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**Research Article**

# Yield and Nutrient Contents of Wheat, and Changes in Selected Soil Properties after 23 Years of Phosphorus Fertilizer Application

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## ABSTRACT

Effective nutrient and fertilizer management practices play a pivotal role in sustaining agriculture and mitigating the effects of climate change. These practices have a direct influence on soil fertility and crop productivity. This study investigated the long-term impacts of different phosphorus (P) fertilizer doses (0, 50, 100, and 200 kg $P_2O_5$ $ha^{-1}$) on biomass yield and grain nutrient levels of wheat crops as well as on selected soil properties. The experiment was established in 1998 at the Research Center of Cukurova University in Adana, Southern Turkey. The wheat seeds were sown in October 2020 and harvested in May 2021. The experimental design was a randomized complete block, comprising 12 plots and replicated three times. Soil samples were collected at depths of 0-15 cm and 15-30 cm and subsequently analyzed for total carbon, organic carbon (OC), total nitrogen (TN), and the number of mycorrhizal spores. At harvest, wheat plant samples were collected for evaluating total dry biomass, grain yield and concentrations of potassium (K), zinc (Zn), total carbon (C), and total nitrogen (N) in the grain. The result showed that there was a linear increase in the total dry biomass of wheat, however, grain yield started to decline at P200. The wheat grain nutrient concentrations did not differ significantly across P doses, except for higher concentrations of Zn and K at P50 and higher values of grain C and N at P100. However, there was a linear decrease in Zn concentration as the applied P dose increased. The soil OC and soil TN at 0-5 cm were significantly changed relative to the control. The highest levels of soil OC and TN were observed at P100. The number of mycorrhizal spores did not significantly change with the P dose, but a decreasing trend was observed at higher doses. In conclusion, based on the observed parameters of wheat grain yield, total biomass production, grain nutrient concentrations, and soil OC storage, the application of 100 kg $P_2O_5$ $ha^{-1}$ outperforms other P doses. However, the agronomic efficiencies, soil nutrient balance, and environmental effects of the applied P dose require more research.
**Keywords:** Phosphorus, Long-term Field Trial, Wheat, Soil Organic Carbon, Biomass Yield, Mycorrhizae.

## INTRODUCTION

Agriculture, food security, and climate change are linked and have an impact on one another. The agricultural sectors contributes to climate change by releasing greenhouse gases, which in turn, impair crop production and food security (Malhi, Kaur and Kaushik, 2021; Yohannes, 2016). Effective nutrient and fertilizer management is vital for addressing climate change and the interconnected challenges (Whitmore *et al.*, 2012). Avoiding excess nutrient applications such as phosphorus (P) and nitrogen (N), can contribute to climate change mitigation. Phosphorus fertilizers have been used to improve crop yield since the first "Green Revolution" (Sahrawat, Abekoe and Diatta, 2001). However, efficient management of P fertilizer is crucial as the P reserves are being depleted (Childers, Corman, Edwards and Elser, 2011). In the soils of the Mediterranean region, including southern Turkey, limited availability of P has necessitated the implementation of extensive fertilization practices to maximize crop productivity (Ortas and Akpinar, 2011). These practices have raised environmental concerns and socio-economic issues relating to the rising cost of chemical fertilizers.

Extended P fertilization in wheat cultivation has diverse impacts on crop yield and grain quality. Saha, Saha, Murmu, Pati and Roy (2014) reported a positive association between yield and P application rate in a 40-year-old long-term field experiment. In contrast, some other studies showed that prolonged P application does not necessarily enhance yield, but does cause a considerable rise in soil P levels (Takahashi and Anwar, 2007). In addition, the P application affects the grain nutrient concentration through its effect on their absorption and translocation. For instance, increasing the application dose of P fertilizer above 50 kg $P_2O_5$ $ha^{-1}$ was reported to reduce grain zinc concentration (Zhang *et al*., 2015).

