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Effect of Increasing Nitrogen and Phosphorus Fertilization Rates on the Micro- and Macronutrient Contents in Wheat Grain Grown on Acidic Soil

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ABSTRACT

Soil acidity is one of the main factors limiting wheat yield and grain quality, as it significantly impacts nutrient solubility, availability, root development, and nutrient uptake by plants. Understanding how fertilization practices interact with soil acidity is essential for sustainable crop production. This study aimed to investigate the influence of increasing nitrogen (N) and phosphorus (P) fertilization rates on the micro- and macronutrient contents of wheat grain grown on an acidic soil. The experiment was conducted under field conditions using a bifactorial design with five levels of nitrogen (0, 40, 80, 120, 160 kg/ha) and five levels of phosphorus (0, 40, 80, 120, 160 kg/ha). After harvest, the grain samples were processed and analyzed for their macronutrient (N, P, K, Ca, Mg) and micronutrient (Fe, Mn, Zn, Cu) contents. The results revealed that increasing N and P fertilization rates significantly influenced the mineral composition of wheat grain. Higher nitrogen rates (120 and 160 kg/ha) increased grain nitrogen content and enhanced zinc and manganese concentrations, indicating an overall improvement in nutritional quality. When P levels are high, it is common to observe a decrease in Cu uptake, suggesting that there are antagonistic interactions between phosphorus and copper when phosphorus levels are high. Phosphorus fertilization favored the accumulation of P and Mg in the grains, with higher phosphorus application rates leading to statistically significant increases compared to the control. These findings highlight the importance of balanced nitrogen and phosphorus fertilization in optimizing both yield and nutrient composition of wheat grain under acidic soil conditions. Proper nutrient management contributes not only to maintaining long-term soil fertility but also to enhancing the nutritional value and quality of wheat as a staple food crop.

Keywords: wheat, nitrogen, phosphorus, micronutrients, macronutrients.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important crop worldwide, serving as a major source of carbohydrates and protein (Pandey et al., 2020; Azad et al., 2021). Romania is a significant wheat-producing country within the European Union, benefiting from favorable agroclimatic conditions and an extensive tradition in cereal cultivation. In 2020, Romania's wheat production reached approximately 6.4 million tons, placing the country sixth among EU wheat producers (Romania Insider, 2021). Maintaining high production levels highlights the importance of understanding nutrient dynamics in wheat, particularly under different fertilization regimes and soil conditions, to ensure both yield stability and grain quality (Yan et al., 2020).

The nutritional value of wheat grain is strongly influenced by its macro- and micronutrient composition, which depends on soil fertility status and fertilizer inputs. Acidic soils, however, restrict the availability of phosphorus and several micronutrients, reducing their uptake and translocation within the plant (Fageria and Moreira, 2011). Nitrogen and phosphorus fertilization, therefore, plays a central role in managing soil fertility, grain quality, and nutrient balance, while also affecting long-term processes such as soil acidification (Zhang et al., 2024).

Long-term fertilization experiments are essential for understanding how continuous applications of nitrogen (N) and phosphorus

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(P) modify soil chemical properties, nutrient cycling, and the nutrient composition of wheat grain over time, as well as the effects on crop yields (Petcu and Petcu, 2006). Repeated N fertilization - especially from ammonium-based sources - often accelerates soil acidification, altering the solubility and mobility of key cations such as Ca, Mg, and Mn (Vigovskis, 2016; Cade-Menun et al., 2024). Similarly, long-term P fertilization can increase total soil phosphorus but also shift it into less plant-available forms in acidic soils due to fixation by Fe and Al oxides (Chen et al., 2021). These cumulative changes contribute to reduced fertilizer efficiency and influence both crop productivity and grain mineral composition.

Romanian long-term experiments confirm that sustained N and P applications significantly affect soil pH, available P, exchangeable base cations, and wheat yield stability, as well as grain nutrient accumulation (Lupașcu et al., 2012; Mărin et al., 2019, 2021). Despite this progress, the combined influence of increasing N and P doses on macro- and micronutrient accumulation in wheat grain grown specifically on acidic soils remains insufficiently documented.

