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Research Article

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The Relationship Between Soil Organic Carbon and Nitrogen Budgets and Climate Change in a Soybean Plantation Under Long-term Phosphorus Dose Applications

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ABSTRACT
This study evaluated the effects of long-term phosphorus (P) fertilization on soil physicochemical properties and mycorrhizal dynamics in a soybean-growing area. The experiment was established in 1998 as a long-term field trial in the Arık soil series at Cukurova University, Adana, Türkiye, and the current evaluation was initiated in 2023. Four different phosphorus doses were applied using a triple superphosphate fertilizer source (Control (0), 50, 100 and 200 kg P_2O_5 ha^{-1}). After harvest, soils were sampled from two depths (0–15 and 15–30 cm) and from both the rhizosphere and non-rhizosphere. These soils were analyzed for physical, chemical, and biological properties, including total carbon, organic soil carbon (SOC), lime analysis, total nitrogen, available phosphorus, mycorrhizal spore density and bulk density. Soil density and especially available P content in rhizosphere soils increased with higher P applications. In contrast, mycorrhizal spore density decreased, particularly in the 0-15 cm rhizosphere layer, suggesting suppression of mycorrhizal presence under high P availability. While soil organic carbon (SOC) and total nitrogen showed an increasing trend, most of the differences were not statistically significant. Overall, long-term phosphorus fertilization negatively affected soil biological indicators; these results once again demonstrate that balanced, sustainable phosphorus management strategies should be adopted to protect soil health and climate resilience.
Keywords: Mycorrhiza spores, soybean plant, phosphorus fertilization, organic carbon, Long-term experiment

INTRODUCTION

All things in the world are composed of elements, and elemental composition is vital for biology and ecology (Michaels, 2003). Many studies show that soil carbon (C), nitrogen (N), and phosphorus (P) content are interrelated (Walker and Adams, 1958; Melillo et al., 2003; Tian et al., 2010; Işık et al., 2020). These elements are very important because they directly affect the soil's chemical, physical, and biological properties. Soil C and N content is of great importance in controlling atmospheric greenhouse gases. Binding atmospheric carbon in soil, a storage medium, reduces atmospheric CO_2 and increases soil fertility. On the other hand, soil N stability is crucial for increasing productivity, as well as reducing economic inputs from fertilizers and minimizing their negative environmental impacts (Qiu et al., 2016). Fertilization is the most important input parameter affecting yield and quality in plant production (Kılıç and Korkmaz, 2012). Phosphorus (P) is the second most consumed fertilizer element after nitrogen in terms of quantity. Phosphorus plays a role in many metabolic activities in plants, including photosynthesis, energy production, and the synthesis of phospholipids and nucleic acids. It is predicted that apatite rock, the source of the P element, will be depleted within the next 50-150 years (Schnug and De Kok, 2016).

While plant production is insufficient in the absence of P, excess P causes eutrophication (algal growth on the surface). Therefore, the efficiency of P use in agricultural production is of great importance. In this context, the research question of our study is whether the effect of continuous (fixed) P dose application on plant growth is economically viable, as this is not fully known. Determining the impact of increasing phosphorus application rates on soybean crops under long-term field conditions and optimizing phosphorus fertilization are important for farmers across many production systems and for the country's soil and agriculture.

