numbers of nodules per plant. NPK treatment recorded the lowest total biomass of a plant that was statistically at par with NPKM, whereas NPKS resulted in increased number of nodules per plant and total biomass of a plant.

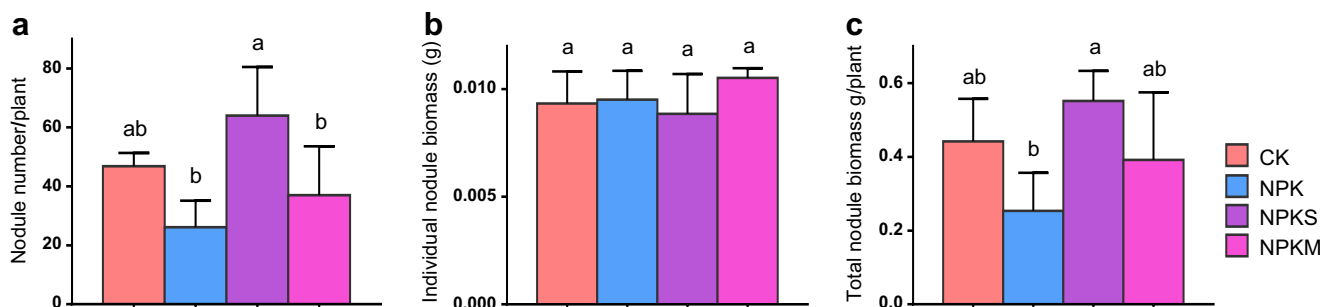


Fig. 4 Impact of inorganic fertilizers containing nitrogen, phosphate and potassium (NPK) and NPK together with straw returning (NPKS) and manure addition (NPKM) on nodulation. CK—No fertilizer (CK). (a)

Nodule number/plant, (b) individual nodule biomass (g), and (c) total nodule biomass g/plant. Same letters on the top of bars indicate no significant differences between the treatments at $P < 0.05$

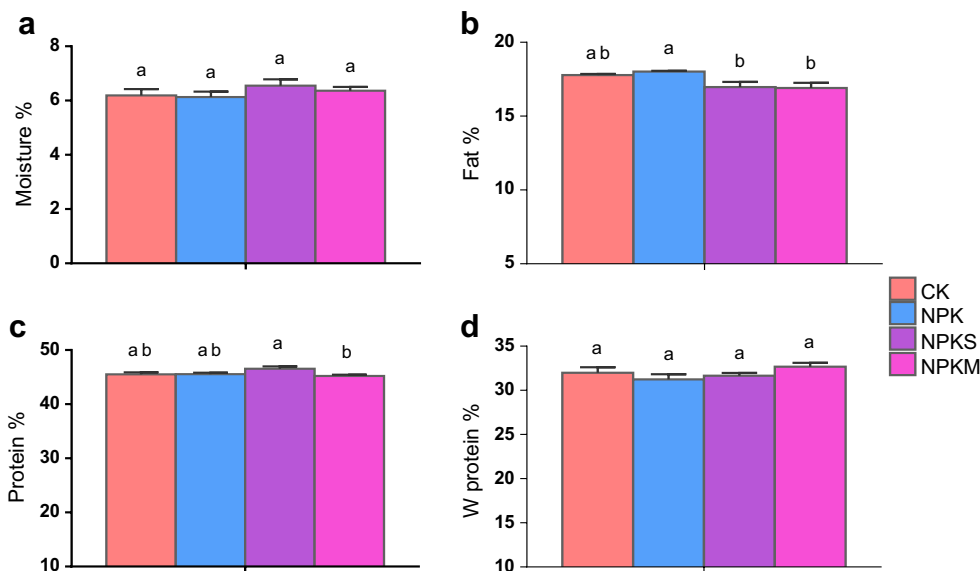
3.5 Soybean seed quality

As shown in Fig. 5, long-term fertilizer treatments did not result in significant difference ($P > 0.05$) in seed water content and water-soluble protein (Wprotein) (Fig. 5 a and d). However, NPKS and NPKM decreased both fat and total protein contents compared with chemical fertilization alone (Fig. 5b), while NPKS resulted in a higher level of total proteins.

