RESEARCH ARTICLE

Effects of combined application of soil amendments on soil nutrient availability, iron speciation transformation, and wheat yield and quality

Bingshen Jiang, Yu Zhao, Xinxin Miao, Yangyang Chai, Jingru Wang, Quanshan Deng, Weina Zhang, Dahong Li*

School of Biological and Food Processing Engineering, Huanghuai University, Zhumadian, Henan 463000, China.

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Soil acidification in arid agricultural regions reduces phosphorus and nitrogen availability and induces aluminum and iron toxicity, thereby constraining crop productivity. Although soil amendments such as quicklime, microbial fertilizers, and biochar are widely used, their individual and combined amendments application effects on phosphorus–iron transformations and crop quality remain poorly defined. This study evaluated the single and interactive amendments application effects of these amendments on soil properties and wheat performance in acidic soil. A pot experiment was conducted with six treatments including control, quicklime alone, quicklime plus microbial fertilizer, and three treatments combining quicklime and microbial fertilizer with graded biochar levels. The results showed that all amendments significantly increased soil pH, soil available phosphorus (SAP), soil available nitrogen (SAN), wheat thousand-grain weight, and soluble protein content. The combined quicklime and microbial fertilizer treatment (QM) was most effective, producing the highest SAN, thousand-grain weight, and soluble protein content, together with a pronounced reduction in soil Fe^{3+} and total iron (Fe). Key soil nutrients showed significant positive correlations with wheat quality traits. Overall, the QM treatment effectively alleviated soil acidity, improved nutrient availability, reduced iron toxicity, and enhanced wheat yield and quality, indicating its potential as a sustainable strategy for acidic soil management. However, these results were based on pot experiments and required validation under field conditions, as well as economic and long-term assessments.

Keywords: combined application; soil amendments; soil nutrients; thousand-grain weight of wheat; soluble protein.

***Corresponding author:** Dahong Li, School of Biological and Food Processing Engineering, Huanghuai University, Zhumadian, Henan 463000, China. Phone: +86 396 285 3022. Email: lidahong27@163.com.

Introduction

Zhumadian, Henan, China is a major wheat-producing region in the country, yet its farmland productivity is increasingly constrained by soil degradation. Intensive cultivation, excessive nitrogen fertilization, and seasonal erosion have accelerated soil acidification, creating a major obstacle to sustainable wheat production [1]. Acidic soils directly impair crop growth by disrupting nutrient availability. At low pH of 4.5 to 6.0, amorphous iron (Fe) and aluminum (Al) oxides acquire a net positive surface charge, which enhances the immobilization of phosphate ions of $H_2PO_4^-$ and HPO_4^{2-} through ligand exchange and electrostatic adsorption. This process forms stable Fe/Al–P complexes and markedly reduces soil phosphorus (P) availability [2]. Field studies indicated that, when soil pH declined below 5.0, Olsen-P concentrations in

Zhumadian fluvo-aquic soils decreased by 40 – 60% relative to neutral soils, severely restricting phosphorus uptake by wheat [3]. In parallel, soil acidification increases Fe solubility and the risk of Fe toxicity. Although total Fe content is typically high, the associated rise in soluble Fe^{3+} activity poses the primary physiological threat. Under acidic conditions, dissociation of surface hydroxyl groups on Fe minerals such as goethite and hematite releases Fe^{3+} into soil solution as $Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3 + 3H^+$, a reaction that intensifies sharply below pH 4.5. Excessive Fe^{3+} disrupts root function, damages photosynthetic systems, and induces broader metabolic disorders in plants [4]. Consequently, P deficiency and Fe toxicity act synergistically to constrain root development, nutrient acquisition, and ultimately wheat yield and grain quality.

To alleviate soil acidity and enhance nutrient use efficiency, amendments such as Quicklime (CaO), microbial fertilizers, and biochar are widely applied. Lime rapidly neutralizes soil acidity but can increase soil compaction and reduce micronutrient availability with prolonged use [5]. Microbial fertilizers promote nutrient solubilization through organic acid production or siderophore release, yet their effectiveness often declines in strongly acidic soils when pH is lower than 5.5 [6]. Due to its porous structure and negative charge, biochar improves soil pH, absorbs ammonium, and enhances soil structure, thereby providing more persistent benefits [7]. Combined application of these amendments is expected to offset individual limitations as that lime can establish a suitable pH environment for microbial activity, while biochar enhances nutrient and water retention [8]. However, the interactive mechanisms underlying these combinations remain poorly resolved, particularly regarding P and Fe transformation processes and their combined effects on wheat quality [9, 10].

