Pol. J. Environ. Stud. Vol. 32, No. 3 (2023), 2125-2139
DOI: *10.15244/pjoes/158765* ONLINE PUBLICATION DATE: 2023-02-27

Original Research

The High Phosphorus Incorporation Promotes the Soil Enzymatic Activity, Nutritional Status, and Biomass of the Crop

Babar Iqbal[1,2#], Ismail Khan[1,3#], Qaiser Javed[1]*, Khulood Fahad Alabbosh[4], Inamullah[5], Zhiguo Zhou[2], Abdul Rehman[6]

[1]School of Environment and Safety Engineering, Jiangsu University, P.R. China
[2] College of Agriculture, Nanjing Agricultural University, Nanjing, P.R. China
[3]College of Water Conservancy, Shenyang Agricultural University, Shenyang, China
[4]Department of Biology, College of Science, University of Hail, Hail, Saudi Arabia
[5] Department of Agronomy, The University of Agriculture, Peshawar-Pakistan
[6]Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan

Received: 30 October 2022
Accepted: 28 December 2022

Abstract

Phosphorus (P) application in the soil improves soil fertility and thus contributes to reproductive organ development, resulting in a higher cotton yield. However, the effect of increasing phosphorus rate on soil nutrient status, phosphorus-related enzyme activities, and its effects on crop productivity needs excellent attention. A consecutive two-year (2017-2018) field experiment containing three phosphorus levels [0 (P1), 100 (P2), and 200 (P3) kg P_2O_5 ha^{-1}] was accomplished by maintaining three replications. During both cropping years, soil samples were gathered from the topsoil (0-20 cm) and subsurface (20-40 cm) during the harvesting stage of the cotton crop. Soil collected at topsoil showed a significant increase in available and total phosphorus contents and urease and acid, alkaline, and neutral phosphatase enzyme activities compared with the sub-soil. The soil nutrients, viz. ammonium, nitrate, total nitrogen, available potassium, and total potassium, were significantly higher in P3 at the topsoil compared to other treatments. Soil enzymes viz. urease and acid, alkaline, and neutral phosphatase activities in the P3 were improved compared to the P1 and P2 applications. On average, the activities of these enzymes were maximum in 2017 at both soil depths. Moreover, the maximum biomass accumulation was observed in vegetative (root, stem, and leaves) and reproductive (bur, lint, and seed) organs with P3 incorporation in the soil. Similarly, an increase in the phosphorus application rate significantly enhanced the plant biomass and seed cotton yield during the 2017 and 2018 cropping seasons. Conclusively, the findings of this study showed that the P3 (200 kg P_2O_5 ha^{-1}) rate enhanced cotton productivity by improving the soil physicochemical properties, which alternately enhanced

#The authors equally contributed
*email: 1000005540@ujs.edu.cn

the phosphorus availability to the crop. Thus, changing these properties of the soil resulted in the enhancement of soil enzyme activities by increasing the cotton yield under field conditions.

Keywords: cotton (*Gossypium hirsutum* L.), soil enzymes, soil nutrients; vegetative, reproductive growth

Introduction

Phosphorus (P) is one of the essential plant nutrients, following nitrogen, and it plays a significant role in plant growth and yield production [1]. However, its deficiency at a particular crop stage may drastically affect the plants' overall productivity, including cotton. Plants uptake P from the soil solution at a specific concentration and only a small proportion of organic P is directly obtained from soils [2]. Therefore, the imbalances in the metabolic activities of the crops, choruses, retarded root growth, and overall stunted plant growth resulted in the P deprivation in the soil by diminishing crop productivity [3]. Furthermore, the cotton plant suffers a severe P denervation in saline soil due to low available P contents [4]. Additionally, phosphorus limitation resulted in the inhibition of the photosynthetic activities, which restricts the movement of the nutrients from source to sink and decreases biomass accumulation and yield of cotton [1, 4]. Conversely, the increased P application improved biomass accumulation by increasing leaf area, leaf biomass, and nutrient accumulation in vegetative and reproductive organs [5]. However, increased leaves and stem biomass improved reproductive organs biomass in field-grown cotton [6].

The concentration of P varies with the depth, such as saturation in the topsoil and low concentration in the subsoil [7]. Usually, phosphorus is not leached from the soil like nitrogen, possibly due to its adsorption to the soil surface with reactive minerals [8]. Cotton plant uptakes phosphorus in inorganic form from the soil is comprised of 100-3000 mg P kg^{-1}, mainly including 30-65% organic forms [9]. However, its availability mainly depends on the soil's temperature, moisture availability, and physicochemical properties [10]. Additionally, many factors, such as planting density, vegetation types, and climatic conditions, affect the activities of phosphatases activities in the soil [11]. Previous studies showed that soil erosion, agricultural practices, and cropping considerably affect phosphatase enzyme activities [12]. Therefore, understanding the phosphorus cycle and its interaction with other factors is a primary source for implementing soil for life, microorganism function, and plant growth. Moreover, the soil quality and environmental constancy depend on the enzyme's activities and alternately affect soil fertility status [13]. Soil enzymes are considered an excellent indicator to change in biological status because of their quick reaction [14]. However, the urease and phosphatase activities are influenced by soil depth [15, 16]. Additionally, these enzymes act as biocatalysts, and several soil biochemical processes are directly or indirectly influenced by these enzymes [16-18]. Furthermore, soil enzyme activities modify soil quality and represent integrated nutrient transformation and soil fertility status [19]. Despite this, soil enzyme activities are influenced by microorganisms having large biomass, high metabolic activity, and short lifespan under favorable environmental conditions [14, 17]. The phosphorus deficit conditions lead the plant roots, fungi, and other microorganisms to promote extracellular phosphatase activity [4, 20].

Cotton (*Gossypium hirsutum* L.) is a significant fiber crop that is produced all over the world. Many countries like China, India, and Pakistan grow cotton as a major cash crop [22]. Cotton has recently grown on nutrient-deficit soils, adversely affecting yield and economic returns [23]. Moreover, the competition of food crops with cotton is another big challenge to expanding its cultivation on a large scale. This adversely affects productivity and farm income [1, 24, 25]. Furthermore, poor fertility conditions of soil also contribute to the challenges of field management and targeted yield [26]. To ensure good yield and quality, field management practice, especially fertilizer allocation, plays a pivotal role in low-fertility soils.

Soil covers the earth's surface, which supports plant growth and helps increase crop productivity [27]. Most of the nutrients are in soil structure, where these nutrients may be released for plant use [28]. These attributes directly or indirectly influence plant growth, including cotton. Modern agriculture is associated with the heavy application of inorganic fertilizers to achieve a high targeted yield. Moreover, resources are required to support intensive farming, which flourishes and nourishes the environment and agroecosystem. However, the excessive use of inorganic fertilizers not only enhances the production cost but also has detrimental effects on the soil ecosystem. Several previous studies focused on the physicochemical properties of soil and P released concerning enzymatic activity. Still, the information on the impact of P on soil attributes and its direct effect on vegetative and reproductive organ development by promoting biomass and seed cotton yield is lacking. However, what is the best P level and its effect on soil nutritional status and enzyme activities at different soil depths, and cotton yield is focused on in this study. The objective of our study was to find out 1) the best phosphorus level for the enhancement of soil enzymatic activities, which increased the nutritional status of the crop; 2) to find out the best phosphorus level for the efficient nutritional management of the crop, which promoted the vegetative organ and reproductive organ development and resulted in higher seed cotton yield.

Materials and Methods

Experimental Site and Conditions

During 2017 and 2018, the experiment was carried out at the Pailou Experimental Station (118°50'E, 32°02'N), Nanjing Agricultural University, Nanjing, Jiangsu, P. R. China. The exact property of the experimental field soil was clay, mixed, thermic, typical Alfisols (Udalfs; FAO Luvisol), and acidic in nature. Moreover, the pre-planting soil physicochemical properties have also presented in Table 1 [1]. The mean monthly air temperature and rainfall data were collected from Nanjing meteorological station and represented in Fig. 1.

Experiment Site and Conditions

Randomized Complete Block Design (RCBD) was used to conduct the experiment consisting of three replications by maintaining three P levels, i.e., (0, 100, and 200 kg P_2O_5 ha^{-1}) during the 2017 and 2018 cropping seasons. The experimental plot area was maintained at 56 m^2 (8.0 × 7 m) with six cotton rows having 76 cm row-row and 15 cm plant-plant distance. Cotton cultivar Lu-54 was grown (as a nursery) in the mid of April, which attained 2 to 3 true leaves stage in a maximum of 30-35 growing days. These seedlings were transplanted to the field on the 20th of May in 2017 and 2018. Fertilizers such as nitrogen, phosphorus, and potassium were applied each at the rate of 225 kg ha^{-1} in the form of urea (46% N), triple superphosphate (44% P_2O_5),

Table 1. Effect of P application rates on cotton biomass of root, stem, leaf, and root/shoot ratio during 2017 and 2018 growing seasons.