3.6 Correlation between seed yield and soil properties, soil enzyme activity, and nodulation

Pearson correlation between seed yield and soil parameters was highly ($P < 0.001$) positive in all cases except in total nitrogen ($P < 0.05$). No correlation existed between seed yield and moisture or pH. The correlation coefficients between seed yield and soil parameters including ammonium, nitrate, available phosphorus, available potassium, total nitrogen, and organic matters in soil were 0.75, 0.88, 0.84, 0.95, 0.66, and 0.67, respectively (Table 2).

Fig. 5 Impact of inorganic fertilizers containing nitrogen, phosphate, and potassium (NPK) and NPK together with straw returning (NPKS) and manure addition (NPKM) on seed quality traits. CK—No fertilizer (CK). Same letters on top of the bars indicate no significant differences between the treatments at $P < 0.05$



A Pearson correlation analysis revealed significant ($P < 0.001$) positive correlation between seed yield of soybean and activity of enzymes acid phosphatase and β -1,4-glucosidase in soil, with correlation coefficients of 0.66 and 0.68, respectively (Table 2). On the other hand, there was no significant correlation ($P > 0.05$) between seed yield and nodulation (Table 2).

4 Discussion

4.1 Soil fertility, soybean seed yield, and quality

Soybean seed yield increased significantly under NPK compared with no fertilizer control. An additional 29.6% increase in soybean seed yield was observed under both NPKS and NPKM. Our results are consistent with a study in Indian mid-Himalayas, which reported that inorganic fertilizers together with manure for 21 years increased soybean yield by 30% and 25%, respectively (Choudhary et al. 2018). Another recent study has demonstrated that up to 75% higher soybean

Table 2 Pearson correlation coefficient matrix to show the relationship between soybean yields and soil properties, soil enzymes activity and nodulation

	Seed yield	H ₂ O	pH	NO ₃ ⁻ -N	NH ₄ ⁺ -N	AP	AK	TN	SOM	AcP	NAG	PhOx	BG	Nodulation
Seed yield														
Water content (H ₂ O)	0.548 ns													
pH	-0.302 ns	0.31 ns												
Nitrate nitrogen (NO ₃ ⁻ -N)	0.753**	0.54 ns	0.07 ns											
Ammonium (NH ₄ ⁺ -N)	0.876**	0.47 ns	-0.34 ns	0.69*										
Available phosphorus (AP)	0.840**	0.53 ns	-0.28 ns	0.46*	0.83**									
Available potassium (AK)	0.952**	0.59*	-0.25 ns	0.83**	0.89**	0.83**								
Total nitrogen (TN)	0.662*	0.49 ns	0.01 ns	0.78**	0.60*	0.57 ns	0.68*							
Soil organic matter (SOM)	0.669*	0.57 ns	-0.07 ns	0.39 ns	0.72**	0.85**	0.71**	0.52 ns						
Acid phosphatase (AcP)	0.659*	0.42ns	0.22ns	0.64*	0.37ns	0.45ns	0.56ns	0.71**	0.37ns					
β-1,4-Nacetyl-glucosaminidase (NAG)	0.378ns	0.05ns	-0.12ns	0.18ns	0.07ns	0.176ns	0.24ns	0.20ns	0.08ns	0.44ns				
β-1,4-glucosidase (BG)	0.683*	0.12ns	-0.50ns	0.14ns	0.54ns	0.66*	0.51ns	0.20ns	0.44ns	0.29ns	0.56ns			
Phenol oxidase (PhOx)	0.355ns	0.43ns	0.30ns	0.65*	0.26ns	0.13ns	0.33ns	0.77**	0.18ns	0.56ns	0.36ns	0.09ns		
Nodulation	0.220ns	0.22ns	0.46ns	0.35ns	0.21ns	-0.07ns	0.11ns	0.09ns	0.08ns	0.14ns	0.18ns	0.19ns	0.433ns	

**Correlation is statistically significant at the $P < 0.001$ level (2-tailed)

*Correlation is statistically significant at the $P < 0.05$ level (2-tailed)

ns not significant

yield can be achieved under combined use of straw mulch and inorganic fertilizers (Akhtar et al. 2019). Similar observations on long-term use of NPK, NPKS, and NPKM have been reported on cereal crops (Zha et al. 2015). Overall, our data suggest that organic amendment is desirable for achieving higher soybean yield in central China plain.