Such long-term interactions between fertilization practices and soil acidity highlight the need for multi-decadal field experiments capable of capturing cumulative changes that short-term studies cannot reveal. For this reason, beginning in the autumn of 1961, long-term fertilization trials were established on the acidic soils of the Livada Agricultural Research Station, providing a unique platform for studying the sustained effects of nitrogen and phosphorus inputs under consistent pedoclimatic conditions.

This study aims to clarify how gradual increases in nitrogen and phosphorus inputs modify nutrient uptake processes and the subsequent accumulation of essential macro- and micronutrients in wheat grain grown on acidic soil, thereby providing a deeper understanding of the long-term interactions between fertilizer regimes, soil chemical constraints, and grain nutritional quality.

To address this objective, the following section describes the experimental site, fertilization treatments, sampling procedures, and analytical methods used in the study.

MATERIAL AND METHODS

The field experiment was conducted at the Livada Agricultural Research and Development Station (ARDS Livada), located in north-western Romania, under a moderate temperate-continental climate. The soil at the experimental site is classified as Luvic Brown soil.

A 5P × 5N bifactorial design was applied to evaluate the response of winter wheat to increasing fertilization rates. Phosphorus (P) was used at five doses: 0, 40, 80, 120 and 160 kg P/ha, and nitrogen (N) was applied at 0, 40, 80, 120 and 160 kg N/ha, resulting in 25 treatment combinations. Treatments were arranged in a subdivided-plot experimental design with three replications (Figure 1).

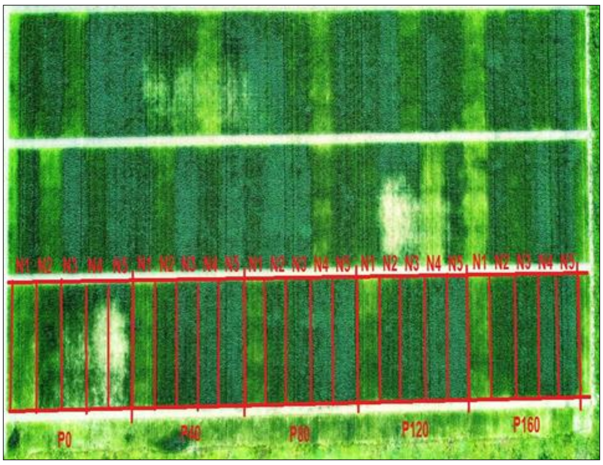

Figure 1. Aerial view of the 5P × 5N bifactorial fertilization experiment in winter wheat (cv. Glosa) at ARDS Livada, showing the arrangement of phosphorus (P0-P160) and nitrogen (N1-N5) treatments, 2020

Ammonium nitrate served as the nitrogen fertilizer, while concentrated superphosphate was used as the phosphorus source.

The experiment was integrated into a crop rotation system, and in 2020 the cultivated crop was winter wheat (*Triticum aestivum* L.), cultivar Glosa.

The aerial image depicts the experimental layout, with the main parcels assigned to the five phosphorus levels (P0-P160). Within each parcel, the plots labeled N1-N5 correspond to the nitrogen treatments. Differences observed in covering density and color indicate the crop's response to nutrient supply.

Seventy-five wheat grain samples were collected and analyzed to assess their macro and micronutrient content. The samples were dried and ground.

Total nitrogen content was determined using Kjeldahl analysis, which includes digestion in concentrated H_2SO_4, distillation under alkaline conditions, and subsequent acid-base titration of the distilled ammonium.

The protein content of the wheat samples was determined using a Grain Analyzer (Granolyzer), which operates on the principle of near-infrared reflectance spectroscopy (NIR). Before analysis, the instrument was calibrated using the manufacturer's standard calibration curves for common wheat (*Triticum aestivum* L.).

Phosphorus content in wheat grain was assessed following mineralization with a mixture of concentrated mineral acids, and quantified colorimetrically by spectrophotometry using the ammonium vanadate-molybdate complex. (Răuță and Chiriac, 1980).

In the same digest, potassium concentration was measured by flame photometry, whereas calcium, magnesium, copper, zinc, manganese, and iron were determined by atomic absorption spectrometry.

The results were subjected to analysis of variance (ANOVA), and differences among treatment means were evaluated using Tukey's multiple comparison test (Ceapoiu, 1968). For all graphical representations, means followed by the same letter do not differ significantly at the 5% level ($P < 0.05$), according to Tukey's HSD test.