Phosphorus level	Root biomass (kg ha^{-1})	Stem biomass (kg ha^{-1})	Root/Shoot ratio	Leaf biomass (kg ha^{-1})
2017				
P_1	655.2 d	955.9c	0.7bc	2081.7 cd
P_2	774.3 c	1289.3b	0.6c	2662.2 b
P_3	1006.5 a	1639.7a	0.6c	3333.3 a
Mean	812.0	1295.0	0.6	2692.3
2018				
P_1	759.0 c	685.3d	1.1a	1483.5 e
P_2	851.1 b	946.0c	0.9ab	1869.5 d
P_3	1056.0 a	1317.3b	0.8bc	2292.2 c
Mean	888.7	982.9	1.0	1881.8

According to the LSD test, means with the alphabet in the same column are statistically significant. P1, P2 and P3 represent the P level, i.e., 0, 100, and200 kg P_2O_5 ha^{-1}.

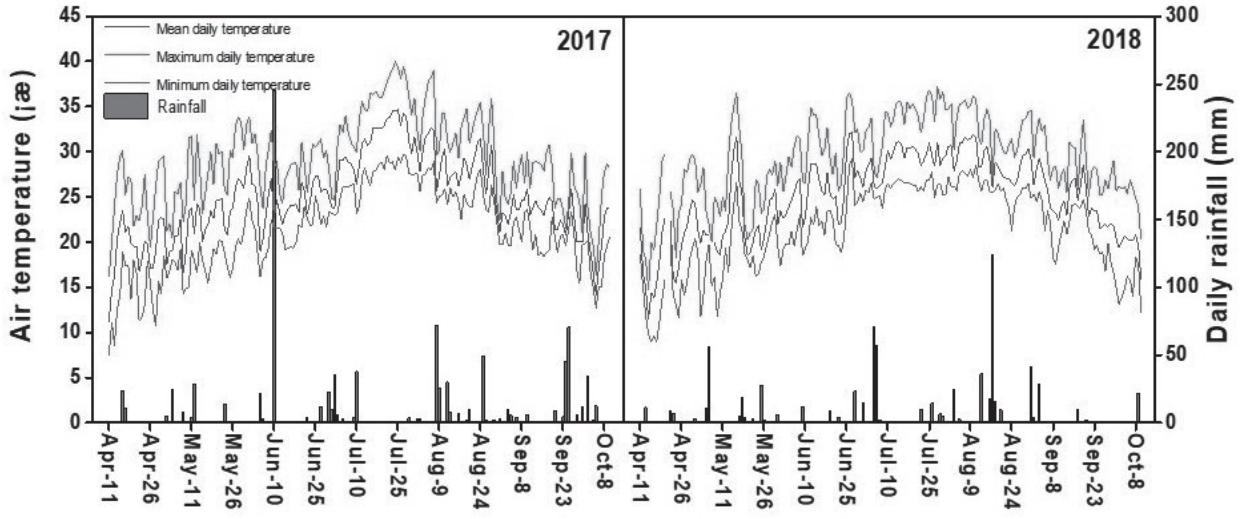

Fig. 1. Meteorological data regarding air temperature and rainfall was presented during each month of the growing crop during the 2017 and 2018 cropping seasons.

and potassium chloride (59% K_2O). The P and K fertilizers were applied as basal placement when transplanting. At the same time, nitrogen was applied in 4 different splits, such as 20%, 25%, 40%, and 15% at transplanting as basal dose, flower initiation stage, bloom stage, and end of the flowering stage, respectively. Other agronomic management measures were used following local agricultural practices.

Seed Cotton Yield Measurement

Seed cotton was picked manually in three pickings by maintaining ten randomly selected plants from each treatment plot with a ±3-day difference during 2017 and 2018 growing seasons. The final yield was obtained by adding all three pickings of seed cotton and presented in kg ha^{-1}.

Plant Biomass Determination

Cotton plants were selected from the field containing three replications and further divided into different plant parts, i.e., root, stem, leaves, and reproductive parts. The reproductive portion was further divided into bur, lint, and seed. All the plant parts were oven dried at 80ºC to maintain a constant weight. The biomass of each part was weighed and expressed in kg ha^{-1} of plant weight.

Soil Sampling and Processing

Soil samples were obtained during the boll opening stage at two distinct depths, i.e., topsoil (0-20 cm) and subsoil (20-40 cm), using an auger [1, 25, 29]. The collected samples were sealed, put in the ice box, and immediately shifted to the laboratory for further analysis. Each sample was divided into two equal parts, of which one was stored at -20ºC and the rest half was air dried and stored in a dark and dry place. Fresh samples were passed through a <2-mm sieve after grinding to a fine powder. Fresh samples were used for ammonium (NH_4^+-N), nitrate (NO_3^--N), and soil enzymes (Urease, acid, alkaline, and neutral phosphatase). While dried soil was used to determine available phosphorus and potassium and total nitrogen, phosphorus, and potassium contents.

Soil Snalysis and Determinations

Soil Nutrients

Available N in soil was determined by the procedure described by Ameur et al. (2018) [30]. Fresh soil of ten grams in 50 mL flask, added 1 mol L^{-1} KCl solution, shake for 1 hr, and filtered. For ammonium (NH_4^+-N) and nitrate (NO_3^--N), the extract was analyzed calorimetrically, and for nitrate via the Griess-Ilosvay technique and analyzed using a flow-injection technique [31].

Available soil P content was determined using the procedure described by [30]. The $NaHCO_3$ extraction method was used to determine available P and further analyzed with a spectrophotometer (UV2550, Shimadzu, Japan) by the procedure described by [31]. However, the previously described method determined the available K content [32]. The methods of [32] determined the soil total P and K.

Using the Kjeldahl technique, total soil N was measured with the Foss Kjeltec analyzer. The soil sample was weighed, put in the tube, and treated with H_2SO_4-H_2O_2 at 350ºC by adding pure water for 15-20 minutes. Then put out all the samples and filter. Added 5 mL filtrate to 50 mL flask. Added 20 mL water and 2-3 drops of DNT to adjust its color. A sodium carbonate solution was added to change the color. When the color disappeared, H_2SO_4 solution was added to achieve its color. In the end, added 5 mL of ascorbic acid solution and waited for 30 minutes. Finally saved, the solution to determine total N as pronounced by [30, 31].

Total P was measured by adding 5 ml of the filtrate to a 50 mL flask. Added 20mL water and 2 to 3 drops of 2-4 dinitrophenol (DNT) solution to adjust its color. Added 2-to 3 drops of sodium carbonate solution to change its color (dark yellow to light yellow), and when the color disappeared, H_2SO_4 solution was added to achieve its color. In the end, added 5mL of ascorbic acid solution and kept it for 30 minutes until its color changed blue. The absorbance was read at 700 nm. In a 50 mL flask, 5 mL soil solution (filtrate) was taken to determine the total K, and the volume was adjusted by adding water. The sample was read using an atomic absorption flame photometer (PerkinElmer Atomic Absorption Photometer Pin AAcle 900T). The final contents were calculated by plotting the data against standard curves.

Enzymatic Activity Measurement

Soil urease assay was determined by the procedure pronounced by [34]. Briefly, add a five-gram sample of fine soil in a flask, 1 mL toluene, and 10 mL of 0.1 M citrate buffer (pH 6.7). Samples were kept in an incubator at 38ºC for one hr. to allow the soil temperature to equilibrate. Then filtered, the soil solution was through filter paper and kept the filtrate for further analysis. Treated 1mL supernatant of soil with 3 mL sodium hypochlorite solution and 4 mL sodium phenol solution. The samples were kept at room temperature for 20 minutes. The released ammonium was measured at 578 nm with a spectrophotometer. Urease assay was expressed as µg NH_4^+-N released per g oven-dried soil during an hour incubation at 38ºC.

The acid phosphatase activity in the fresh soil samples was determined by the method described by the previous researchers [17, 18]. The acid phosphatase was assayed by adding 1 g fresh soil, 0.25 mL methylbenzene, 4 mL universal buffer, and 1 mL

p-nitrophenyl phosphate solution and incubating the mixture for 1hr at 37ºC. Later, added 4 mL of calcium chloride solution (0.5 mol L^{-1}) and sodium hydroxide (0.5 mol L^{-1}) solutions sequentially. The mixture was shaken and filtered. Each sample also contained a blank sample in which 4mL calcium chloride, 4 mL sodium hydroxide, and modified universal buffer were added, and then the sample was filtered; the released p-Nitrophenol was read at 400nm using a spectrophotometer [17, 18].