The improvement in soybean yield under long-term fertilizer application is closely related to soil fertility. Soybean yield is significantly impacted by organic matter, total nitrogen, ammonium, nitrate, available phosphorus, and available potassium in soil (Anthony et al. 2012). The combination of inorganic fertilizers and organic amendment increases soybean yields to a greater extent because organic amendment not only adds extra nutrients to the soil but also increase soil buffering capacity and reduce nutrient leaching (Shi et al. 2017; Radersma and Smit 2011). Organic amendment also increased soil enzyme activity, especially in the rhizosphere, which created a favorable environment for the roots of plants. Higher enzyme activity may help degrade soybean allelochemicals, which means a fast turnover of soil C and influence soybean yields (Darmanti et al. 2015). Organic carbon and other organic matters in soil along with macronutrients containing N, P, and K are good indicators of soil fertility, which affects wheat yields (Sarhat 2015). Based on our observation, these soil fertility indicators are also suitable for assessing soybean yield potential in central China.

Long-term NPK did not significantly change the soybean seed quality in terms of water-soluble proteins, proteins, and fat. On the other hand, long-term NPKS and NPKM reduced fat in seeds, and NPKM also reduced total proteins while NPKS increased the protein content. Hisani et al. (2015) have also observed that organic fertilizers and mulches provide benefits for soybean plants in improving the quantity and quality of crop production. Schrader and Briskin (1989) have also reported that there is a possible alteration of soybean seed protein quality under different mineral nutrition. The impact of NPKS and NPKM on soybean seed quality may be due to a larger dilution effect by yield enhancement or a stronger undesirable higher P and K under these conditions (Krueger et al. 2015). Our results together with previous studies (Bellaloui et al. 2010; Bellaloui et al. 2015) demonstrate that soybean seed quality can be altered by soil management. Accordingly, soil management should be recommended based on the balance between yield and seed quality and investment and income.

4.2 Impact of organic amendment on nodulation

Long-term NPK, NPKS, and NPKM do not change the number of nodules and nodule biomass of each soybean plant compared with no fertilizer control. In fact, chemical fertilizers generally reduce the formation of nodules (Ohyama et al. 2017). The increase of phosphorus is beneficial to

nodulation (Yusuf and Silas 2016). However, increase of inorganic nitrogen in soil reduces nitrogen fixation, likely due to the fact that nodulation and nitrogen fixation of legumes are primarily regulated by nitrogen demand. Interestingly, we found that the number and biomass of nodules under NPKS were significantly higher than under NPK. This is surprising because the soil inorganic nitrogen under NPKS was higher than under NPK. The reason is currently unknown, but may be due to the stimulation of soybean, nitrogen demand outweighed by the increase in soil inorganic nitrogen. Our results indicate that long-term inorganic fertilizers and organic amendment result in higher soybean yield not by increasing nitrogen fixation as shown by the insignificant correlation between the yield and soybean nodulation (Table 2), and the number and biomass of nodules are usually positively correlated with the amount of legume nitrogen fixation (Tajima et al. 2007). Future studies should examine alternative fertilizer regimes that could both increase nitrogen fixation and yield.

5 Conclusions

A 27-year fertilization field experiment revealed significant benefits of NPKS and NPKM to both soil fertility and soybean yield. However, NPKS and NPKM may have an undesirable effect on seed quality. A major impact of NPKS and NPKM on soil property is the increase in activity of the enzyme's phenol oxidase and β -1,4-glucosidase in the rhizosphere. Organic amendment significantly reduced fat proportion. Significant ($P < 0.01$) positive correlations were observed between crop yields and the abundance of ammonium, nitrate, available phosphorus, available potassium, total nitrogen, and organic matters in soil, and between crop yields and the activity of acid phosphatase and β -1,4-glucosidase in soil. Our data suggest that organic amendment coupled with inorganic fertilizers may be the best fertilization strategy for crop rotation systems in central China to achieve high soybean yield in soybean-wheat systems.

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Availability of data and material The datasets generated and/or analyzed during the current study are available from the corresponding author, upon reasonable request.

Code availability Not applicable.

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Declarations

Conflicts of interest The authors declare no competing interests.

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