Phosphorus fertilizers remain in the soil in an unavailable form at a rate of 80%, accumulating over time and potentially creating adverse effects, primarily on microbial activity. Phosphorus is taken up by plants in the form of HPO_4^{-2} and $H_2PO_4^-$ (orthophosphate ions), depending on the soil pH. The project topic is the prediction that phosphorus raw material sources, which are increasingly scarce worldwide, may lead to a future phosphorus fertilizer supply problem. In this context, it is crucial to know the amount of phosphorus fertilizer required by plants. However, the effectiveness of phosphorus fertilizers depends on the presence of nitrogen. Meanwhile, in the context of increasing climate change, the carbon sequestration potential of phosphorus fertilizers and knowledge of plant-soil carbon-nitrogen budgets are of ecological and economic importance. In this context, the use of plant species and varieties that bind atmospheric nitrogen is also essential. Mycorrhizal symbiosis is the most common symbiotic relationship known between plant roots and mycorrhizal fungi. Fossil evidence indicates that symbiosis between plant roots and mycorrhizal fungi occurred approximately 400 million years ago (Balemi and Negisho, 2012) and (Egerton-Warburton et al., 2003). Numerous studies have reported that mycorrhizal fungi can increase the host plant's uptake of phosphorus (P), improve growth, and increase water and nutrient uptake. Mostly, the interaction between plants and mycorrhizae is a fundamental relationship that enhances the physical, chemical, and biological properties of rhizosphere soils. The study examined the development of nitrogen-fixing plants such as soybeans under continuous phosphorus application and its effect on the soil carbon-nitrogen budget. The project is among the Green Deal's priority areas in scientific and ecological terms. The primary objective of this study is to determine the effects of long-term increasing phosphorus dose applications on the growth, yield, and nutrient uptake of soybeans. Furthermore, by examining the relationships among phosphorus, soil properties, and mycorrhizal symbiosis, the study aims to contribute to the development of optimal P management strategies. The findings are consistent with similar studies in the literature and provide important information in terms of agricultural productivity and sustainability.

MATERIALS AND METHODS

The study was established in 1998 as a fixed trial in the Arık soil series at the Research and Application Farm, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Çukurova University, and research is still ongoing in the same area. The soil characteristics of the Arık series are presented in Table 1. The soybean crop was planted in June 2023 and harvested in November 2023. The soybean variety (*Glycine max. L*) used was Arısoy was used as a phosphorus fertilizer at rates.

In the study, phosphorus fertilization (Triple Super Phosphate (TSP)) was performed before each production season at 0 kg P2O5 ha-1 (P0), 50 kg P_2O_5 ha^{-1} (P50), 100 kg P_2O_5 ha^{-1} (P100), and 200 kg P_2O_5 ha^{-1} (P200). For soybean plants, 200 kg N ha^{-1} and 50 kg K ha^{-1} were applied as base fertilization in three replications. Soil samples were taken from different soil depths (0-15 and 15-30 cm) and different regions (rhizosphere and non-rhizosphere) at harvest and prepared for analysis.

At harvest, rhizosphere and non-rhizosphere soil samples were collected from the 0-15 and 15-30 cm soil depths. Bulk density samples were also taken. The subsamples were taken to the laboratory for further analysis.

Table 1. Some physicochemical properties of the Arık soil series. *(Dingil et al., 2008)*

Soil Properties	Unit	Results
pH	(sat.)	7.63
EC	(mmhos cm^{-1})	0.06
$CaCO_3$	(%)	27.2
O.M.	(%)	1.17
Texture (%)	Sand	17
	Silt	28
	Clay	55
Texture Class	-	C
P_2O_5	(kg ha^{-1})	71.1

The available phosphorus in the soil was determined using the Olsen method, extracted with this method, and measured at 880 nm using a spectrometer. (Olsen, 1954). The inorganic carbon (IC) content of the soil was determined by measuring %$CaCO_3$ using a digital Scheibler calcimeter (Ülgen and Yurtsever, 1995). Nitrogen (N) and carbon (C) content of soil samples taken from the field were determined using a Fisher-2000 model CN analyser following the dry combustion method (Grant et al., 2001). The organic carbon content was determined by subtracting the inorganic carbon from the total carbon.

OC (Organic Carbon) = TC (Total Carbon) –IC (Inorganic Carbon)

Soil spore counts were analyzed using the wet-sieving method as described by Gerdemann and Nicolson. Mycorrhiza spores were isolated from soil samples by wet sieving (53 µm mesh); following this, the samples were centrifuged with sucrose and decanted as per the technique, then counted with a 25× stereo microscope (Gerdemann and Nicolson, 1963).

Soil analysis data were subjected to statistical analysis using the ANOVA method. The LSD test will be performed using the SAS software package.