Similarly, the neutral and alkaline phosphatase activities were also determined by the methods described in the previous experiments conducted by [34]. For the further experiment, four g of air-dried soil was collected in a 40 mL tube. Added and homogeneously mixed the soil with 0.8 mL methylbenzene (toluene). Then added, 4mL phenyl phosphate and 4mL relevant buffer solution (Neutral phosphatase and alkaline phosphatase). At the same time, set control for every soil sample by replacing 4 mL phenyl phosphate with 4mL pure water. Tubes containing treated and control samples were covered because toluene is poisonous. The samples were incubated for one day at 37ºC. After incubation, added 40 mL of water was shaken and filtered. Kept the filtrate for enzyme assay. Added 1mL filtrate to 10mL tubes with 1mL borate saline buffer having pH 9.0 each. Borate saline buffer mixing 80mL of 0.05 M borax solution with 20 mL of 0.2 M boric acid solution and shake. Then added to the 10 mL tubes 0.1 mL 2.5% potassium ferricyanide, 0.5% 4-Aminoantipyrine, and 7.8 mL water to get a 10 mL solution. The solution was shaken and then waited 30 minutes. Lastly, measured its optical density (OD) was at 57 0nm, and the results of the neutral and alkaline phosphatase assays were represented as mg p-nitrophenol g^{-1} d^{-1}.

Data Analysis

The statistical tool sigma plot 12.5 was used to analyze variance in all data (ANOVA). Data are shown as means ($n = 3$) of the represented treatments. Treatment means were separated by statistical differences using an LSD t-test at the (P<0.05) level. Origin 9.1, Sigmaplot 12.5, and R 4.1.1 software were used to draw the graphs.

Results and Discussion

Seed Cotton Yield

The P levels significantly (P<0.05) affected the seed cotton yield at both growing seasons; however, the effect of year on seed cotton yield was statistically insignificant. Mean data during the two years, i.e., 2017 and 2018 cropping seasons, showed a substantial increase in seed cotton yield by 23.9-30.8% in the P2 treatment while by 34.5-52.3% in the P3 treatment as compared to P1 treatment during 2017 and 2018, respectively [1]. Phosphorus is a multifunctional plant macronutrient used to boost crop development and output; however, the reduced supply in the P content resulted in the crop stunted growth and decreasing yield [1, 25, 34]. The P treatment significantly impacted the soil's nutritional state and the accessibility of vital nutrients to cotton plants. The most excellent way to mitigate the problem of dwindling resources is to create viable technologies that increase P-use efficiencies to accommodate plant requirements. The essential component in improving soil nutritional status and increasing cotton yield is management practice, namely more significant P fertilization to the crop. Therefore, the main focus in the present study was the application of adequate P rates in the soil, which enhanced the soil's nutritional status and how it mitigates the biogeochemical soil cycle and impacts the vegetative, reproductive organ biomass, and final yield of the cotton crop. Cotton yield was higher under a high P rate and inclined by the increased P rate [28]. This increment in cotton yield was promoted due to high P uptake from the soil compared to control plots. The higher P uptake was also associated with cotton's higher vegetative and reproductive organ biomass. The soil with increased P binding is essential to the crops since P distribution to the plants consumes more significant resources than P production in crop growth and development [34,35], which results in poor agricultural P-use efficiency. However, ideal fertilizer management favors crop growth and yield and helps improve soil health. In a good-conditioned soil-plant system, roots mainly uptake nutrients from different depths, supporting a well-organized nutrient supply to the areal parts of the crops.

Plant Biomass

Table 1. represents the data concerning the biomass accumulation of cotton (root, stem, root/shoot ratio, and leaves) affected by different P levels and years. The accumulated biomass in the crop was significantly (P<0.05) affected by P levels and year. Mean data during the two years, i.e., 2017 and 2018, showed an increase in biomass accumulation by 65.1-71.9% and 84.9-89.2% in the root, 58.3-52.0% and 74.1-72.4% in the stem, and 62.5-64.7% and 78.2-79.4% in leaves in P3 and P2 treatment compared to P1, during 2017 and 2018, respectively. The root/shoot ratio was decreased with the increasing P level. The accumulation of biomass in cotton boll (bur, lint, and seed) was affected by different P levels and years are presented in Table 2. The increases in biomass of bur, lint, and seed were 63.5-65.1% and 80.2-78.1%, 71.0-65.2% and 76.1-78.4%, and 71.5-69.0% and 75.4-73.1% in P3 and P2 treatment compared with P1 during 2017 and 2018, respectively. P application improved the biomass of bur, lint, and seed and additionally increased the proportion of bur and ratio of lint to seed; however, it decreased the proportion of seed biomass in both the cropping seasons. In 2017 relatively higher biomass accumulation

Table 2. Effect of phosphorus application rates on the biomass allocation in cotton's reproductive parts (Bur, lint, and seed) during 2017 and 2018 growing seasons.

Phosphorus level	Boll biomass (kg ha^{-1})			Proportion (%)			
	Bur	Lint	Seed	Bur	Lint	Seed	Lint/seed ratio
2017							
P_1	1343.7 c	1328.6 c	1773.6 c	30.2	29.8	40.1	74.3
P_2	1676.4 b	1743.5 b	2353.1 b	29.0	30.2	40.8	74.0
P_3	2114.1 a	1870.1 a	2480.1 a	32.7	28.9	38.4	75.2
Mean	1711.4	1647.4	2202.3	30.6	29.6	39.8	74.5
2018							
P_1	1653.6 c	1416.7 c	1890.2 c	33.3	28.6	38.1	75.0
P_2	2120.4 b	1807.9 b	2591.7 b	32.5	27.7	39.8	69.6
P_3	2545.8 a	2172.4 a	2739.0 a	34.1	29.1	36.7	79.3
Mean	2106.6	1799.0	2407.0	33.3	28.5	38.2	74.6

Means showing different letters within the same column are statistically significant according to LSD test. Whereas P1 = 0, P2 = 100, and P3 = 200 kg P_2O_5 ha^{-1}.

was observed in the crop stem and leaves. In 2018, a significant increase was recorded in root biomass, root/shoot ratio, bur, lint, and seed cotton yield. The root is the central part of the plant and plays a critical role in absorbing several nutrients from the soil and supply to the other parts of the plant. Most plants showed higher flexibility in root growth development in response to P supply from the soil [6]. The accumulation of biomass in cotton was strongly affected by the nutrient supply and soil nutritional status. The current study resulted in a strong P application impact on the biomass of vegetative organs (root, stem, and leaf) (Table 1) which might be due to the better soil nutritional status, root structure, and environmental condition. However, the biomass accumulation of root plants is mainly controlled by the environment and soil characteristics [36]. Besides the soil's nutritional status, biomass accumulation is strongly affected by the deficit nutrient supply to the root system [1, 34]. Therefore, P uptake is mainly concerned with the biogeochemical cycle in the soil and crop growth [28]. Cotton yield, biomass, and accumulation increased with adding P compared to P deficit conditions. Overall, biomass accumulation occurred due to nutrient supply and improved with a higher P application rate. Dry matter production is mainly affected by the amount of fertilizer and its placement depths [25, 34]. Our results indicated that P3 enhanced the dry matter accumulation of cotton's vegetative and reproductive organs and significantly promoted the aboveground biomass (root, stem, and leaf), which strongly interacts with the reproductive organs (bur, lint, and seed), as shown in table 1 and 2, respectively. Thus, the changes in the soil nutrient profiles and enzymatic activities mainly impact the nutrient supply to the crops by increasing dry matter accumulation and partitioning to the reproductive organs under appropriate fertilizer application [34]. However, this biomass accumulation was recorded higher in the reproductive organs, followed by the vegetative organ biomass, i.e., leaves and stem [1, 26, 34].

Soil Properties

Soil Available Nitrogen, Phosphorus, and Potassium

Phosphorus application rate exerted a significant effect on soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) at all soil depths in 2017 and 2018 (Fig. 2). The NH_4^+-N was a 12.5-7.1% increase in the P3 and 7.2-5.4% in the P2 at topsoil while 12.8-8.3% increase in the P3 and 6.2-5.2% increase in the P2 at subsoil as compared to control plots (P1) during both cropping seasons, respectively. Overall, the increased P application rates drastically affected the level of NH_4^+-N in topsoil and subsoil in all tested samples. However, the years 2017 and 2018 did not vary significantly either at P level or soil depth (Fig. 2a). Similarly, NO_3^--N content was 67.9-21.9% and 29.2-14.3% increase in P3 and P2 at topsoil, while 73.9-29.2% and 36.2-15.0% increase in the P3 and P2 treatment at subsoil compared to control, during 2017 and 2018, respectively. Increased soil depth decreased the soil NO_3^--N in all samples and in cropping years (Fig. 2b).