RESULTS AND DISCUSSION

The nutrient composition of wheat grain is a crucial indicator of both crop health and grain quality. Macronutrients such as nitrogen (N), phosphorus (P), calcium (Ca), and magnesium (Mg) play essential roles in wheat growth and development.

Nitrogen is a fundamental element in wheat physiology because it is directly involved in the synthesis of structural and metabolic compounds that determine grain yield and quality.

Grain nitrogen concentration is tightly associated with protein content, and therefore with several technological traits used to classify wheat for end-use quality (Haile et al., 2012).

Similar to the observations of Cade-Menun et al. (2024), who reported that long-term N-NH_4 fertilization promotes soil acidification, our results also show a consistent decline in soil pH with increasing nitrogen inputs.

In our experiment, soil pH decreased from 5.87 in the unfertilized control to 5.09 at the highest nitrogen application rate.

Despite this acidification, fertilization with 120 and 160 kg N/ha led to statistically significant increases in grain nitrogen content.

Under phosphorus fertilization, slight decreases in grain nitrogen content were observed (Figure 2).

This trend has also been reported in other studies and is commonly attributed to a relative dilution of nitrogen (Takahashi and Anwar, 2007; Mehdi et al., 2025).

Moreover, the higher nitrogen accumulation in the grain at these application levels translated into correspondingly higher protein content, confirming the strong positive relationship between nitrogen availability, grain nitrogen concentration, and protein synthesis in wheat (Figure 3).

This positive relationship between high nitrogen supply and increased grain protein content is also strongly supported by recent studies.

For example, a meta-analysis by An et al. (2024) showed that nitrogen rates of 240-300 kg N/ha significantly increase total protein and its fractions in wheat.

Phosphorus fertilization caused small but statistically significant increases in the average phosphorus content of wheat grains, especially at higher application rates (80, 120, and 160 kg/ha) compared to the control, as shown in our graphical data (Figure 4).

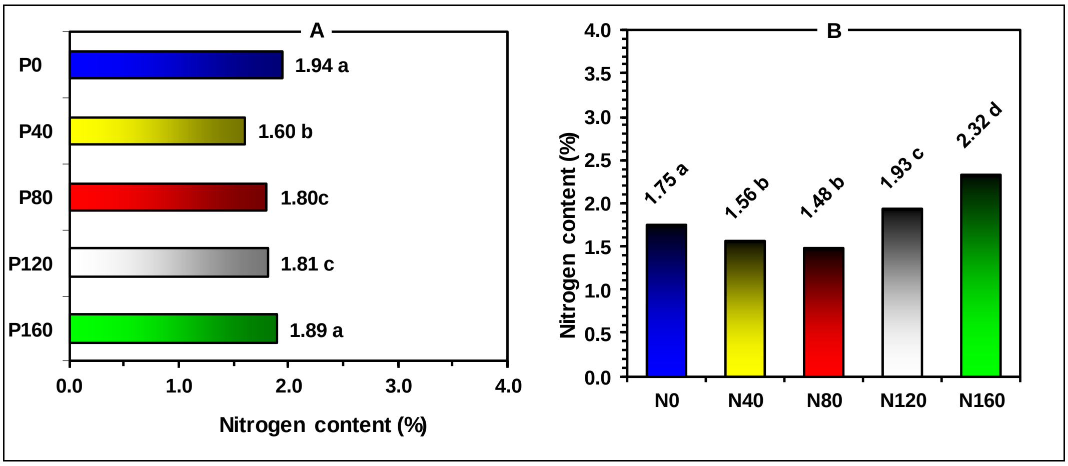

Figure 2. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Nitrogen Content in Wheat Grains