RESULTS AND DISCUSSION

Soil Bulk Density

Increasing phosphorus dose levels significantly increased soil bulk density at both depths (0–15 cm: $p<0.0005$; 15–30 cm: $p<0.0002$) (Table 2). The highest soil bulk density at both depths was observed with the P200 application, with values of 1.46 g/cm³ at 0–15 cm and 1.46 g/cm³ at 15–30 cm. Compared to the control treatments, increased P fertilization increased soil bulk density. This result can be attributed to the chemistry of $Ca(PO_4)_2$, which is expected to increase the soil structure formation and compaction. In this concept, Huang et al. (2025) indicated that calcium carbide slag promoted the formation of hydrated calcium silicate, filling pores and improving the mechanical properties of the cured soil, resulting in a denser, more stable structure, as observed under microscopy.

Table 2. Soil bulk densities depend on different phosphorus doses

Treatments	0-15	15-30
P_0	1.29 ±0.05 b	1.31 ±0.04 b
P_{50}	1.43 ±0.03 a	1.45 ±0.03 a
P_{100}	1.45 ±0.01 a	1.47 ±0.01 a
P_{200}	1.46 ±0.01 a	1.46 ±0.01 a
Significant	P<0.0005	P<0.0002

The data represent the meaning of three replicates. ± indicates standard deviations. Letters indicate differences between means in the Tukey test. N.S. = Not statistically significant.

Soil Phosphorus Content

The effect of increasing P application rates on soil extractable P_2O_5 content is shown to have resulted in statistically significant differences ($p<0.0001$) in both soil depths (0-15 and 15-30 cm) and in different zones (rhizosphere and non-rhizosphere) (Figure 1).

The highest values were obtained with the P200 application at all depths and rhizosphere regions. The highest values were obtained as follows: 154.93 for R 0-15, 117.51 for R 15-30, 147.90 for NR 0-15, and 104.53 for NR 15-30. This demonstrates that long-term continuous mineral fertilization increases soil phosphorus content in the short term. Krey et al. (2013) reported that mineral fertilizer application provided more P to the soil than the control treatment. It was also reported that organic fertilizers improve soil quality more than mineral fertilizers over the long term. Furthermore, in our previous work reported by (Işik et al., 2023), increasing P application increased soil P content. In general, A negative correlation (r = -0.5484 to 0.3708) between $CaCO_3$ and phosphorus content in soil at different depths was observed, as expected.

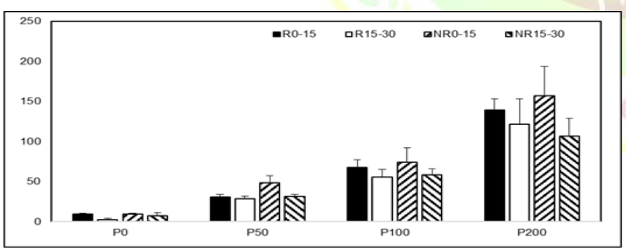

Figure 1. Effect of increasing doses of P applications on soil P_2O_5 content (ton ha^{-1}).

Soil $CaCO_3$ and Inorganic Carbon (IC) Contents

Soil $CaCO_3$ content also indicates the soil's IC content. The effect of increasing P application rates on soil inorganic carbonate content under field trial conditions is shown in Table 4. A statistically significant difference was observed in soil $CaCO_3$ at depths of R 0-15 cm and NR 15-30 cm, but no statistically significant difference was observed. Statistically significant differences were only observed between the averages at depths R 0–15 and NR 0–15 ($p<0.05$). No significant differences were found at other depths. Low $CaCO_3$ content was generally obtained with P0 application at both soil depths and regions. The highest value at the R 0–15 depth was 26.00% with P50; at the R 15–30 depth, it was 26.75% with P100; at the NR 0–15 depth, it was 27.21% with P50; and at the NR 15–30 depth, it was 26.68% with P200. When examining the effect of increasing P application doses on soil IC ratio, a statistically significant difference was observed at the R 0-15 cm and NR 15-30 cm depths, similar to the $CaCO_3$ ratio; however, this difference was not considered meaningful (Table 4). At NR 0–15, a depth difference was found only between applications, which was statistically significant (p=0.0114). The highest value for R 0–15 was obtained in the P50 application (3.12%), while for R 15–30 cm, the highest value was obtained in the P100 application (3.21%). It was concluded that phosphorus applications did not cause a significant change in $CaCO_3$ content overall. This finding can be explained by the fact that the soil's calcium carbonate content is a physical property that does not change in the short term (Özbek et al., 1995).