Maintaining optimal soil fertility (quality) can support plant growth while reducing reliance on external inputs. Soil organic carbon (OC) plays an essential role in the sustainable maintenance of soil quality and the proper functioning of agroecosystems (Ramesh *et al*., 2019). Achieving effective soil OC management requires attention to soil management, agronomic practices, and nutrient application. To facilitate carbon storage in the soil, it is essential to nourish plants with essential nutrients such as P. This enables plants to enhance photosynthesis efficiency, leading to increased $CO_2$ absorption and the deposition of organic materials into the soil (Himes, 1997; Lal, 2004). Therefore, the dynamics of soil OC and soil N are impacted by how P fertilizer is managed (Işık, Öztürk, Akşahin, Demirkol and Ortaş, 2020; Ortas and Bykova, 2020). Phosphorus also affects the symbiotic relationship between host plants and mycorrhizal fungi; as a higher P level reduces the infection rate and sporulation of the mycorrhizae (Ortas, 2012).

A long-term field trial is needed to better understand how P fertilizer affects wheat productivity, grain qualities, soil OC, and total N dynamics, as well as mycorrhizal fungi abundance. Despite these facts, there is limited information about the specific patterns and extent of these parameters' responses to prolonged P application (Ortas and Islam, 2018). Therefore, this study was conducted to evaluate the long-term (over 23 years) effects of different P fertilizer application rates on: (1) the total biomass yield and grain nutrient levels of the wheat crop; and (2) the changes in selected soil properties (soil OC, total N, and indigenous mycorrhizal spore counts).

## MATERIALS AND METHODS

### Description of Experimental Site

A long-term field experiment was established in 1998 and continued through 2020/21 (23 years) at Cukurova University agricultural research center, department of Soil Science and Plant Nutrition site. The site is situated in the Mediterranean region of Southern Turkey, at 37.0133° Latitude and 35.3587° Longitude, with an elevation of 31 meters above sea level. The area has a Mediterranean climate type, with a long-term average annual temperature of 19.1°C, and precipitation of 670.8 mm. The main rainy season is between November and April. The soils of the study area have a clay textural class with 53.5% clay, pH (7.57), organic matter (0.89%), available P (15.35 mg $kg^{-1}$), and total N (0.09%).

### Design of Experiment

The experimental design was a randomized complete block design (RCBD) with four doses of phosphorus, such as 0, 50, 100, and 200 kg $P_2O_5$ $ha^{-1}$. These doses were labeled as P0, P50, P100 and P200, respectively. The experiment had three blocks and 12 plots, each with a 200 m2 area (20 x 10 m). Wheat seeds (Adana-99 variety) were planted in October 2020 and harvested in May 2021. Triple superphosphate (TSP) was used as a source of P fertilizer, and all P doses were applied as a basal application by broadcasting.

### Data Collection and Lab Analysis

During harvesting, plant samples from a 2x1 $m^2$ area were collected to measure the above- and below-ground dry biomass and grain nutrient concentrations of wheat crop. Soil samples were also taken at 0-15 and 15-30 cm soil depths to determine the organic carbon, total nitrogen, C:N ratio, and number of mycorrhizal spores. The soil inorganic carbon was estimated from a $CaCO_3$ value that was determined by Calcimeter. The difference between total carbon and inorganic carbon was used to determine the soil organic carbon. The soil C:N ratio was also calculated as the ratio of soil OC to soil total N. The total carbon and nitrogen for both soil and grain samples were analyzed by the dry combustion method using Fisher CN-2000 analyzer. Using ICP, the DTPA extraction method (Lindsay and Norvell, 1978) was used to determine the amounts of K and Zn in the grains.

### Statistical Analysis

The wheat crop and soil data were checked for conformity with the assumptions of ANOVA using Shapiro-Wilk's test for normality and Levene's test for homogeneity of variance. Data analysis was conducted using R programming version 4.3.0 statistical software (R Core Team, 2023). For comparing treatment means, the Least Significant Difference (LSD) test ($P < 0.05$) was employed.

## RESULTS AND DISCUSSION

### Dry Biomass Yield of Wheat Crop as Affected by P Application Doses

Phosphorus fertilizer has a promising positive effect on the growth and productivity of wheat crops particularly in cases where the initial soil P level is low. However, its long-term application is expected to have an impact on the nutrient stoichiometric balance and the efficiency of nutrient utilization, thereby affecting crop yield. The current study found that prolonged application of P had a significant impact on the dry biomass and grain yield of wheat (Figure 1).