Soil depth and available phosphorus (P) content were observed to be inversely proportional. Fig. 2c) shows variation in the available P in the soil at different soil depths during 2017 and 2018. In the treatment, P3 rather than P2 accumulated more available P in topsoil as compared to the control. However, in year comparison,

P3 application resulted in a 29.8-23.9% increase in available P in the topsoil while a 24.7-19.9% increase in subsoil compared to P2 and P1 at different soil depths during 2017 and 2018, respectively. The topsoil resulted in higher available content compared to subsoil samples. The available P content was higher in 2018 as compared to 2017. The available K contents at different P levels and soil depths were presented in Fig. 2d during both cropping seasons. The effect of P levels on the available K was significantly affected ($P<0.05$) at different soil depths. Available K at various soil depths increased by increasing P level and soil depth. Available K perceived was 17.1-15.7% in the P3 and 8.5-7.8% in the P2 at topsoil while 17.2-17.5% and 9.7-10.0% increase in the P3 and P2 at subsoil as compared to control (P1) during 2017 and 2018, respectively. The topsoil recorded higher available K content compared to subsoil samples. In 2017, the available K content was recorded as lower compared to 2018. Phosphorus fertilization level led to significant changes in the nutrient profile of NH_4^+-N, NO_3^--N, available P, and K in the soil (Fig. 2). It was reported that nutrient availability and uptake was strongly affected by the root in the soil [38]. These interactions strongly supported the uptake of total P

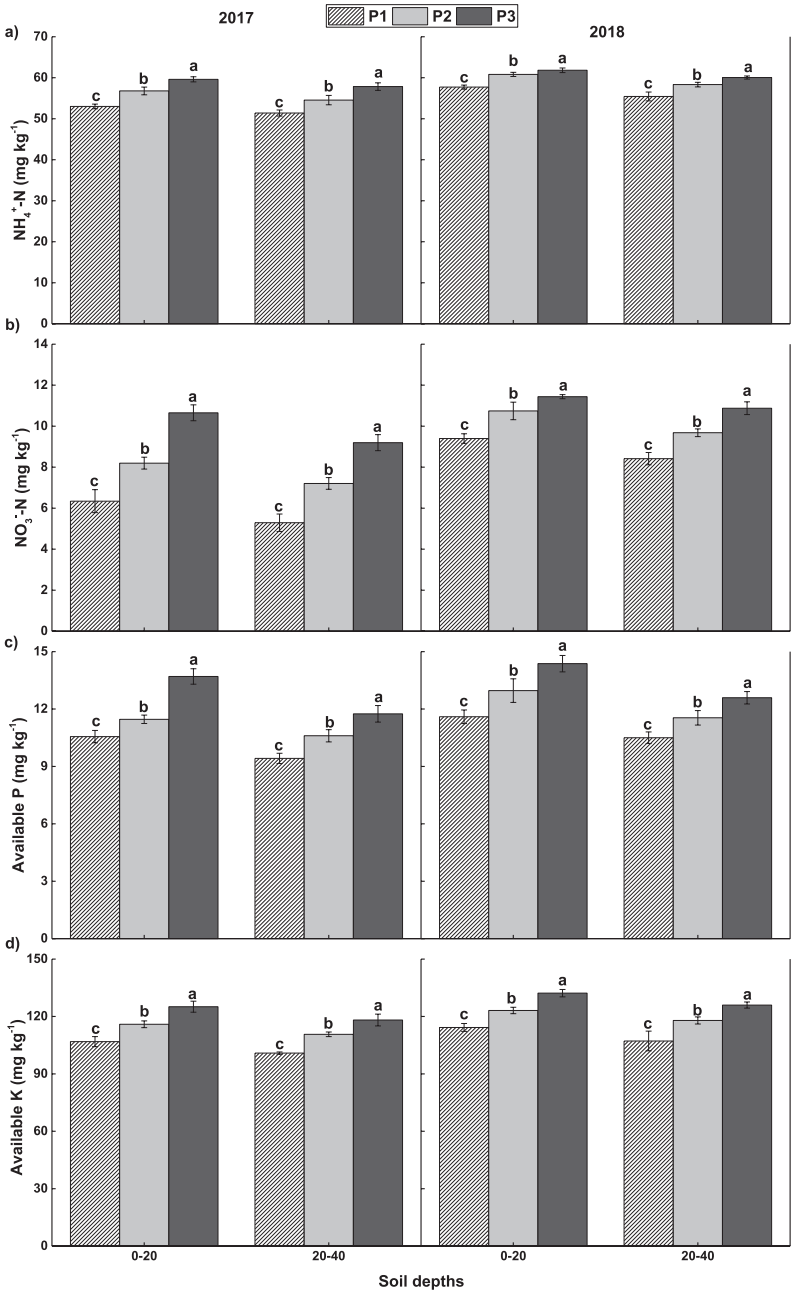

Fig. 2. The P application rates on a) NH_4^+-N, b) NO_3^--N, c) available P, and d) available K contents in the topsoil (0-20 cm) and subsoil (20-40 cm) during cotton production in both 2017 and 2018 growing seasons. The bars represent different P levels and indicate standard errors of the mean ($n = 3$), while different letters on the columns represent the statistical difference at $P<0.05$. Whereas; P1, P2, and P3 indicate the P levels i.e., 0, 100, and 200 kg P_2O_5 ha^{-1}.

from the soil to the plants [1, 25]. In the current study, P treatment significantly influenced the amount of N, P, and K in the soil, resulting in a significant increase in the biomass of cotton vegetative and reproductive organs (Table. 1, 2). A strong relationship was recorded between P application rates and N and K accumulation in the soil and plants. Hence, it is in the more excellent support of increased crop yield [1]. Similarly, previous results indicated that the addition of inorganic fertilizers increased the nutrients profiles in the soil as compared to the control [26,37], which increased net ammonification (NH_4^+-N), net nitrification (NO_3^--N) rate, available P and K, hence, readily available to the plants which supported the higher plant biomass and enhancing the yield of the crop. The present study resulted in a higher availability of nutrients on the surface soil compared to the subsoil. The plants' higher uptake, increased the biomass and yield [22, 23]. P availability is a critical limiting factor; therefore, adding P fertilizer to cotton plants improved the soil available P content due to high water content, reducing the P available form. Contrary to other nutrients, P is quickly adsorbed by soils, so plants consume P compounds appearing in the soil upon interacting with P fertilizers [37, 38]. However, applying more P rates resulted in the accumulation of higher available P, which increased the crop's biomass and yield [39]. The present study revealed that the available K in soil decreased with the increase of soil depth in cotton (Fig. 2). With the increased P concentration in soil, available K alternately increased, and surface soil had more K contents compared to the sub-surface of soil. This surface soil K promotes crop growth and increases the biomass in the plant organs. [34, 37] vigorous plants resulted in a higher K absorption with

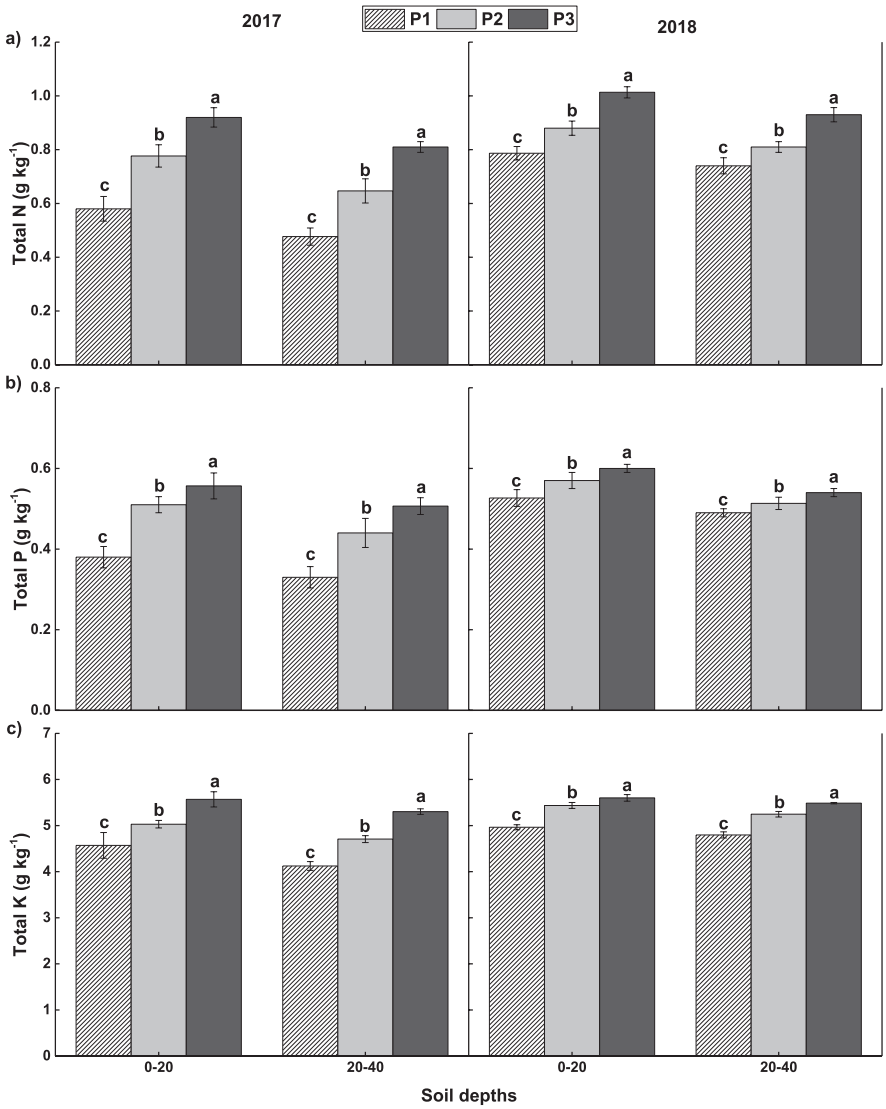

Fig. 3. Effect of P application on total a) nitrogen, b) phosphorus and c) potassium content in the topsoil (0-20 cm) and subsoil (20-40 cm) during cotton production during both the 2017 and 2018 growing seasons. The bars represent different P levels and indicate standard errors of mean *(n* = 3), while different letters on the columns represent the statistical difference at $P<0.05$. Whereas; P1, P2, and P3 indicate the P levels, i.e., 0, 100, and 200 kg P_2O_5 ha^{-1}.

more P content and positively correlated with the seed cotton yield. The increased concentration of available K in topsoil resulted in higher uptake of plants [40]. Similarly, the P rate also influences the availability of K in the soil [40]. Our results confirmed that higher nutrient content in the topsoil layer than the subsoil layer influenced the available K in the soil, promoting biomass and yield in cotton crops.

