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Proper Delay of Phosphorus Application Promotes Wheat Growth and Nutrient Uptake under Low Phosphorus Condition

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Abstract: It is widely known that root morphology has different response mechanisms at different phosphorus (P) levels. However, the effects of P application times on wheat (*Triticum aestivum* L.) root morphology and, therefore, on growth and nutrient uptake are still unclear. In this study, we investigated the feedback from the physiological indexes of wheat on different P application times after planting. Compared with the P application at planting, the shoot biomass with delayed P application for 21 days (d) increased by 11.8% ($p < 0.05$). Compared with the P application at planting, the shoot P uptake with delayed P application for 14–21 days increased by 38.4–71.2%, while the shoot N uptake and K uptake with delayed P application for 21 days increased by 16.0% and 14.1% ($p < 0.05$). Compared with the P application at planting, P use efficiency, N use efficiency, and K use efficiency with delayed P application for 21 days increased by 16.4%, 12.4%, and 12.4%. Delayed P application for 14–21 days promoted wheat shoot growth, nutrient (P, N, and K) uptake, and their use efficiencies. This is particularly important for optimizing the P fertilizer input and nutrient management for wheat growth.

Keywords: delayed P application; nutrient uptake; P deficiency; phosphatase; wheat growth

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1. Introduction

Phosphorus (P) is one of the vital nutrients for plant growth. It constitutes the backbone of essential biomolecules, such as nucleic acids, membrane phospholipids, and ATP, and is involved in many key biological processes regulating a variety of physiological activities [1,2]. Rock phosphate, apatite, and other raw materials are mostly found in finite deposits in China, the United States, and Morocco [3]. Deficient P-induced yield reduction has been found on 30–40% of the world's arable land [4,5]. The plant–available P from a phosphate fertilizer applied to the soil is easily fixed by adsorption by calcium, iron, magnesium, and aluminum plasma after the fertilizer application, resulting in a seasonal P use efficiency (PUE) by plants of only 15–20% in agricultural fields [4,6]. In addition, cereal crops, such as wheat, barley, corn, and rice, have largely low inorganic P (P_i) fertilizer use efficiency, averaging less than 10% [5,7]. It is well known that the aboveground biomass of plants is more affected by P deficiency than the underground biomass. Shen et al. [8] suggested that maintaining root biomass and root length was the main strategy for wheat to cope with extreme P deficiency. It is reported that the root-to-shoot ratio of plants under low P conditions is generally higher than under high P conditions [9].

Most P is taken up from soils by the root system. In response to a low mineral P environment, there are two main mechanisms triggered to ensure that plants take up more

nutrients: altered root morphology and differential increases in P-mobilizing exudation. Fine roots are more crucial than coarse roots for the plant to explore and obtain nutrients [10]. Studies have shown that appropriate P deficiency can increase plant root growth, especially the growth of fine roots [8,11]. It is reported that the mean nitrogen (N) and P concentrations were 11.0 and 0.9 g kg^{-1}, respectively, for fine roots (<2 mm in diameter), whereas the mean N and P concentrations were 6.5 and 0.6 g kg^{-1} in larger roots (2–5 mm in diameter) [12]. Fine root production is the largest component of root production [13]. The proportion of fine roots in total root length increased under appropriate low P conditions, thus promoting nutrient uptake by the roots [8,14]. It was the fine root growth that was first inhibited under severe P deficiency [8]. High root tissue density (RTD) represented a self-protective investment strategy for plants, and the robust tissues were used to resist physical stress, mechanical damage, and herbivory [15–17].