To address these uncertainties gaps, a pot experiment was conducted in this research by using acidic dryland soils collected from Zhumadian, Henan, China. Six treatments comprising single, binary, and ternary combinations of Quicklime, microbial fertilizer, and biochar were systematically evaluated to assess the effects of these amendments on key soil properties including pH, organic carbon, available N and P, Fe fractions of Fe^{2+}, Fe^{3+}, total Fe, and to clarify the mechanisms linking amendment-induced changes in soil chemistry with wheat yield and soluble protein content. By identifying the most effective amendment strategy, this study provided a scientific basis for improving wheat productivity and quality in acid-affected regions with broader relevance to the Huang-Huai Plain in central China.

Materials and methods

Soil sample collection

The soil used for the pot experiment was collected from the surface layer (0 – 20 cm) of acidic farmland in Weitun Village, Runan County, Zhumadian, Henan, China. The soil samples were transported to the laboratory of the School of Biological and Food Engineering, Huanghuai University, Zhumadian, Henan, China. Visible impurities including plant roots and brick fragments were removed by sieving through a 10-mesh sieve followed by air-drying.

Soil amendments

Three types of amendments were used in this research. Lime was obtained from Zhuhua Lime Factory (Liudian, Henan, China) and applied at 2.4 g/pot, which was equivalent to 960 kg/ha under field conditions. This rate was determined through preliminary trials to raise soil pH to 5.5 – 6.0, a range favorable for microbial activity and wheat growth [11]. A microbial fertilizer was purchased from Baoding Keliufeng Biochemical Technology Co., Ltd. (Baoding, Hebei, China) containing *Bacillus subtilis* and *Paenibacillus mucilaginosus* with a viability of $\geq 5 \times 10^8$ CFU/g. It was applied at 0.375 g/pot, corresponding to 150 kg/ha following the manufacturer's recommendations. Biochar was produced in the laboratory by pyrolyzing wheat straw at 500°C under limited oxygen conditions. The resulting biochar had a pH of 9.8 and a specific surface area of 250 m^2/g.

Sample grouping and treatments

The experiment followed a completely randomized design with six treatments and three replications (n = 3). The treatments were control (CK) with no amendment, quicklime alone (Q) (2.4 g), quicklime (2.4 g) + microbial fertilizer (QM) (0.375 g), QM + biochar (QMLB) (7.5 g), QM + biochar (15 g) (QMMB), QM + biochar (30 g) (QMHB). A biochar application gradient was established to assess dose-dependent effects with rates of 7.5, 15, and 30 g per pot, which were equivalent to 6.25-, 12.5-, and 25-ton biochar/ha in field, respectively, and fell within the range commonly used in similar studies [5]. The pot experiment was conducted in plastic pots measuring 23.3 cm in diameter and 25 cm in height, each filled with 6.0 kg of prepared soil. Amendments were thoroughly mixed into the soil according to the assigned treatments. Forty (40) "Xunmai 8105" wheat seeds (*Triticum aestivum* L. cv.) obtained from Henan Jinliangtian Agricultural Technology Co., Ltd. (Zhengzhou, Henan, China) were sown uniformly in each pot. During the growth period, plants were managed under routine conditions including regular irrigation and pest and disease control. To minimize soil compaction, shallow tillage to a depth of approximately 5 cm was performed every two weeks to maintain soil aeration and promote root development.