Soil Total Nitrogen, Phosphorus, and Potassium

The contents of total nitrogen (N) in the soil were significantly increased by applying phosphorus levels at the two depths (Fig. 3a). Increased P levels increased the contents of total N in the soil. Lower total N was recorded in the control (P1) plot in both depths. Higher total N content recorded was 58.6-28.8% and 33.9-11.9% in the P3 and P2 treatment at topsoil, while 69.9-25.7% and 35.7-9.5% at subsoil compared to control plots, during both cropping seasons. Higher P application recorded higher total N content in soil; the trend was P3>P2>P1. Higher total N content was found in the soil with P2 and P3 treatments compared to control as soil depth increased. In 2017, the recorded total N in soil was lower compared to 2018 in cotton production.

Total phosphorus (P) in soil was directly proportional to the increase in P level. Soil total P content in the two soil layers (Fig. 3b) and available P content was similar. The P3 exhibited higher total P content in topsoil, followed by P1 and P2 treatments. The P3 level resulted in a higher 46.5-13.9% and 34.2-8.2% increase in the P3 and P2 treatment at topsoil, while 53.5-10.2% and 33.3-4.8% increase occurred at subsoil as compared to control plot during 2017 and 2018, respectively. Like the total P, total K content also decreased with the increase in soil depth; however, increasing P application increased the soil K contents irrespective of year. Fig. 3c) presented the effect of P levels at different soil depths on total soil K. During 2017 and 2018, 21.9-12.8% and 10.1-9.5% increases occurred at P3 and P2 in the topsoil while 28.6-14.4% and 14.1-9.4% increase occurred in the subsoil as compared to control plot, respectively. Treatment P1 showed a lower content of total K in the subsoil. In 2017 the total K in topsoil and subsoil was recorded higher than in 2018. Total N in the soil increased with the application of P due to the formation of soil aggregates [41]. In the present study, a higher total N in the topsoil resulted in the higher interaction of root microorganisms with decreased uptake of N compared with the P [42]. However, these increased N availabilities in the soil is due to the immobilization of N by soil microorganisms [34]. Thus, it might be concluded that the P application influenced N uptake by the root. Additionally, total P in the surface soil increased due to less mineralization of P in the cotton crop. In the current study, P3 treatment exhibited a higher total P, probably because of P accumulation in the top layer of soil with the slow P mineralization and less utilization [43]. Topsoil had a greater total K compared with the subsoil (20-40cm) layer (Fig. 3). The availability and application of P results in the enhancement of total K contents in the surface soil [42]. Increased application of inorganic fertilizers caused P immobilization in the soil. Still, the availability of water content in the surface soil absorbed these nutrients where plants might get more nutrients compared to the sub-surface of soil. The water contents, organic matter, growth stages, and P rates are interrelated directly to each other in cotton production [25, 34, 36].

Soil Enzymes Activities

The activities of urease and phosphatases (Acid, alkaline, and neutral) significantly increased with increasing P rate in both soil depths. Fig. 4a) presented the soil urease of topsoil and subsoil. The P levels and soil depth considerably affected ($P<0.05$) soil urease activity in 2017 and 2018. Statistically, higher urease activity was observed in the P3 than in the P2 and P1 treatments. In 2017 and 2018, higher urease activities of 173.64 and 160.53 μg g^{-1} h^{-1} were observed in the topsoil layer in P3 rate, while lower 90.77 and 82.88 μg g^{-1} h^{-1} were recorded at the subsoil layer in P1 treatment. In the cropping years 2017 and 2018, in comparison to P2 and P1 treatments, higher acid phosphatase activity of 22.1 and 23.48 mg P-Nitrophenol g^{-1} h^{-1}, 37.61 and 33.54 mg P-Nitrophenol g^{-1} h^{-1} alkaline phosphatase and 43.54 and 35.54 mg P-Nitrophenol g^{-1} h^{-1} neutral phosphatase, respectively, were observed in topsoil in the P3 treatment, respectively. Lower acid, alkaline, and neutral phosphatase activity were observed in the subsoil in the P1 treatment. In 2017 the recorded acid and alkaline phosphatase activity were lower compared to 2018 (Fig. 4b, c), while neutral phosphatase activity was vice versa (Fig. 4d). It was found that P fertilizer application was more effective in elevating the soil enzyme activities in the topsoil compared to the subsoil (Fig. 4). A similar phenomenon existed in the soil, which was mainly concerned due to the more excellent supply of P [37]. However, the available P increased in the soil with the addition of more P from fertilizer application (Fig. 2c) which might be due to the higher activities of phosphatase enzymes in the soil. In stressed and polluted environments, enzymes can determine and notice soil quality [34]. However, the soil depth and nutrient contents strongly affected the urease activity and contents [31]. Soil urease activity was also influenced by the time when urease activity was noticed more in May-September than in April [30]. The urease enzyme reduced the urea present in the soil [34]; however, our study noticed a slightly different activity of this enzyme activity. The higher P application improved the urease and phosphatase (acid, alkaline, and neutral) activities in the 0-20cm and 20-40cm soil depths. However, an increase was observed in the topsoil than in the subsoil. The possible reason might be the availability of more P that ultimately supported

microbes and plant roots with good nutritional conditions. Similarly, the inflation of the microbe's activity with the addition of P fertilizer also resulted in the fluctuation of these enzymes' activities [25, 30, 34, 42]. The other reason behind this phenomenon might be the plant root system, mainly dispersed in the topsoil of 0-30cm [28]. Soil urease and phosphatase activities were observed as directly related, and it concluded that the total soil NP contents increased due to N and P transformations [18,20]. These findings concluded that P limitation inhibits the activities of the enzymes, which alternately influence the nutrients contents in the soil and diminish its movement inside the crop body by decreasing the seed cotton yield.

Relationship between Soil Nutrients Contents and Enzymes Activities

The enzyme activity was shown to have a positive linear relationship with the available P concentration in the soil. The slopes of fitted lines regarding the urease activity (r^2 = 0.9286-0.8957), acid (r^2 = 0.7325-0.9219),