Acid phosphatase (ACP) and alkaline phosphatase (ALP) are the key driving factors of the biogeochemical degradation of organic P (P_o) [18], promoting the conversion of soil P_o to P_i [19]. Acid phosphatase (ACP) in soil includes ACP secreted by plants and microorganisms [20], while alkaline phosphatase (ALP) is associated with the metabolism of bacteria, fungi, and earthworms [21,22]. Delgado–Baquerizo et al. [23] studied the data from 224 globally distributed drylands and found that P_i concentration was correlated with ACP and that the effect of abiotic factors (e.g., labile organic matter fraction) on soil P_i was mediated. Bergkemper et al. [24] found that the abundance of genes encoding ALP was higher than the abundance of genes encoding ACP in acidic soils, which emphasized the importance of ALP in soil P_o mineralization. If N is sufficient but P is deficient in plants, a high N to P ratio will stimulate the process of transcription, and a low N to P ratio will inhibit the transcription of genes encoding ALP [25–27]. The ACP activity varies among crops. The ACP activity of faba bean rhizosphere increased from the lowest P treatment (3.5 mg kg^{-1} Olsen P) to 123 mg kg^{-1} Olsen P, and then decreased with a further increase in P supply, while the ACP activity of maize was not affected in P treatments [28]. However, rhizosphere ACP was found to have a positive linear correlation with maize shoot P concentration, but rhizosphere ACP of wheat was not [8,14]. When plants lack sufficient P for optimal growth, faba bean releases significant amounts of ACP into the rhizosphere to increase the plant availability for P [9]. Therefore, the increase of ACP and ALP in the rhizosphere has an important role in soil nutrient activation, nutrient uptake, and utilization by the crop.

Phosphorus plays a vital role in plant nutrient uptake. Many studies have shown that early P nutrition is very important for P absorption in crops [2,29–32]. The higher rate of P uptake in the early stages of the crop life cycle, the more P could be transferred to grains during the grain-filling period for optimum crop yield [29,30]. However, early on, it can be further subdivided, and the uptake of P by crops at different times is different. Nadeem et al. [32] studied the changes in available P sources for maize growth at the seedling stage. Phosphorus uptake by roots significantly increased from the 5th d after planting, and both sources of P (originated from soils and seed) supplied roots and shoots; 96% of phytate was hydrolyzed and 60% of total seed P was exported toward newly growing seedlings up to the 7th d after planting; 92% of total seed P was transported to newly growing seedlings till the 17th d after planting, and then ceased to be a significant source of P for growth. Therefore, it is very important to study the appropriate P application time in the crop seedling stage. Phosphorus content also affects N and potassium (K) nutrient uptake. Phosphorus is one of the components of N metabolism enzymes in crops. Rideout and Gooden [33] believed that delaying P application time prolonged the growth cycle of tobacco, but had no significant impact on the biomass, P, N, or K uptake during tobacco early post-transplant growth. When P application was delayed for 14 d, the shoot biomass, root biomass, shoot N and P concentrations, and N/P ratio of barley did not change, but the shoot P uptake was significantly reduced; when P application was delayed for 21 d, N and P concentrations, and N P ratio in the shoot of barley did not change, but the shoot and root biomass, N and P uptake in the shoot decreased; when P application was delayed for

28 d, N concentration in the shoot of barley increased, P concentration and N/P ratio in the shoot did not change, while the shoot N and P uptake were reduced [34]. As the nutrient absorption by crops is affected by different fertilization times, it is of great importance to apply phosphate fertilizers at the appropriate time.

Phosphorus fertilizer is usually applied as base fertilizer for most of the crops, as the critical period of P in gramineous plants is seedling. However, sufficient P supplement can inhibit root growth resulting in a reduced ability for nutrient and water uptake by plant roots. There is a lot of research on the effects of P application at different growth stages of crops, but few studies focus on the effects of different P application times on nutrient uptake at the wheat seedling stage. Considering that at the very early stage, plants mainly use nutrients in seeds, rather than nutrients from soils, it is necessary to study the appropriate P application times at the crop seedling stage. Furthermore, exploring the effect of different P application times on nutrient uptake in wheat seedlings is of great significance for promoting the later growth of wheat and nutrient uptake. In this study, we are going to investigate the effects of P application time on (1) wheat growth, (2) root morphology, (3) soil phosphatase activity, (4) nutrient utilization, and (5) nutrient uptake. The results of the study will provide a scientific basis for improving nutrient use efficiencies in wheat, especially for P.