Table 3. Statistical differences in P_2O_5, $CaCO_3$, IC, and mycorrhizal spore count due to increasing phosphorus application rates at different soil depths (0-15 cm and 15-30 cm) and zones (rhizosphere and non-rhizosphere).

IC R0-15	IC R15-30	IC NR0-15	IC NR15-30	P_2O_5 NR0-15	P_2O_5 NR15-30
*	N.S.	N.S.	*	***	***

The data represent the meaning of three replicates. ± indicates standard deviations. Letters indicate differences between means in the Tukey test. N.S. = Not statistically significant.

According to Table 4, the total carbon content showed a significant difference only at 15–30 cm depth (p=0.0008). No statistically significant difference was found in organic carbon values. Differences were not crucial at other depths and in different regions. The highest carbon concentration at 15–30 cm depth was 4.65% with the P200 application. Differences in the other areas were minor and not statistically supported. Nevertheless, the highest values were frequently recorded with the P200 application, indicating that phosphorus applications may support long-term soil organic matter accumulation.

The differences in organic carbon concentration and total organic carbon were found to be statistically insignificant. Soil samples collected from 0-15 and 15-30 cm depths in the rhizosphere and from areas outside the rhizosphere after harvest were analyzed by dry combustion to determine total soil carbon concentration. After determining the inorganic carbon content from soil IC, soil organic carbon was determined. As shown in Figure 2, increasing phosphorus application increased organic carbon content at 0-15 cm compared with 15-30 cm.

Table 4. Total carbon (TC), inorganic carbon (IC), and organic carbon (%C) concentration in soil depending on different phosphorus doses.

Treatments	R		NR	
	0-15 cm.	15-30 cm.	0-15 cm.	15-30 cm.
	TC (%)			
P_0	4.39 ±0.09	4.21 ±0.12 b	4.29 ±0.10	4.11 ±0.17
P_{50}	4.57 ±0.14	4.55 ±0.22 a	4.43 ±0.19	4.29 ±0.21
P_{100}	4.55 ±0.17	4.32 ±0.11 ab	4.17 ±0.38	4.30 ±0.12
P_{200}	4.45 ±0.28	4.52 ±0.23 ab	4.40 ±0.12	4.13 ±0.30
Significant	N.S.	p<0.05	N.S.	N.S.
	IC (%)			
P_0	3.00 ±0.05	3.04 ±0.17	3.27 ±0.55	3.05 ±0.01
P_{50}	3.21 ±0.15	3.26 ±0.40	3.34 ±0.18	3.28 ±0.20
P_{100}	2.75 ±0.60	2.88 ±0.60	3.14 ±0.12	3.07 ±0.16
P_{200}	2.64 ±0.46	2.73 ±0.44	3.22 ±0.13	3.20 ±0.09
Significant	N.S.	N.S.	N.S.	N.S.
	OC (%)			
P_0	1.38 ±0.15	1.17 ±0.21	1.01 ±0.52	0.98 ±0.08
P_{50}	1.36 ±0.08	1.29 ±0.19	1.08 ±0.29	1.00 ±0.04
P_{100}	1.79 ±0.51	1.44 ±0.63	1.04 ±0.49	1.23 ±0.24
P_{200}	1.81 ±0.29	1.79 ±0.44	1.18 ±0.06	0.92 ±0.31
Significant	N.S.	N.S.	N.S.	N.S.

The data represent the meaning of three replicates. ± indicates standard deviations. Letters indicate differences between means in the Tukey test. N.S. = not statistically significant.