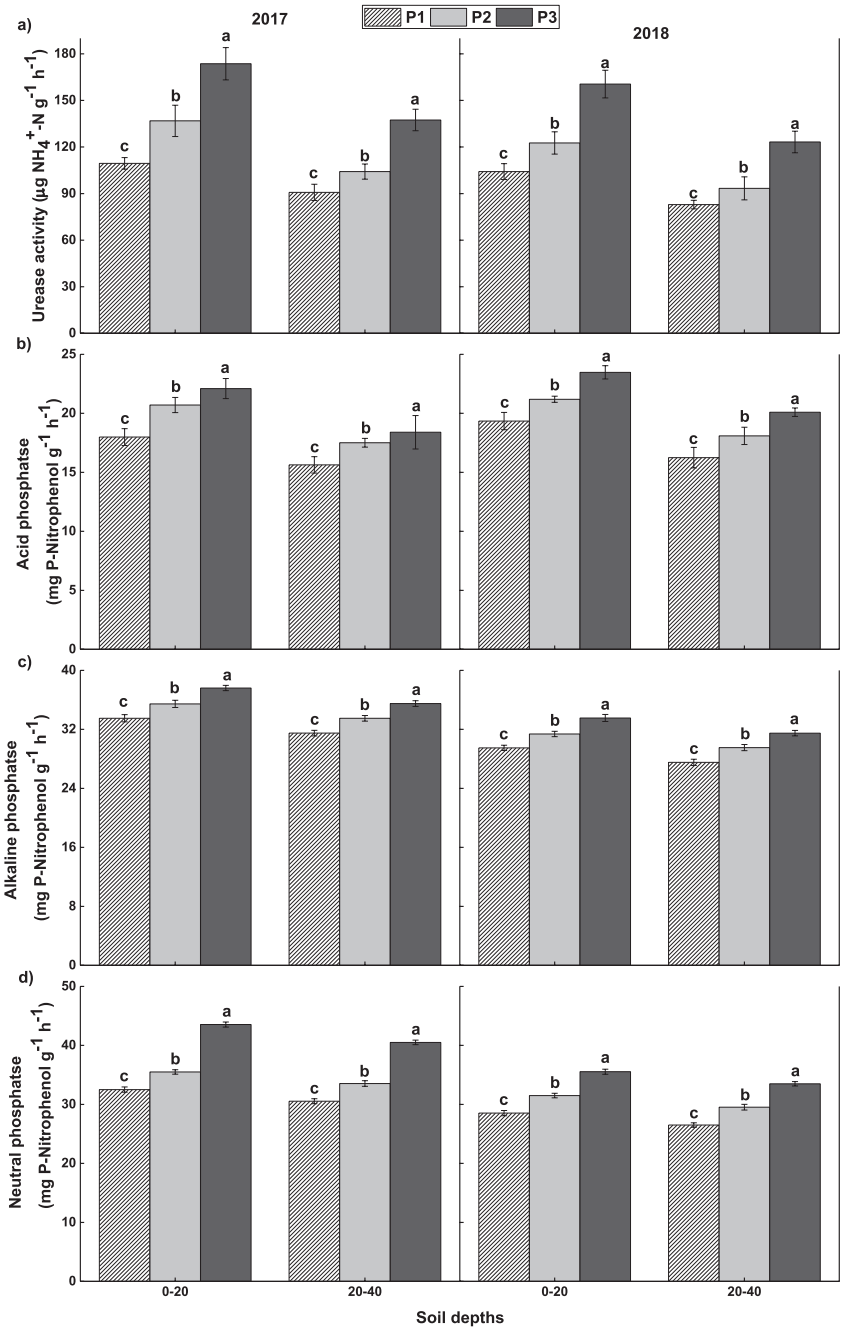

Fig. 4. Effect of P application on the activity of a) urease, b) acid, c) alkaline and d) neutral phosphatase enzymes in the topsoil (0-20 cm) and subsoil (20-40 cm) during cotton production in both 2017 and 2018 growing seasons. The bars represent different P levels and indicate standard errors of mean (n = 3), while different letters on the columns represent the statistical difference at P<0.05. Whereas; P1, P2, and P3 indicate the P levels, i.e., 0, 100, and 200 kg P_2O_5 ha^{-1}.

alkaline (r^2 = 0.9328-0.8760), and neutral phosphatase (r^2 = 0.8773-0.8216) activity during both cropping seasons, are representing at Fig. 5(a, b, c, d). The PCA-Biplot revealed significant changes in soil nutrient contents and enzymatic activities across P levels during cotton growing seasons (Fig. 6). All variables for soil nutrients and enzyme activity were classified into distinct categories. Over the two years, the cumulative variance of distribution functions approached 95.0% (Dim1 reached 91.8-91.3%, and Dim2 reached 3.2-3.7%). According to the findings, the increase in phosphorus application rates significantly impacted NH_4^+-N, NO_3^--N, AP, AK, TN, TP, TK, urease, alkaline, and neutral phosphatase activities during the cotton cropping seasons. Whereas the correlation between soil nutrients (NH_4^+-N, NO_3^--N, AP, AK, TN, TP, and TK) and enzyme activities (urease, acid, alkaline, and neutral phosphatase) showed a significant positive correlation (Fig. 7), but the strength of this correlation varied during both cropping seasons. Conclusively, plant activities greatly depend on the nutritional status in the soil and uptake by the plants. In contrast, the present study revealed that the higher P application rates (P3) in the soil proved sufficient for the cotton crop to keep the

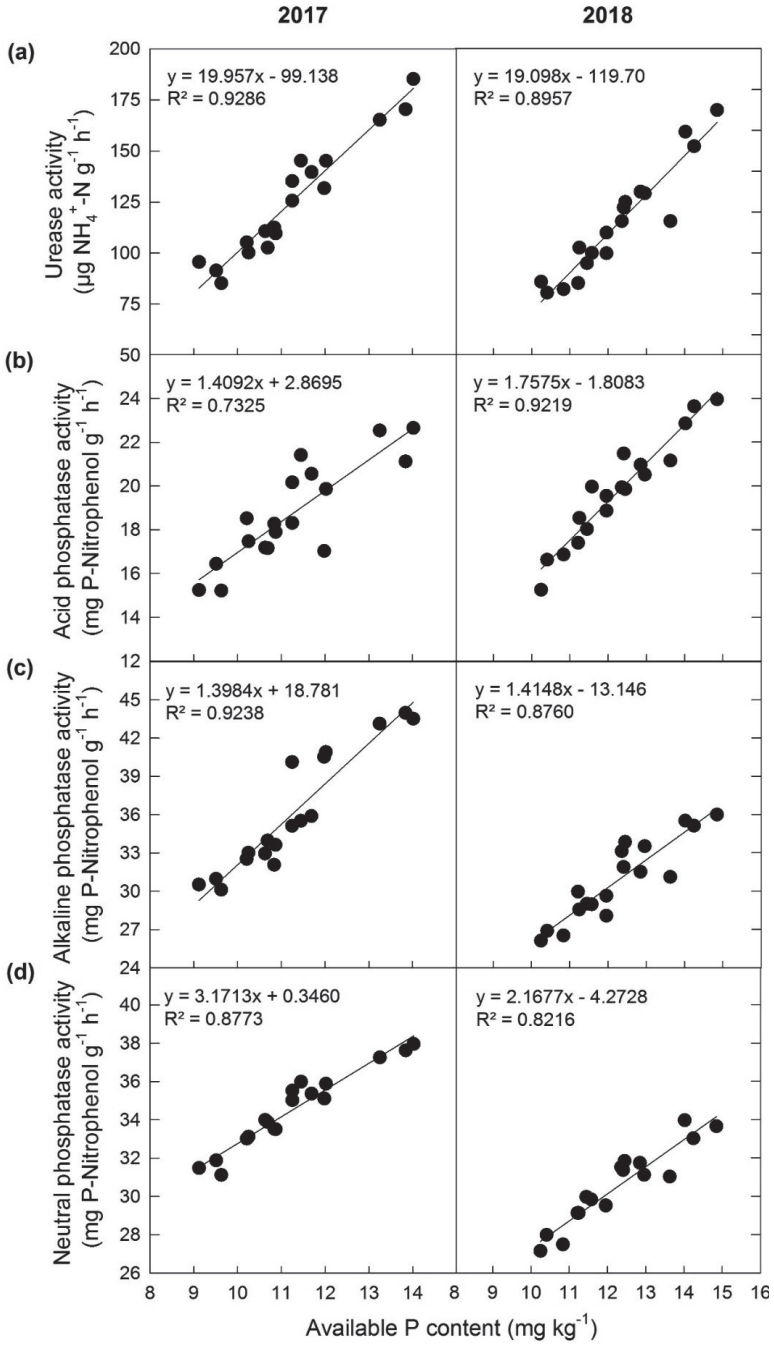

Fig. 5. Relationship between soil available P and enzymatic activities in the soil concerning the a) Urease, b) acid phosphatase, c) alkaline phosphatase, and d) neutral phosphatase in the soil during the 2017 and 2018 growing season of the crop.

plant productivity at the targeted rate. Conversely, the biogeochemical soil cycle, like the enzymatic activities (urease, acid, alkaline, and neutral phosphates), has a strong relationship with the soil available and total N, P, and K contents which improves the availability of these nutrients to the crop and enhances the productivity. However, soil P contents correlated more highly with the soil enzymatic activities. Therefore, soil nutritional status and qualitative characteristics strongly depend on soil enzymatic activity function [18, 20, 34]. Moreover, a higher seed cotton yield production in P3 treatment might result from good nutritional conditions and