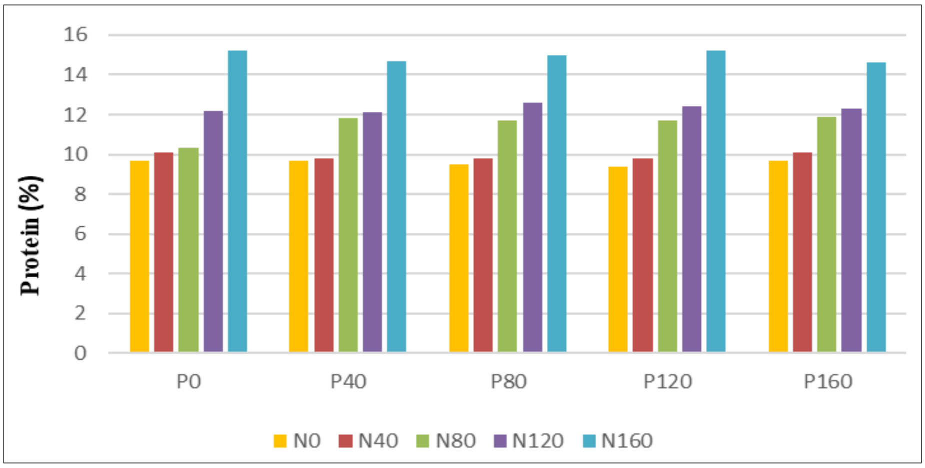

Figure 3. Effects of Phosphorus and Nitrogen Fertilization on Protein Content in Wheat Grains (adapted from Kurtinecz and Banateanu, 2020)

Recent studies also support the effect of combined nitrogen and phosphorus fertilization on phosphorus accumulation in wheat grains (Harutyunyan et al., 2023).

The relatively stable potassium concentration observed in the wheat grains suggests that the grains exhibit K^+ homeostasis, consistent with the regulated transport and buffering mechanisms that plants deploy to maintain internal potassium levels even under variable external supply (Luan et al., 2017).

Remarkably, the only statistically significant increases occurred under intermediate nitrogen doses (N80 and N120), compared to the control (Figure 5).

This pattern implies that, when fertilising with nitrogen, it may be more effective to aim for an optimal (rather than maximal) N level to maximize not only yield, but also the nutritional quality of the grains (in this case, their potassium content).

The decline in calcium content in the wheat grain with increasing phosphorus dose (from 102 mg/kg in the control to 67 mg/kg in P120-fertilised grains) reflects the antagonistic interaction between P and Ca (Figure 6).

High external phosphorus supply can reduce Ca accumulation in grains, possibly by affecting Ca uptake or its partitioning within the plant.

This interpretation is supported by the findings of Wierzbowska and Bowszys (2008), who reported that higher phosphorus fertilisation levels significantly reduced the calcium content in spring wheat grain, indicating that excessive P inputs can adversely affect Ca nutrition.

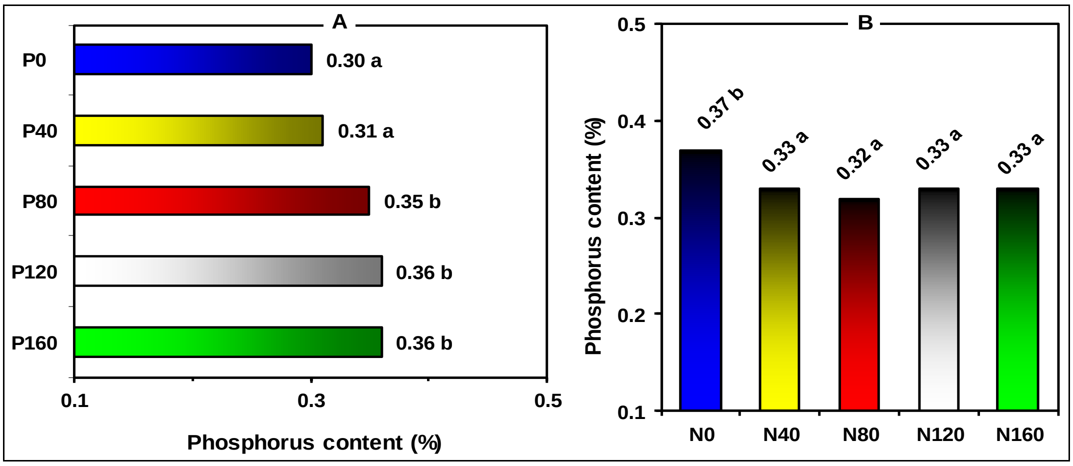

Figure 4. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Phosphorus Content in Wheat Grains