The highest and lowest total dry biomass of 13.51 t $ha^{-1}$ and 6.86 t $ha^{-1}$ were obtained at P200 and at control, respectively (Figure 1). The applied P doses showed a significant increase in total biomass production relative to P0; however, no significant differences were observed among the various doses. Compared to the control, P50 and P200 resulted in significantly higher straw and total dry biomass production, whereas P100 and P200 showed significantly higher grain yield and root biomass. The higher total dry biomass at P200 was attributed to higher straw biomass. The grain yield obtained at P100 dose was 15% higher than at P50 and 4% higher than at P200. Consequently, applying higher P doses beyond P100 did not improve yield but increased straw biomass and reduced the harvest index. In the previous studies conducted in 2013/14 (Akpinar and Ortas, 2023) and in 2016/17 (Bykova, 2018), similar trends were observed in yield and total biomass production, except for higher grain yields recorded at P200.

The soils of the study area are known for their higher lime and clay content and moderate P fixation capacity (Ortas and Akpinar, 2011). Increasing continuous P availability through fertilizer application may contribute to the observed increase in yield and biomass production. In support of this study, Tang, Li, Ma, Hao and Li (2008) also found that wheat yield increased with P dose in a long-term (15 years) P fertilization trial. The yield reduction at the maximum P dose may be attributed to long-term P accumulation in the soil, leading to reduced crop responses to subsequent P applications. Similar research findings also indicate that long-term application of higher doses of P did not significantly improve grain yield but increased the residual soil P level (Liu, Hu, Yang, Abdi and Cade-Menun, 2015).

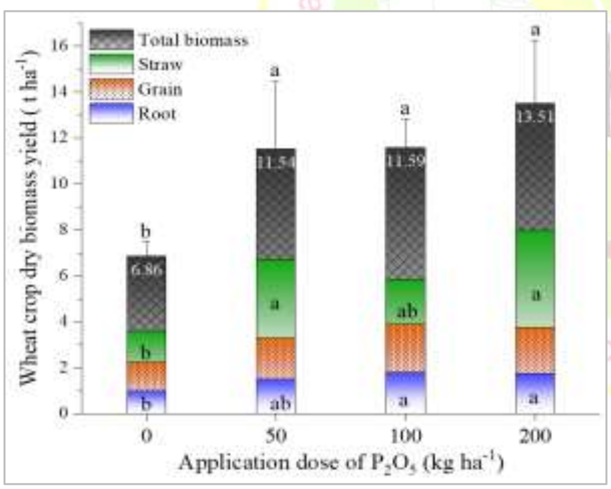


**Figure 1**. Effect of long-term P application on wheat biomass (total, straw, grain, and root: from top to bottom) at different doses. Bars with the same letter indicate no significant difference at $P < 0.05$.

**Response of Nutrient Concentrations in Wheat Grains to P Application Doses**

Grain K, Zn, C, and N concentrations were determined to examine the influence of frequent P application doses on grain quality with respect to nutrient accumulation. The results are indicated in Figure 2, and it was found that the long-term application of the P dose did not have a significant impact on the concentrations of grain nutrients. However, there was a decreasing trend in grain Zn level as the P dose increased from P50 to P200 (Figure 2 A).

Relative to the control and P200, grain Zn concentration at P50 was higher by 15.58% and 22.12%, respectively. In general, there is a negative P–Zn interaction with increasing P addition to soil (Sánchez-Rodríguez, Del Campillo and Torrent, 2017). High P levels in the soil can disrupt the plant's ability to efficiently utilize and transport Zn into the sink, resulting in decreased the Zn level in the grains (Ortas and Islam, 2018; Yang, Tian, Lu, Cao and Chen, 2011). A meta-analysis study also showed that P application reduced wheat grain Zn concentration by 16.6% (Zhang *et al*., 2021).

The grain K, C, and N concentrations did not show a consistent trend across the P doses. The highest grain K concentration (0.412%) was observed at P50, and the lowest K (0.386%) was at P0 (Figure 2 A). The results showed that higher P application rates led to marginally higher grain K concentrations despite the lack of statistically significant differences. Grain total C and N followed the same trend, and the highest values for N (2.29%) and C (40.95%) were obtained in P100 (Figure 2 B). For the same experiment, the highest grain C and N in P200 were reported (Akpinar and Ortas, 2023; Işik, Akşahin and Ortaş, 2021). However, the current study showed that P100 produced higher grain C, and N. This indicates that the response of grain nutrient contents to P doses may change over time. Moreover, the higher grain yield obtained at P100 may result in reduced N and C concentrations in the grains due to the dilution effects.