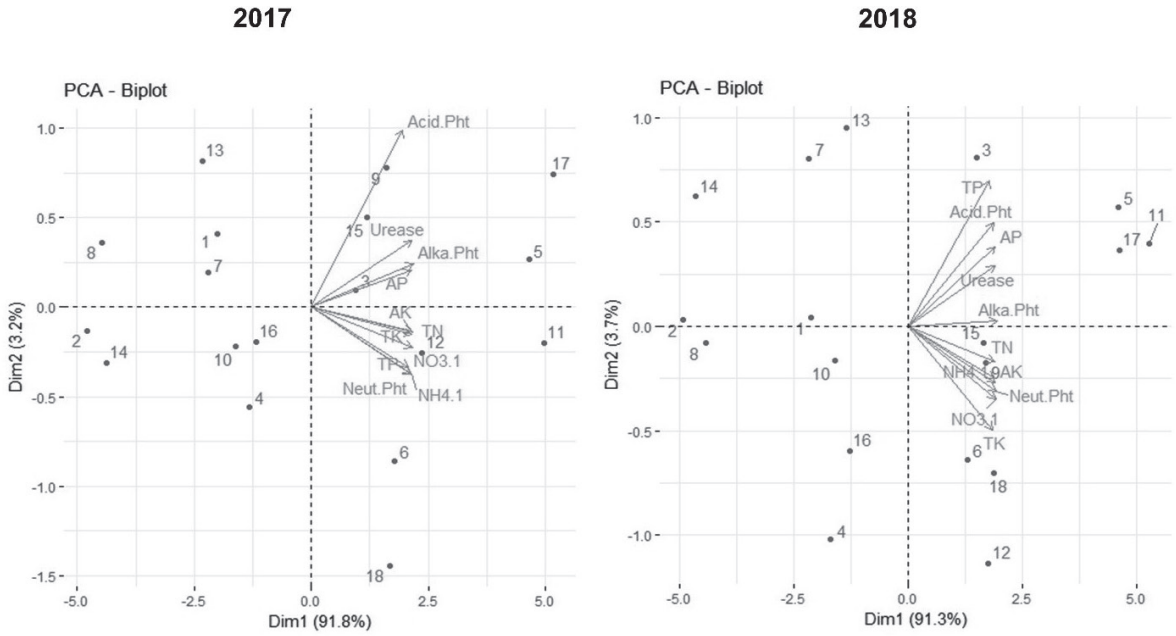

Fig. 6. Principal component analysis (PCA) of all soil enzymes and soil nutrients phosphorus treatments at 0-40 cm soil depth for cotton over the two-year study. NH_4^+-N, NO_3^--N, TN (Total N); AP (available phosphorus); TP (total P); AK (available potassium); TK (total potassium); urease; Acid Pht. (acid phosphatase); Alka. Pht. (alkaline phosphatase); and Neut. Pht. (neutral phosphatase) represent the data from the cotton seasons, respectively.

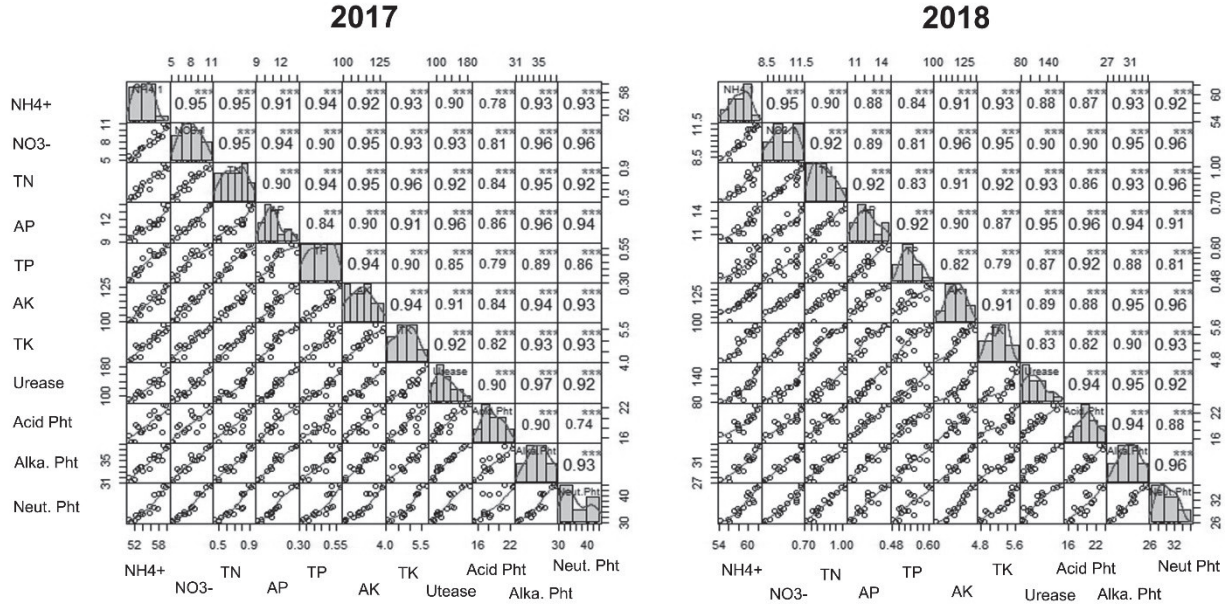

Fig. 7. Correlation among soil nutrient (NH_4^+-N, NO_3^--N, AP, AK, TN, TP and TK) and enzyme activities of urease, acid phosphatase, alkaline phosphatase, and neutral phosphatase in the soil during 2017 and 2018 cropping seasons. The bars and line, shown with observation (soil nutrient contents and enzyme activities), are regarded as correlation coefficients as 1 (r = 1). Other line graphs and correlation coefficients represent the comparative values to r = 1. Where *** = very highly significant (P<0.001).

enzymatic activities in the soil, promoting nutrient uptake in the crop body. Furthermore, a higher P application balanced the P rate in the soil caused by heavy nutrient feeder plants.

Conclusions

The present study showed a significant increase in ammonium, nitrate, available potassium, total nitrogen, and total potassium by increasing the P application rates (from 0 to 200 kg P_2O_5 ha^{-1}). Similarly, the urease and phosphatase activities enhanced with the increased P rates and strongly influenced the nutrient in the soil profile. Conversely, soil available and total phosphorus increased with higher phosphorus and decreased considerably with the depth of the soil. The results of the current study suggested that the P application rates affects the accessibility of nutrients, mainly the P availability, which enhances the enzymatic activities and alternately promotes the nutritional status of the soil by enhancing crop productivity and resulting in higher root, stem, leaf, and boll biomass. Based on the above results, it was concluded that higher P application (P3) favored the growth and biomass accumulation of vegetative and reproductive organs of cotton and had more positive effects on the seed cotton yield. Therefore, ensuring a reasonable P rate may be an excellent strategy to establish ideal reproductive biomass leading to a high seed cotton yield.

Author Contributions

Conceptualization, B.I.; Z.Z.; methodology, B.I.; and I.K.; formal analysis, Q.J.; investigation, A.R.; resources, I.U.; and Z.Z; data curation, B.I.; and I.K.; writing original draft preparation, B.I.; writing-review and editing, Z.Z.; Q.J.; and K.F.A.; funding acquisition, Z.Z, and K.F.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by National Key R&D Program of China(2021YFD1700900), Central Public-interest Scientific Institution Basal Research Fund(Y2022GH10) and the Agricultural Science and Technology Innovation Program of the Chinese Academy of Agricultural Sciences (No. CAAS-ASTIP202101).

Data Availability Statement

All data supported this research article is already included in this manuscript.

Acknowledgments

We are thankful for financial support from the Special Fund of National Public Welfare Industry (Agriculture) R&D Program (201503136), China Agriculture Research System (CARS-15-14) and Jiangsu Collaborative Innovation Center for Modern Crop Production (JCIC-MCP).

Conflict of Interest

The authors declare no conflict of interest.

References

1. IQBAL B., KONG F., ULLAH I., ALI S., LI H., WANG J., KHATTAK W.A., ZHOU Z. Phosphorus application improves the cotton yield by enhancing reproductive organ biomass and nutrient accumulation in two cotton cultivars with different phosphorus sensitivity. Agronomy **10** (2), 153, **2020**.
2. TIAN J., BOITT G., BLACK A., WAKELIN S.A., CONDRON L.M., CHEN L. Accumulation and distribution of phosphorus in the soil profile under fertilized grazed pasture. Agric. Ecosyst. Environ. **239**, 228, **2017**.
3. CHEN M., ALIM N., ZHANG Y., XU N., CAO X. Contrasting effects of biochar nanoparticles on the retention and transport of phosphorus in acidic and alkaline soils. Environ. pollut. **239**, 562, **2018**.
4. ROMERO-PERDOMO F., BELTRÁN I., MENDOZA-LABRADOR J., ESTRADA-BONILLA G., BONILLA R. Phosphorus nutrition and growth of cotton plants inoculated with growth-promoting bacteria under low phosphate availability. Front. Sustain. Food Syst. **4**, 618425, **2021**.
5. DEB D., KLOFT M., LASSIG J., WALSH S. Variable effects of biochar and P solubilizing microbes on crop productivity in different soil conditions. Agroecol. Sustain. Food Syst. **40**, 145, **2016**.
6. CHASTAIN D.R., SNIDER J.L., CHOINSKI J.S., COLLINS G.D., PERRY C.D., WHITAKER J. (2016). Leaf ontogeny strongly influences photosynthetic tolerance to drought and high temperature in *Gossypium hirsutum*. J. Plant Physiol. **199**, 18, **2016**.
7. HEUER S., GAXIOLA R., SCHILLING R., HERRERA-ESTRELLA L., LÓPEZ-ARREDONDO D., WISSUWA M. Improving phosphorus use efficiency: a complex trait with emerging opportunities. Plant J. **90**, 868, **2017**.
8. ZHANG Q., SONG Y., WU Z., YAN X., GUNINA A., KUZYAKOV Y. Effects of six-year biochar amendment on soil aggregation, crop growth, and nitrogen and phosphorus use efficiencies in a rice-wheat rotation. J. Clean. Prod. **242**, 118435, **2020**.
9. BRENNER J., PORTER W., PHILLIPS J.R., CHILDS J., YANG X., MAYES M.A. Phosphorus sorption on tropical soils with relevance to earth system model needs. Soil Res. **57**, 17, **2019**. doi: 10.1071/SR18197.
10. DAI Z., LIU G., CHEN H., CHEN C., WANG J., AI S. Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. ISME J. **14**, 757, **2019**.