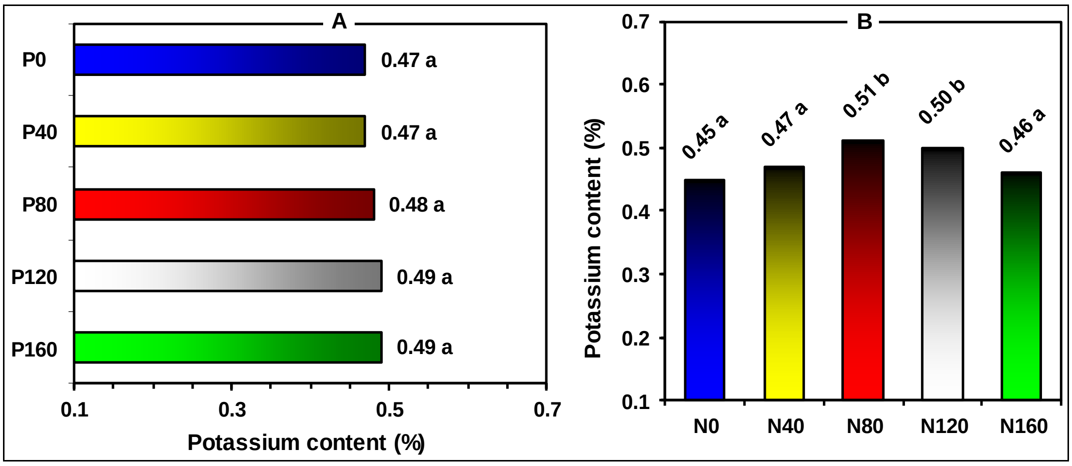

Figure 5. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Potassium Content in Wheat Grains

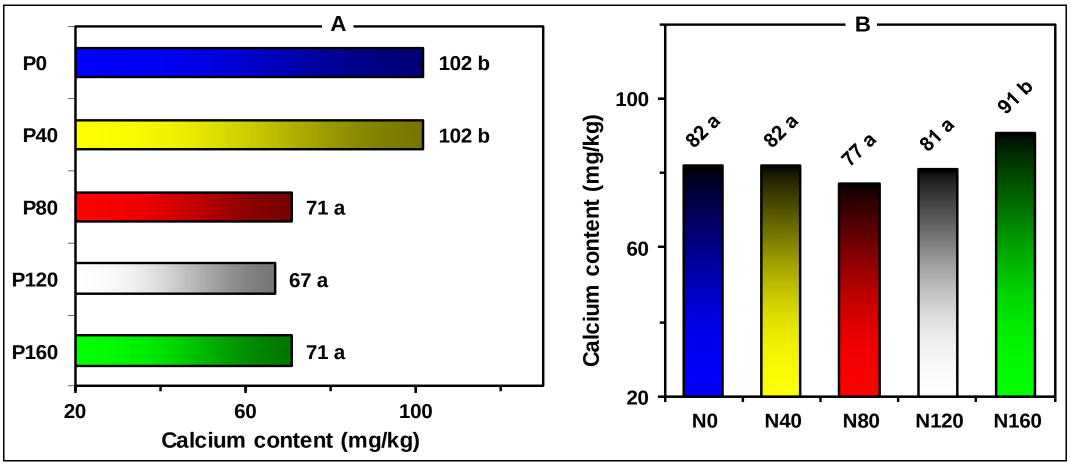

Figure 6. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Calcium Content in Wheat Grains

In the nitrogen fertilisation treatments, a consistent decrease in the mean magnesium content of the wheat grain was observed as the N dose increased (Figure 7). Such reductions may be attributed to nutrient dilution or to N-Mg antagonism, where high nitrogen supply impairs magnesium uptake or redistribution. Similar findings have been reported by Kutman et al. (2011), who showed that elevated nitrogen levels can lead to lower concentrations of magnesium in wheat tissues. Additionally, Fageria (2001) discussed nutrient interactions in crops and highlighted that excessive nitrogen fertilisation may reduce the uptake of other essential nutrients, including magnesium.