**Dynamics of Soil Organic C, Total N, and Mycorrhizal Spores with Varying P Doses**

The P application doses had a significant effect on the soil organic carbon (SOC) at 0-15 cm soil depth but were insignificant at 15-30 cm depth (Table 1). All applied P doses were significantly higher than the control (P0) with respect to the SOC at 0-15 cm but insignificant among each other. At P100, the higher SOC (1.49%) was found in the topsoil depth, however, 1.64% SOC was measured at 15-30 cm depth. The higher P level enhances plant biomass productions, including roots, and this might contribute to higher SOC factions. This finding is in agreement with the results of studies that recently reported in the same field conditions that long-term P application had a positive impact on SOC under a wheat cropping system (Akpinar and Ortas, 2023; Ortas and Bykova, 2020; Ortas and Lal, 2012).

The soil TN was significantly affected by P dose application at 0-15 cm depth, while it was not significant at 15-30 cm depth (Table 1). There was no consistent pattern observed in the TN concentration with different P doses. The highest soil TN (0.118%) was obtained at P100 in the top 0-15 cm, and the lowest TN (0.102%) was recorded at P50 in the subsoil. Generally, soil TN was higher in the top 0-15 cm. The soil C:N ratio was also significantly affected by P fertilizer at both depths and increased as the P dose increased. A higher C:N ratio was determined at P200 (Table 1). This could be the contribution of higher crop residues and biomass produced at higher P doses. It was generally expected that the particulate fraction of SOC wound increased with organic sources from crop residues with a higher C: N ratio, mainly in the wheat cropping system. A similar trend from the same experiment in 2013/14 was found by Akpinar and Ortas (2023).

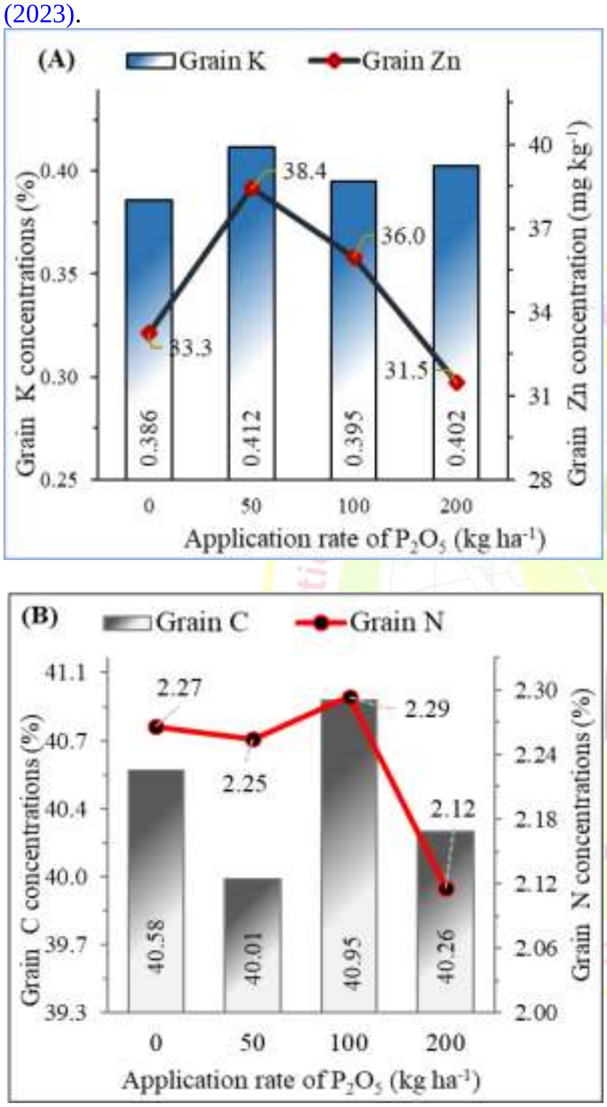


**Figure 2.** Effect of P application dose on concentrations of K and Zn (A), and total carbon and nitrogen (B) in wheat grain.