Pot sample collection and preparation

At physiological maturity, soil and plant samples were collected. Soil samples were obtained from the 0 – 20 cm layer of each pot using a three-point sampling method. After debris removal and homogenization, the composite soil sample from each pot was divided into two portions with one portion being air-dried and ground to pass through a 20-mesh sieve for determination of pH, soil available phosphorus (SAP), and Fe fractions, while the other portion was ground to pass through a 100-mesh sieve for analysis of soil alkali-hydrolyzable nitrogen (SAN) and soil organic carbon (STOC). Above-ground plant biomass was harvested, and grains were manually threshed. Grains were oven-dried at 55°C to constant weight to determine moisture content, after which thousand-grain weight was calculated. A subset of dried grains was then ground into a fine powder for soluble protein content analysis [9].

Sample analysis

All soil and plant measurements were conducted according to standard procedures described in Soil Agricultural Chemical Analysis [10]. Soil pH was measured by using a PHS-3C pH meter (Shanghai Yi Dian Group Co., Ltd., Shanghai, China) with the potentiometric soil:water ratio of 1:2. SAN was determined by the alkali diffusion method. SAP was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and quantified using the molybdenum blue method with a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing, China). STOC was measured by the potassium dichromate volumetric method with dilution heating [10]. Fe^{2+} and Fe^{3+} contents were determined by using the o-phenanthroline method [11]. Thousand-grain weight was measured gravimetrically [12], and grain soluble protein content was quantified by using the Coomassie Brilliant Blue G-250 assay [9].

Data processing and statistical analysis

Data processing and visualization were performed by using Microsoft Excel 2019 (Microsoft, Redmond, WA, USA). SPSS 20.0 (IBM, Armonk, NY, USA) was employed for statistical analysis. Treatment effects were assessed by one-way analysis of variance (ANOVA). When statistically significant differences were detected ($P < 0.05$), mean values were compared using Duncan's multiple range test. Pearson correlation analysis was applied to evaluate relationships between soil properties and wheat quality parameters. All results were expressed as mean ± standard deviation (SD) based on three replicates.

Results

Impacts of combined application of soil amendments on soil chemical properties

The results showed that all amended treatments exhibited significantly higher pH values than the control (P < 0.05). Q, QM, QMLB, QMMB, and QMHB treatments increased soil pH by 0.85, 1.03, 0.76, 0.75, and 0.66, respectively. QM treatment produced the strongest ameliorative effect on soil acidity. STOC differed significantly among treatments (P < 0.05). QMHB treatment resulted in the highest STOC content followed by QMMB, whereas CK, Q, QM, and QMLB showed no significant differences. In contrast, SAN content responded differently. QM treatment produced the highest SAN concentration, reaching 94.1 mg/kg followed by Q treatment at 79.6 mg/kg. No significant differences were detected among the remaining treatments. SAP content in CK was 42.1 mg/kg. All amended treatments significantly increased SAP compared to that in CK (P < 0.05). Specifically, SAP increased by 28.9%, 24.6%, 19.8%, 17.2%, and 16.1% under the Q, QM, QMLB, QMMB, and QMHB treatments, respectively. Notably, SAP levels under Q, QM, QMLB were significantly higher than those under the other amended treatments (Table 1).

Table 1. The effects of combined application of soil amendments on soil pH, STOC, SAN, SAP.

	pH	STOC (g/kg)	SAN (mg/kg)	SAP (mg/kg)
CK	4.7^{d}	8.45^{c}	66.23^{c}	42.1^{c}
Q	5.5^{b}	8.78^{bc}	79.60^{b}	54.2^{a}
QM	5.7^{a}	8.51^{c}	94.12^{a}	52.5^{a}
QMLB	5.4^{c}	8.55^{c}	69.72^{c}	48.9^{b}
QMMB	5.4^{c}	9.29^{b}	68.93^{c}	49.4^{b}
QMHB	5.3^{c}	11.26^{a}	68.45^{c}	53.8^{a}

Note: Different lower-case letters in the same column indicated significant differences among treatments at P < 0.05.

Impacts of soil amendments on different Fe fractions in soil

All amended treatments significantly reduced total Fe content compared with CK (P < 0.05). Reductions of 28.0%, 6.8%, 21.1%, 18.7%, and 14.2% were observed in Q, QM, QMLB, QMMB, and QMHB treatments, respectively. Q treatment achieved the greatest reduction of 28.0%, whereas QM treatment showed the smallest decrease of 6.8% (Figure 1).