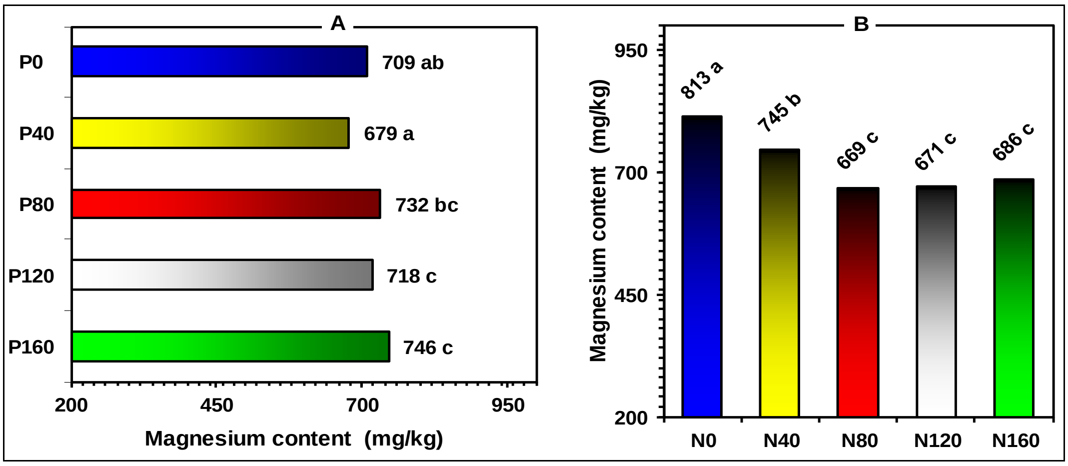

Figure 7. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Magnesium Content in Wheat Grains

Copper, zinc, manganese, and iron are essential micronutrients required for various physiological and biochemical processes in wheat, such as enzyme activation, photosynthesis, respiration, and grain development; therefore, their quantification in the grain provides valuable information on plant nutrition and the potential nutritional quality of the harvested crop (Ahmed et al., 2024).

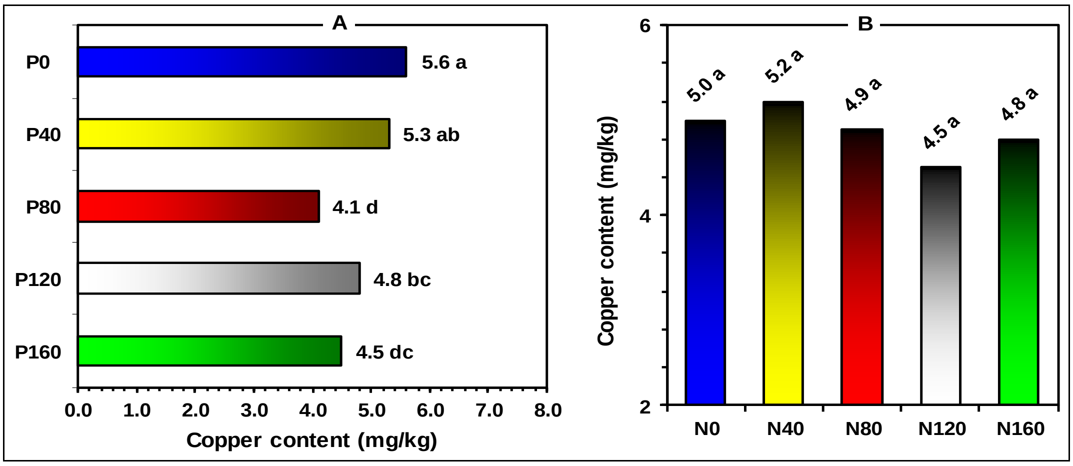

Figure 8. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Copper Content in Wheat Grains

From Figure 8, it can be observed that there is a statistically significant decrease in copper content in wheat grains as phosphorus fertilization doses increase, which can be attributed to the antagonistic interaction between phosphorus and copper (Pandey et al., 2020). Excess phosphorus in the soil reduces copper availability by forming insoluble copper phosphate complexes, thereby limiting Cu uptake and translocation to the grain.

Some relevant studies have shown that increasing nitrogen fertilization in acidic soils can improve grain uptake of micronutrients (Mn, Zn, Fe), but the effect is not always linear and heavily depends on other management factors (Chauhan et al., 2019; Naeem et al., 2023).

Our results showed a significant increase in grain zinc and manganese at that higher nitrogen application rate, demonstrating that elevated nitrogen fertilization promotes Zn and Mn accumulation in wheat grain (Figures 9 and 10). Increasing phosphorus fertilization rates caused only small variations in the zinc and manganese contents of wheat grains, with no statistically significant differences compared to the unfertilized variant.