Table 5. Total nitrogen (%N) concentration and N content in soil depending on different phosphorus doses

Treatments	R		NR	
	0-15 cm.	15-30 cm.	0-15 cm.	15-30 cm.
	N (%)			
P_0	0.12 ±0.00	0.11 ±0.00	0.11 ±0.00	0.10 ±0.01
P_{50}	0.12 ±0.00	0.11 ±0.01	0.09 ±0.01	0.09 ±0.00
P_{100}	0.12 ±0.02	0.10 ±0.01	0.10 ±0.01	0.09 ±0.01
P_{200}	0.12 ±0.02	0.12 ±0.01	0.11 ±0.01	0.10 ±0.00
Significant	N.S.	N.S.	N.S.	N.S.
	Mg N ha^{-1}			
P_0	2.11 ±0.15 b	2.08 ±0.16 b	2.09 ±0.02	1.99 ±0.15 b
P_{50}	2.51 ±0.10 ab	2.36 ±0.20 b	2.03 ±0.17	1.91 ±0.06 b
P_{100}	2.61 ±0.37 a	2.30 ±0.19 b	2.18 ±0.14	2.06 ±0.20 ab
P_{200}	2.75 ±0.22 a	3.30 ±0.94 a	2.31 ±0.20	2.31 ±0.10 a
Significant	P<0.0439	P< 0.0492	N.S.	P< 0.0293

The data represent the meaning of three replicates. ± indicates standard deviations. Letters indicate differences between means in the Tukey test. N.S. = not statistically significant.

When examining organic carbon concentration values, P200 yielded 1.54% for R 0-15, 1.46% for R 15-30, 1.33% for NR 0-15 with P0, and 1.23% for NR 15-30 with P100 applications. For total organic carbon values, the highest values for R 0-15, R 15-30, and NR 0-15 regions were obtained in the P200 application as 33.63 Mg OC ha^{-1}, 32.29 Mg OC ha^{-1}, and 25.69 Mg OC ha^{-1}, respectively, and in the NR 15-30 region, it was obtained as 1.23 Mg OC ha^{-1} in the P100 application.

When examining the effect of different phosphorus doses on %N concentration, the differences in mean %N were not statistically significant. When nitrogen concentration values were examined, they were obtained with 0.13% for R 0-15, 0.150% for R 15-30, 0.11% for NR 0-15, and 0.103% for NR 15-30 in the P200 application. When total nitrogen amounts were examined, only in the NR 0-15 region were the differences between the averages found to be statistically insignificant. When total nitrogen amounts were examined, they were obtained in the P200 application as 2.75 Mg N ha^{-1} for R 0-15, 3.30 Mg N ha^{-1} for R 15-30, 2.31 Mg N ha^{-1} for NR 0-15, and 2.31 Mg N ha^{-1} for NR 15-30.