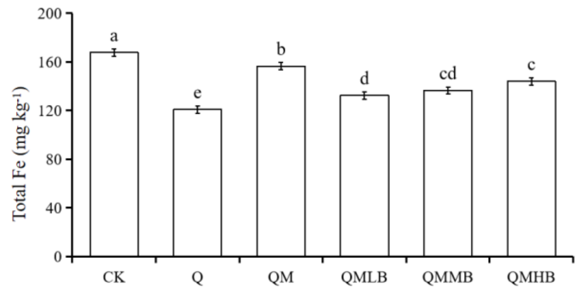

Figure 1. Total iron (Fe) contents in different treatments of the pot experiment. Different small case letters indicated significant differences among treatments (P < 0.05).

Similarly, Fe^{3+} content was significantly lower in all amended treatments than CK (P < 0.05). Relative to CK, Fe^{3+} decreased by 47.8%, 13.9%, 27.0%, 29.3%, and 34.4% under Q, QM, QMLB, QMMB, and QMHB treatments, respectively. Consistent with total Fe trends, Q treatment showed the largest reduction of 48.0%, while QM treatment displayed the smallest decrease of 13.9% (Figure 2).

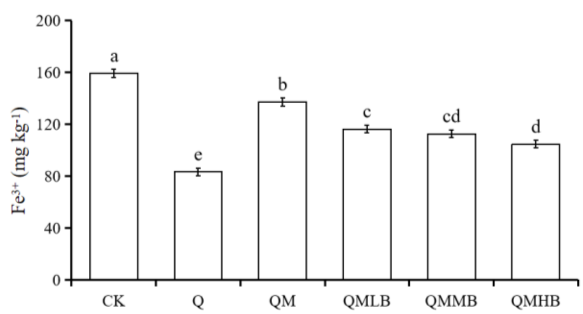

Figure 2. Ferric Iron (Fe^{3+}) contents in different treatments of the pot experiment. Different small case letters indicated significant differences among treatments (P < 0.05).

All amended treatments produced significantly higher ferrous Fe (Fe^{2+}) levels than CK (P < 0.05) with increases of 350.0%, 129.0%, 91.7%, 185.0%, and 372.0% in Q, QM, QMLB, QMMB, and QMHB, respectively. The largest increase occurred in Q at 350.0% and QMHB at 372.0%, whereas QMLB showed the smallest increase of 91.7% (Figure 3).

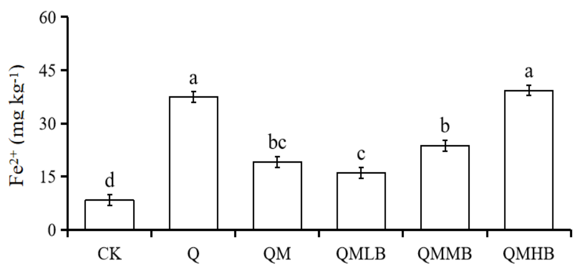

Figure 3. Ferrous iron (Fe^{2+}) contents in different treatments of the pot experiment. Different small case letters indicated significant differences among treatments (P < 0.05).

Effects of soil amendments on wheat quality

(1) Wheat thousand-grain weight

All amendments significantly increased thousand-grain weight compared to CK (P < 0.05) with the increases of 4.9%, 8.3%, 4.7%, 4.9%, and 3.6% in Q, QM, QMLB, QMMB, and QMHB, respectively. QM achieved the highest value (Figure 4).

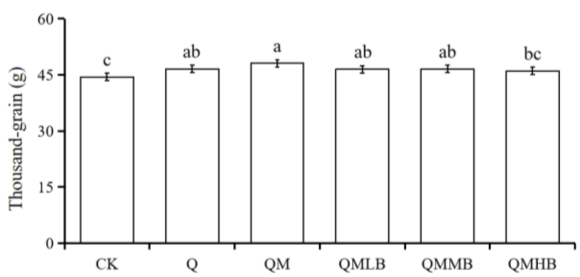

Figure 4. Wheat thousand-grain weight in different treatments of the pot experiment. Different small case letters indicated significant differences among treatments (P < 0.05).