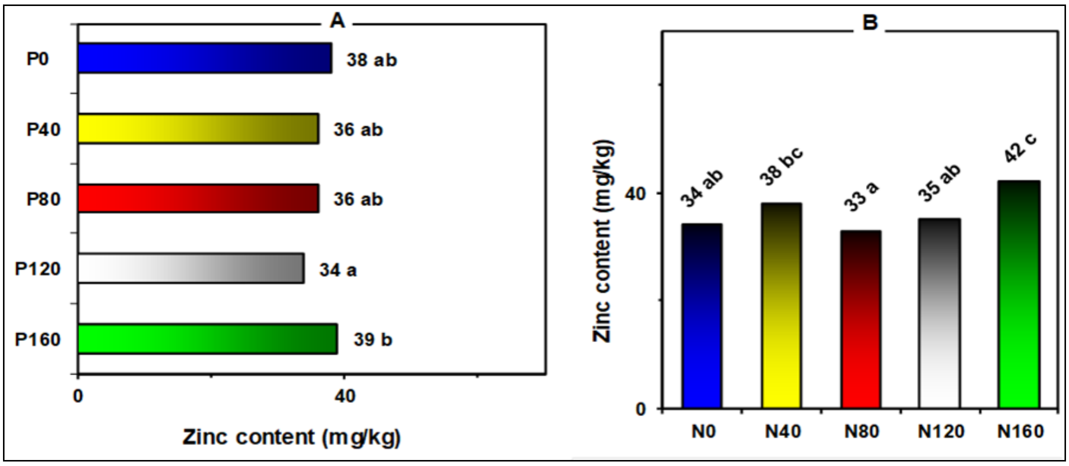

Figure 9. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Zinc Content in Wheat Grains

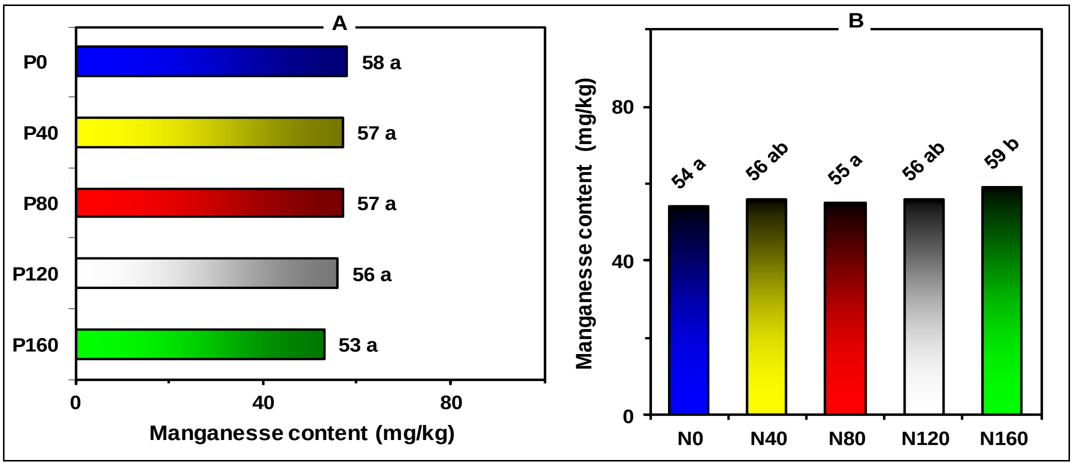

Figure 10. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Manganesse Content in Wheat Grains

As shown in Figure 11, the mean iron content in wheat grains did not exhibit statistically significant changes in response to nitrogen fertilization, whereas under phosphorus fertilization, the highest values were recorded at the P40 and P80 application rates.

Figure 12 demonstrates a reciprocal synergistic effect of prolonged fertilization with progressively increasing nitrogen and phosphorus rates, particularly at elevated doses, indicating potential yield enhancement through nutrient interactions (Kurtinecz and Banateanu, 2020). To mitigate the well-documented inhibitory effects of excessive nitrogen - characterized by reduced nitrogen use efficiency and diminished grain yield - nitrogen was applied in up to four split doses at the highest fertilization level, thereby optimizing nutrient uptake and minimizing adverse effects (Zhang et al., 2021).