Mycorrhizal fungi have a significant impact on mineral nutrient uptake and plant growth. In this study, the wheat crop was not externally inoculated with this fungus. However, to ascertain the effects of P doses on indigenous mycorrhizal spores, the relationship between the applied P dose and the number of native mycorrhizal spores was determined. The study showed that there was no significant effect on spore counts. On average, more mycorrhizal spores (43 spores 10 g soil$^{-1}$) were recorded in the control plot. A decreasing trend in spore numbers was observed as the P dose increased. The number of spores was generally higher in the surface soil depth than in the 15-30 cm depth. The mycorrhizal dependency of plants and the number of mycorrhizal spores in the soil are reported to decrease when the soil or applied P level is higher (Ortas, 2012; Ortas and Islam, 2018; Treseder, 2004). The decrease in spore count might be attributed to the ineffective symbiotic association of the host plant and the fungus with a higher P dosage.

**Table 1.** Effect of long-term P application on selected soil properties

| $P_2O_5$ dose (kg ha$^{-1}$) | Soil depth (cm) | Soil OC (%) | Soil TN (%) | C: N ratio | Mycorrhizal spore numbers per 10 g soil |
|---|---|---|---|---|---|
| 0 | 0-15 | 1.05 $^{b}$ | 0.108 $^{b}$ | 9.75 $^{b}$ | 43.00 |
| 50 | | 1.38 $^{a}$ | 0.115 $^{ab}$ | 12.01 $^{a}$ | 28.00 |
| 100 | | 1.49 $^{a}$ | 0.118 $^{a}$ | 12.61 $^{a}$ | 18.67 |
| 200 | | 1.47 $^{a}$ | 0.115 $^{ab}$ | 12.81 $^{a}$ | 26.67 |
| LSD (P<0.05) | | 0.19 | 0.0105 | 1.423 | 25.76 (NS) |
| 0 | 15-30 | 1.27 | 0.107 | 11.84 $^{b}$ | 18.67 |
| 50 | | 1.30 | 0.102 | 12.83 $^{ab}$ | 17.33 |
| 100 | | 1.64 | 0.105 | 15.58 $^{a}$ | 13.33 |
| 200 | | 1.61 | 0.104 | 15.46 $^{a}$ | 19.00 |
| LSD (P<0.05) | | 0.449 (NS) | 0.016 (NS) | 2.969 | 14.01 (NS) |

*NS=Not significant

## CONCLUSIONS

In a 23-year-old field experiment, the objectives of this study were to explore the long-term effects of different doses of P (0, 50, 100, and 200 kg P2O5 ha$^{-1}$) fertilizer on the total dry biomass yield of wheat, the levels of nutrients in the grain, and certain soil properties. The results showed that P application doses had a significant impact on wheat crop biomass. The total dry biomass yield of wheat increased linearly with the P application dose. However, the grain yield and root biomass started to decline at P200. The study also revealed that grain K, Zn, C, and N concentrations were unaffected considerably by the long-term P dose application. While larger grain C and N concentrations were found in the P100 treatment plots, it was noticed that grain, Zn, P, and K levels were relatively higher in the P50 treatment. However, the concentration of Zn in the grain showed a decreasing trend as the dose of P fertilizer increased. This indicates that, as supported by many studies, a higher P dose has an antagonistic effect on the concentration of Zn in the grain. The P doses had a varying effect on soil properties. The soil OC and TN at 0-15 cm depth, increased significantly with P application doses relative to the control. The higher biomass production might contribute to the increased SOC. However, at the 15–30 cm soil depth, the impact of P dosages on SOC was insignificant. There was no discernible difference between P50, P100, and P200 in terms of the soil total N at the 0-15 cm depth. The application of P dosage had no appreciable impact on the soil TN at a depth of 15 to 30 cm depth. Despite the lack of a noticeable difference between the applied P doses, adding P fertilizer increased the soil C to N ratio. The number of mycorrhizal spores did not show significant changes with increasing P doses. The control plot showed higher spore counts; however, as the dosage of P increased, the spore numbers tended to decline. In conclusion, our study suggests that a long-term application of 100 kg $P_2O_5$ $ha^{-1}$ is recommended based on wheat productivity, grain nutrient concentration, and soil OC storage. However, further investigation is needed to determine the efficiency of P, the soil nutrient balance, and the environmental impacts of the applied P doses.

## CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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