(2) Wheat soluble protein content

Soluble protein contents were significantly higher in all amended treatments than that in CK (P < 0.05) with the increases of 17.9%, 29.7%, 23.5%, 24.4%, and 25.9% in Q, QM, QMLB, QMMB, and QMHB, respectively. Although QM showed the highest value, differences among QM, QMLB, QMMB, and QMHB were not significant (Figure 5).

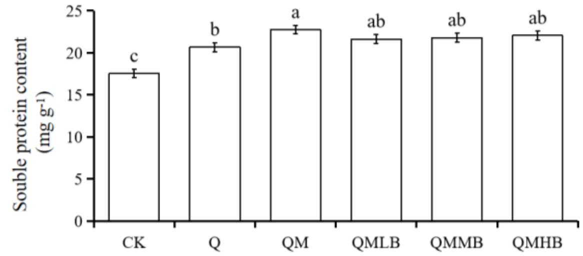

Figure 5. Soluble protein contents in different treatments of the pot experiment. Different small case letters indicated significant differences among treatments (P < 0.05).

Linear correlations between soil nutrients and wheat thousand-grain weight/soluble protein content

The results showed that soil pH and SAP were extremely significantly and positively correlated with wheat thousand-grain weight and soluble protein content (P < 0.01), while SAN was also positively correlated with thousand-grain weight (P < 0.01) (Table 2). These results indicated that soil pH, SAP, and SAN contributed positively to improving wheat grain weight and protein content.

Discussion

Effects of soil amendments on soil phosphorus availability

The pot experiment showed that all soil amendment treatments significantly increased P availability in acidic soil relative to the control that had the lowest available P among treatments. Q produced the highest available P, while differences among the combined treatments of QM, QMLB, QMMB, and QMHB were not statistically significant. These results indicated that both quicklime alone and quicklime combined with microbial fertilizer and/or biochar effectively mitigated P fixation in acidic soils. Mechanistically, the treatments enhanced P availability through complementary pathways, which included that single quicklime application increased soil pH, thereby reducing the positive surface charge of Fe and Al oxides, weakening phosphate adsorption, and promoting the release of fixed P [13]. Further,

Table 2. Correlation analysis between soil nutrients and wheat thousand-grain weight/soluble protein content.

	pH	SAP	SAN	STOC	Fe^{2+}	Fe^{3+}	Total Fe
Thousand-grain weight	0.8590**	0.5088*	0.6634*	0.0084	0.0675	0.1115	0.1104
Soluble protein content	0.8251**	0.6155*	0.2242	0.1158	0.2083	0.2305	0.1747

Note: *: $P < 0.05$. **: $P < 0.01$.

quicklime combined with microbial fertilizer added a biological mechanism. *Bacillus* spp. in the microbial fertilizer secreted phosphatases that mineralized organic P, while metabolic byproducts such as oxalic and citric acids chelated Fe/Al ions, further solubilizing Fe and Al bound phosphate [5, 6]. Together, these chemical and biological effects acted synergistically to increase P availability. When biochar was added to the quicklime-microbial fertilizer system, phosphorus availability was further improved, although not by much. Biochar contributed to the phosphorus cycle indirectly through its alkalinity helping regulate pH, porous structure retaining soluble phosphorus and reducing its loss, and large specific surface area providing a favorable habitat for microorganisms and enhancing phosphatase activity [14]. These findings were consistent with previous reports demonstrating that combined amendments effectively enhanced SAP. Increased P availability likely promoted nitrogen uptake and assimilation in wheat, thereby supporting protein synthesis and photosynthesis and ultimately improving grain nutritional quality [15, 16].