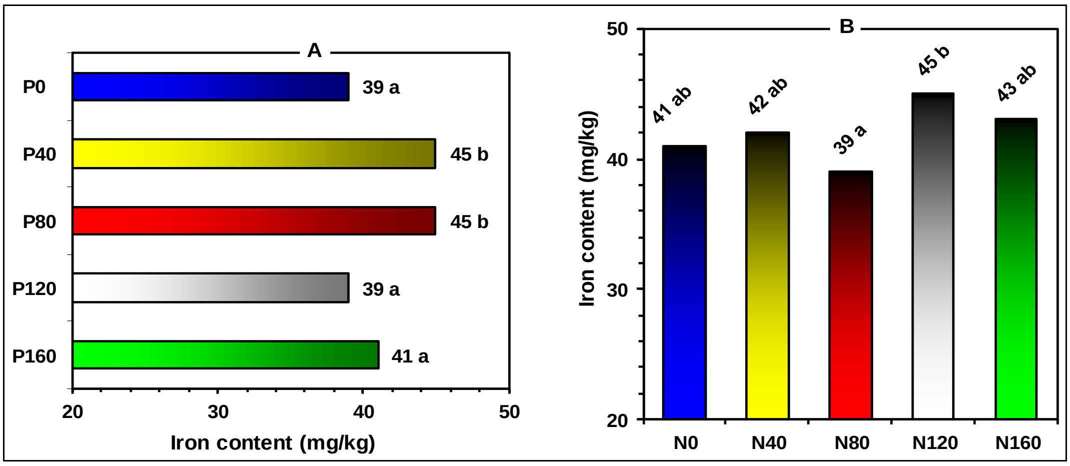

Figure 11. Effects of Phosphorus (A) and Nitrogen (B) Fertilization on Iron Content in Wheat Grains

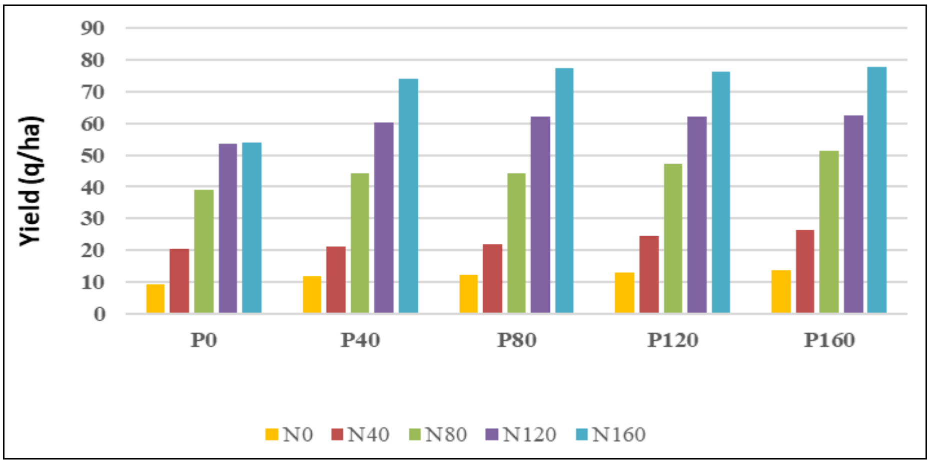

Figure 12. Effect of Progressive Nitrogen and Phosphorus Fertilization Rates on Wheat Yield (adapted from Kurtinecz and Banateanu, 2020)

CONCLUSIONS

This long-term study was conducted on acidic brown luvic soil to evaluate the effects of progressively increasing nitrogen (N) and phosphorus (P) fertilization on wheat grain nutrient content and quality. The results showed that nitrogen fertilization significantly increased grain nitrogen content and protein concentration, highlighting its crucial role in improving wheat nutritional value, whereas phosphorus fertilization had no significant effect on grain protein. Consequently, nitrogen application remains one of the key technological measures for achieving a desirable grain protein concentration.

Compared to the control, nitrogen fertilization resulted in a statistically significant increase in calcium, zinc, and manganese content. Phosphorus fertilization resulted in significant increases in phosphorus and magnesium content in wheat grains, while calcium and copper contents significantly decreased compared to the control.

The highest wheat yields were recorded with higher nitrogen and phosphorus application rates, phosphorus playing a major role due to the phosphorus reserves already accumulated in the soil.

These findings underscore the need to continue long-term research on progressive nitrogen and phosphorus fertilization to better assess crop quality and yield responses and to support the development of sustainable fertilizer management strategies under changing environmental conditions.

ACKNOWLEDGEMENTS

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