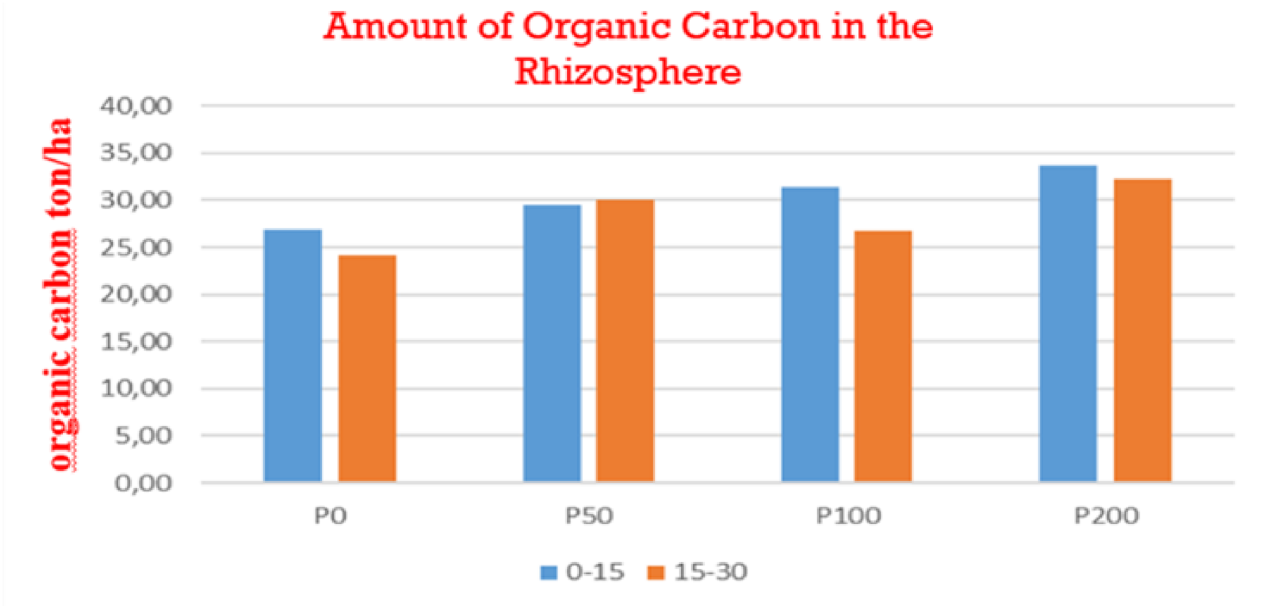

Figure 2. Total organic carbon content in soil after harvest.

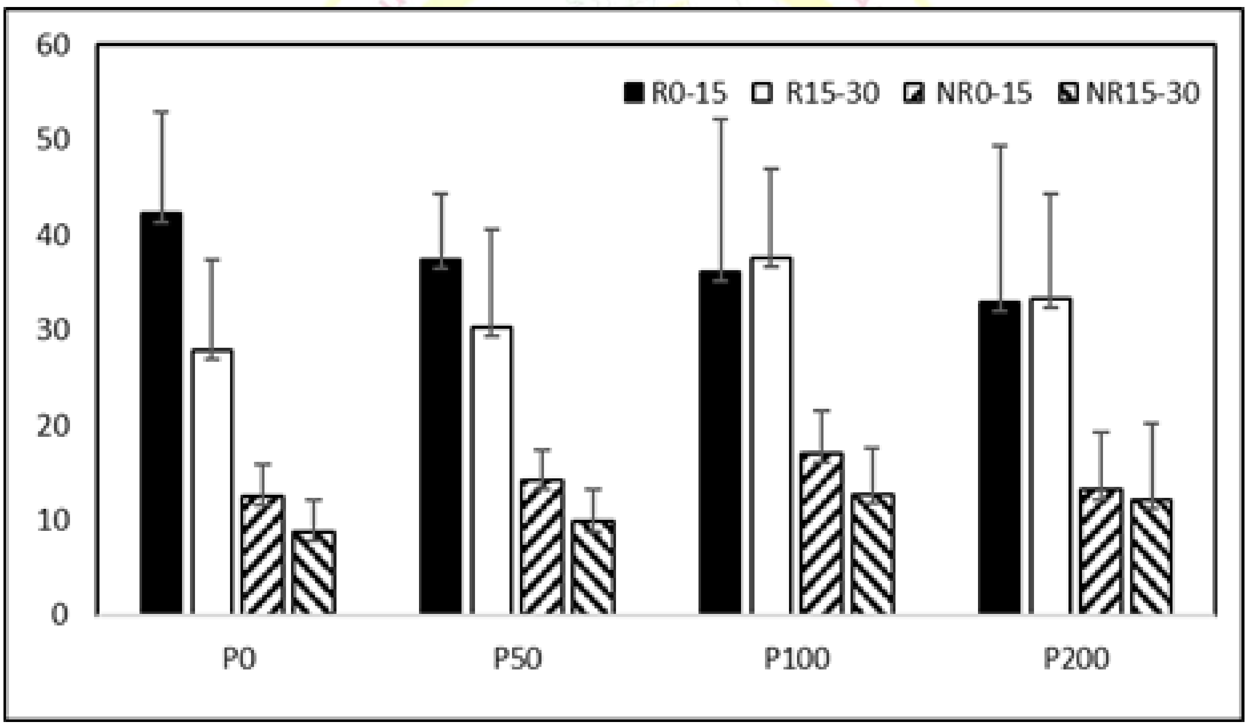

Figure 3. Effect of increasing phosphorus doses at different depths on the number of mycorrhizal spores (10 g^{-1} soil).