Effects of soil amendments on soil Fe fractions

Amendment application substantially modified soil Fe speciation and availability. Compared with CK that exhibited high total Fe and Fe^{3+} levels, all treatments reduced these fractions while increasing the concentration of plant-available Fe^{2+}. Q produced the greatest reduction in total Fe and Fe^{3+}, whereas the largest increase in Fe^{2+} occurred in Q and QMHB. These results indicated that the amendments alleviated potential Fe toxicity and improved Fe nutrition by shifting Fe from Fe^{3+} to Fe^{2+}. This transformation could be attributed to several mechanisms. Sole lime application raised soil pH and modified redox potential (Eh), favoring Fe^{3+} reduction to Fe^{2+} while promoting Fe^{3+}precipitation as hydroxides, thereby lowering soluble, toxic Fe^{3+} levels [17]. In lime combined with microbial fertilizer, microbes augmented quicklime's effects. Microorganisms produced organic acids such as oxalic and citric acid that chelated or reduced Fe^{3+}, and some possessed enzymatic pathways enabling direct Fe^{3+} reduction, thus providing a biological route for Fe transformation. Quicklime-microbial fertilizer–biochar composite further enhanced Fe^{2+} formation as biochar created locally reductive microenvironments and provided a large surface area for Fe^{3+} adsorption, thereby facilitating Fe^{2+} stabilization [18]. The pronounced increase in Fe^{2+} in QMHB highlighted its role in promoting reductive conditions. The conversion of toxic Fe^{3+} to beneficial Fe^{2+} represented a key linkage between soil chemistry and plant performance. In acidic CK soils, excess Fe^{3+} could induce toxicity and restrict P uptake. In contrast, higher Fe^{2+} levels in amended soils, as the primary assimilable Fe form, supported the synthesis of Fe-containing proteins and enzymes such as cytochrome oxidase, thereby optimizing nitrogen metabolism and grain quality. Overall, Fe speciation regulation by soil amendments was critical for mitigating Fe toxicity and enhancing Fe bioavailability in acidic soils [19, 20].

Effects of soil amendments on wheat yield and quality and future perspectives

The results of this research demonstrated that QM most effectively increased soil pH, wheat thousand-grain weight (a yield indicator), and grain soluble protein content (a quality indicator), while QMHB produced the largest gain in soil organic carbon (STOC). Further, Q yielded the highest SAP. These results indicated that all amendments could alleviate major soil constraints in line with previous studies [21]. Among them, QM exhibited the most favorable synergy for enhancing both yield and quality. This advantage likely reflected an optimal balance of lime rapidly neutralizing soil acidity, fostering a conducive environment for inoculated microbes, which enhanced nutrient mobilization (particularly nitrogen) and might secrete growth-promoting substances that directly supported plant physiology and protein synthesis. While QMHB effectively increased soil carbon, it might have subtly altered nutrient availability or microbial communities under pot conditions, limiting its impact on yield and quality relative to QM. It is important to emphasize that these conclusions are based on a short-term pot experiment. Translating these findings into practical agricultural recommendations requires several steps, which include that the performance of QM must be validated, and optimal application rates must be determined through long-term, multi-location field trials. Further, a comprehensive sustainability assessment is needed, encompassing economic feasibility for farmers and long-term effects on soil health, microbial ecology, and carbon dynamics. Furthermore, understanding the mechanisms underlying observed responses including plant physiological processes, microbial interactions, and interactions with management practices such as irrigation and fertilization is essential for optimizing this strategy. This study showed that quicklime, microbial fertilizer, and biochar all improved acidic soils, but the QM combination was most effective for enhancing soil pH, nutrient availability, and wheat yield and quality. This approach provided a targeted strategy for reclaiming acidic soils in the Huang-Huai region, pending validation through larger-scale, long-term studies.

Conclusion

The results of this study indicated that Q, M, QM, and B, used alone or in combination, could significantly improve soil properties and wheat yield. Amendments increased soil pH by 0.66 – 1.03 units, SAP by 16.1 – 28.9%, SAN by 3.4 – 42.1%, thousand-grain weight by 3.6 – 8.3%, and grain soluble protein content by 17.9 – 29.7%, while reducing Fe^{3+} and total Fe contents. The QM treatment showed the strongest synergistic effect, achieving the highest pH and largest gains in yield and quality. Correlation analysis revealed significant positive relationships between soil pH, SAP, and both thousand-grain weight and soluble protein, while SAN correlated only with thousand-grain weight, indicating that alleviating acidity and mobilizing P were primary drivers of the observed yield and quality improvements. The combined lime and microbial fertilizer application was a promising strategy for remediating acidic soils and enhanced wheat productivity and grain quality. Further field trials and long-term studies would be needed to confirm its practical effectiveness, economic viability, and sustainability.

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