11. ESTRADA-BONILLA G.A., DURRER A., CARDOSO E.J. Use of compost and phosphate-solubilizing bacteria affect sugarcane mineral nutrition, phosphorus availability, and the soil bacterial community. Appl. Soil Ecol. **157**, 103760, **2021**. doi: 10.1016/j.apsoil.2020.103760.
12. DE WIEL C.C.M.V., DER LINDEN C.G.V., SCHOLTEN O.E. Improving phosphorus use efficiency in agriculture: opportunities for breeding. Euphytica **207**, 1, **2016**.
13. EDUAH J.O., NARTEY E.K., ABEKOE M.K., BREUNINGMADSEN H., ANDERSEN M.N. Phosphorus retention and availability in three contrasting soils amended with rice husk and corn cob biochar at varying pyrolysis temperatures. Geoderma **341**, 10, **2019**.
14. ROBERTS K., DEFFOREY D., TURNER B. L., CONDRON L. M., PEEK S., SILVA S., KENDALL C., PAYTAN A. Oxygen isotopes of phosphate and soil phosphorus cycling across a 6500 year chronosequence under lowland temperate rainforest. Geoderma **257**, 14, **2015**.
15. GUL S., WHALEN J.K., THOMAS B.W., SACHDEVA V., DENG H. Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. Agric. Ecosyst. Environ. **206**, 46, **2015**.
16. JIN Y., LIANG X., HE M., LIU Y., TIAN G., SHI J. Manure biochar influence upon soil properties, phosphorus distribution and phosphatase activities: A microcosm incubation study. Chemosphere **142**, 128, **2016**.
17. KHADEM A., RAIESI F. Response of soil alkaline phosphatase to biochar amendments: changes in kinetic and thermodynamic characteristics. Geoderma **337**, 44, **2019**.
18. LIANG Y., LI M., PAN F., MA J., YANG Z., LING T. Alkaline phosphomonoesteraseharboring microorganisms mediate soil phosphorus transformation with stand age in Chinese Pinus massoniana plantations. Front. Microbiol. **11**, 571209, **2020**.
19. LIU S., MENG J., JIANG L., YANG X., LAN Y., CHENG X. Rice husk biochar impacts soil phosphorous availability, phosphatase activities and bacterial community characteristics in three different soil types. Appl. Soil Ecol. **116**, 12, **2017**.
20. MA J., HE P., XU X., HE W., LIU Y., YANG F. Temporal and spatial changes in soil available phosphorus in China (1990-2012). Field Crop. Res. **192**, 13, **2016**.
21. MAHMOUD E., EL BAROUDY A., ALI N., SLEEM M. Spectroscopic studies on the phosphorus adsorption in salt-affected soils with or without nano-biochar additions. Environ. Res. **184**, 109277, **2020**.
22. CONSTABLE G., BANGE M. The yield potential of cotton (*Gossypium hirsutum* L.). Field Crop. Res. **182**, 98, **2015**.
23. DENG Q., CHENG X., HUI D., ZHANG Q., LI M., ZHANG Q. Soil microbial community and its interaction with soil carbon and nitrogen dynamics following afforestation in central China. Sci. Total Environ. **541**, 230, **2016**.
24. KINTCHÉ K., GUIBERT H., SOGBEDJI J., LEVÊQUE J., BONFOH B., TITTONELL P. Long-term mineral fertiliser use and maize residue incorporation do not compensate for carbon and nutrient losses from a Ferralsol under continuous maize-cotton cropping. Field Crop. Res. **184**, 192, **2015**.
25. MAI W., XUE X., FENG G., YANG R., TIAN C. Can optimization of phosphorus input lead to high productivity and high phosphorus use efficiency of cotton through maximization of root/mycorrhizal efficiency in phosphorus acquisition? Field Crop. Res. **216**, 100, **2018**. doi: 10.1016/j.fcr.2017.11.017.
26. AMIN A., NASIM W., MUBEEN M., NADEEM M., ALI L., HAMMAD H.M. Optimizing the phosphorus use in cotton by using CSM-CROPGRO- cotton model for semi-arid climate of Vehari-Punjab, Pakistan. Environ. Sci. Pollut. Res. **24**, 5811, **2017**. doi: 10.1007/s11356-016-8311-8.
27. LEE C.H., WU C.H., SYU C.H., JIANG P.Y., HUANG C.C., LEE D.Y. Effects of phosphorous application on arsenic toxicity to and uptake by rice seedlings in As-contaminated paddy soils. Geoderma **270**, 60, **2016**.
28. LIU J., LIU X., ZHANG Q., LI S., SUN Y., LU W. Response of alfalfa growth to arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria under different phosphorus application levels. AMB Expr. **10**, 1, **2020**. doi: 10.1186/s13568-020-01137-w.
29. SOIL SCIENCE DIVISION STAFF. Soil Survey Manual: USDA Handbook No. **18**, **2017**. Washington, DC: Government Printing Office.
30. AMEUR D., ZEHETNER F., JOHNEN S., JOCHLINGER L., PARDELLER G., WIMMER B. Activated biochar alters activities of carbon and nitrogen acquiring soil enzymes. Pedobiologia **69**, 1, **2018**.
31. KHAN I., CHEN T., FAROOQ, M., LUAN C., WU Q., WANNING D., XU S., LI-XUE W. The residual impact of straw mulch and biochar amendments on soil physiochemical properties and yield of maize under rainfed system. Agron. J. **113**, 1102, **2021**.
32. ULLAH R., ZAFAR A., BILAL M., HABIB F., IQBAL J., BASHIR F., NOOR S., AKRAM M.Q., NIAZ A., BAIG K.S., RAUF A., FATIMA L., AKHTAR I., ALI B., ULLAH M.I., AL-HASHIMI A., ELSHIKH M.S.J., ALI S., SAEED-UR-REHMAN H. Method development and validation for the determination of potassium (K_2O) in fertilizer samples by flame photometry technique. J. King Saud Univ. - Sci. **34**, 102070, **2022**.
33. CORDERO I., SNELL H., BARDGETT R.D. High throughput method for measuring urease activity in soil. Soil Biol. Biochem. **134**, 72, **2019**.
34. KONG F., LING X., IQBAL B., ZHOU Z., MENG Y. Soil phosphorus availability and cotton growth affected by biochar addition under two phosphorus fertilizer levels. Arch. Agron. Soil Sci. 1955355, **2021**.
35. SAHANDI M.S., MEHRAFARIN A., BADI H.N., KHALIGHI-SIGAROODI F., SHARIFI M. Improving growth, phytochemical, and antioxidant characteristics of peppermint by phosphate-solubilizing bacteria along with reducing phosphorus fertilizer use. Ind. Crops Prod. **141**, 111777, **2019**.
36. IQBAL A., GUI H., ZHANG H., WANG X., PANG N., DONG Q. Genotypic variation in cotton genotypes for phosphorus-use efficiency. Agronomy **9**, 689, **2019**.
37. IZHAR SHAFI M., ADNAN M., FAHAD S., WAHID F., KHAN A., YUE Z. Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (*Triticum aestivum* L.) in calcareous soil. Agronomy **10**, 1224, **2020**.
38. JIANG Y., REN C., GUO H., GUO M., LI W. Speciation transformation of phosphorus in poultry litter during pyrolysis: insights from X-ray diffraction, Fourier transform infrared, and solid-state NMR spectroscopy. Environ. Sci. Technol. **53**, 13841, **2019**.
39. YAN C., ZHAN H., YAN S., DONG S., MA C., SONG Q., GONG Z., BARBIE M. Effects of straw retention and phosphorous fertilizer application on available phosphorus

content in the soil solution during rice growth. Paddy Water Environ. **14**, 61, **2016**.

40. SURIYAGODA L., SIRISENA D., SOMAWEERA K., DISSANAYAKE A., DE COSTA W., LAMBERS H. Incorporation of dolomite reduces iron toxicity, enhances growth and yield, and improves phosphorus and potassium nutrition in lowland rice (*Oryza sativa* L). Plant Soil **410,** (1), 299-312, **2017**.
41. MENSAH J.A., KOCH A.M., ANTUNES P.M., KIERS E.T., HART M., BÜCKING H. High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. New Phytol. **25,** (7), 533, **2015**.
42. KAMAU S., KARANJA N.K., AYUKE F.O., LEHMANN J. Short-term influence of biochar and fertilizer-biochar blends on soil nutrients, fauna and maize growth. Biol. Fertil. Soils **55**, 661, **2019**.
43. KIM J.A., VIJAYARAGHAVAN K., REDDY D.H.K., YUN Y.S. 2018. A phosphorus-enriched biochar fertilizer from bio-fermentation waste: a potential alternative source for phosphorus fertilizers. J. Clean. Prod. **196**, 163, **2018**.