Effect of Phosphorus Application on Mycorrhizae Spore Numbers

Figure 3 shows the effect of increasing P application doses on soil mycorrhizal spore counts. Increasing P application rates did not result in a statistically significant difference in spore counts (except for NR at 0-15 cm). The decrease in spore count with increasing P applications led to a decline in mycorrhizal spore counts. There is a negative correlation between them. Furthermore, the extra-rhizosphere region contains fewer spores than the rhizosphere. As is known, mycorrhizae attach to plant roots in the rhizosphere, so it is normal to find fewer mycorrhizal spores on plant roots. This is an expected situation. As is known, mycorrhiza does not function under conditions where P is sufficient. Spore counts are higher in the rhizosphere region compared to the non-rhizosphere region. These results are consistent with the literature, which reports that phosphorus suppresses mycorrhizal symbiosis (Bagyaraj et al., 2015). As is known, when phosphorus is optimal or excessive, the infection efficiency of mycorrhizal fungi decreases and, in some cases, even has a negative effect.

A negative correlation (r = -0.92) at a depth of 0-15 cm shows that mycorrhizal activity is inversely proportional to phosphorus availability. A strong positive correlation was observed between depths (r = 0.75, $p < 0.05$). This finding indicates that phosphorus doses applied to deeper soil layers increase the number of mycorrhizal spores.

When the effects of increasing phosphorus (P) doses on spore counts (spores/10 g soil) at different application and soil depths were evaluated, it was determined that in the R 0-15 application, the spore count decreased from 42 spores/10 g soil at the P0 dose to 37, 36, and 32 spores/10 g soil at the P50, P100, and P200 doses, respectively.

In the R15-30 application, the spore count was measured as 28 spores/10 g soil at the P0 dose, 30 spores/10 g soil at the P50 dose, 38 spores/10 g soil at the P100 dose, and 33 spores/10 g soil at the P200 dose, with the highest value obtained at the P100 dose.

In the NR 0-15 application, spore counts increased from P0 to P100 in the range of 13–17 spores/10 g soil and were recorded as 14 spores/10 g soil at the P200 dose.

Similarly, in the NR15-30 application, the spore count was determined to be 9, 10, 13, and 12 spores/10 g soil at doses P0, P50, P100, and P200, respectively. Spore counts ranged from 9–42 spores/10 g soil, with the P100 dose yielding the highest counts in many applications.

The research findings revealed that, in addition to correlation analysis, principal component analysis was also performed on the data. According to the principal analysis, the research findings indicate that the data are predominantly associated with P50 and P100 applications, at 81.17%. Almost all soil parameters measured are significantly different from the control applications. This supports our hypothesis.

CONCLUSION

Under long-term experimental conditions, increasing P fertilization doses increased soil P_2O_5 and organic C content. P doses did not significantly affect soil IC content and lime content. High P doses (P100 and P200) significantly increased total N and C accumulation, contributing to carbon sequestration. Soil P content, density, and total carbon values increased with P doses. However, no significant change was observed in more stable soil properties such as $CaCO_3$ and IC content.

The number of mycorrhizal spores decreased with increasing P applications; the rhizosphere region, in particular, created a more active environment in terms of mycorrhizal density. Excessive chemical fertilization is not beneficial for soil health and sustainable production and can negatively affect soil physical properties and, to some extent, biological properties. Our research findings indicate that P100 application is optimal for soybean cultivation under the required soil conditions.

CONFLICT OF INTEREST

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

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