2. Materials and Methods

2.1. Experimental Set-Up

The experiment was conducted in a greenhouse of Northwest A&F University (34°15′51″ N, 108°4′3″ E), Yangling, Shaanxi, China. Wheat (*Triticum aestivum* L.) cultivar "Xiaoyan 22" was grown in a calcareous loam soil with low P availability, which was collected from an abandoned land in Xixiaozhai Village (34°17′58″ N, 108°1′17″ E), Yangling, Shaanxi, China. The soil sample was air-dried and sieved to 2 mm. The basic physicochemical properties of the soil were determined according to the methods described in [35]. Information on the basic physical and chemical properties of soils is shown in Table 1. Each pot was filled with 1 kg of air-dried soil. There were 5 types of P application times: P fertilizer was supplemented at planting (P0), 7 days after planting (P7), 14 days after planting (P14), 21 days after planting (P21), and no P fertilizer application (NP). The phosphorus application rate was 100 mg kg^{-1} soil (as $NH_4H_2PO_4$). To ensure sufficient nutrients for plant growth, the soil was also supplemented with basal nutrients at the following rates (mg pot^{-1}): N 200 mg kg^{-1} (as CH_4N_2O, $NH_4H_2PO_4$), K 100 mg kg^{-1} (as KCl), Mg 4 mg kg^{-1} (as $MgSO_4$), Fe 2 mg kg^{-1} (as $FeSO_4$), Mn 2 mg kg^{-1} (as $MnSO_4$), Zn 2 mg kg^{-1} (as $ZnSO_4{\cdot}7\,H_2O$), Cu 0.5 mg kg^{-1} (as $CuSO_4{\cdot}5H_2O$), B 0.1 mg kg^{-1} (as H_3BO_3), Mo 0.1 mg kg^{-1} (as $(NH_4)_6MO_7O_{24}{\cdot}4H_2O$), Cl 89 mg kg^{-1}(as KCl), S 8 mg kg^{-1}(as $MgSO_4$, $FeSO_4$, $MnSO_4$, $ZnSO_4{\cdot}7\,H_2O$, $CuSO_4{\cdot}5H_2O$).

Table 1. Soil physical and chemical properties.

Soil Texture	Total N (g kg^{-1})	Total P (g kg^{-1})	Olsen–P (mg kg^{-1})	Total K (g kg^{-1})	SOC (g kg^{-1})	pH	EC (ds m^{-1})
Loam	1.90	0.76	5.44	32.3	10.0	8.05	0.11

Wheat seeds were pregerminated on the wet filter paper at 25 °C, with humidity of 50% in an incubator for 24 h. Six uniformly germinated seeds were moved to pots (16.0 cm in diameter on the top, 12.8 cm in diameter on the bottom, 17.5 cm in height, with a volume of 2 L) on 30 November 2021. The seedlings were thinned to three plants for each pot when the plants reached 5 cm in height. Eight replicates were used for each P treatment, and the pots were arranged in a completely randomized design. All pots were watered every 3 d to weight to maintain 75% field capacity. During the period of wheat growth in the greenhouse, the plants were under natural light at room temperature.

2.2. Harvest and Measurements

Plants were harvested at jointing stage 40 d after planting on 30 December 2021. During the harvesting time, shoots were cut at the soil surface and oven–dried at 65 °C for 3 d. The weight of the aboveground part of each treatment was measured by analytical balance (PL303, Mettler Toledo Technology (China) Co., Ltd., Shanghai, China). Soils adhered to roots were immersed in 0.2 mM $CaCl_2$ solution and then gently shaken to collect the suspension solution of rhizosphere soil [8,14]. After sampling the rhizosphere exudates, the roots of one plant from each pot were collected, washed off soil particles, and placed in individual plastic bags. The bags were stored at 4 °C for 2 d before measuring root morphology [36].

Cleaned root samples were dispersed in water in a transparent tray (25 × 20 × 3 cm) and then scanned with a root scanner (MicrotekScanmakeri800 plus) to obtain digital images, which were analyzed using a Win–RHIZO system (Basic, Regent Instruments Inc., Quebec, Canada) to obtain total root length (TRL), fine root length (FRL) (diameter of 0–2 mm) and total root volume (RV). The scanned roots of each treatment were oven–dried and measured.

Plant materials (0.200 g) were mixed with a mixture of 5 mL of concentrated sulfuric acid and 5 mL of 30% v/v H_2O_2. The mixed solution was then digested at 375 °C until the solution became clear and transparent. Total nitrogen (TN) was determined by the Kjeltec Nitrogen Analyzer (Kjeltec8400, Foss Co., Ltd., Hillerød, Denmark); total P was analyzed by the vanadate–molybdate–yellow method [37]; total potassium by the flame photometric method by atomic absorption spectrophotometer [37].

2.3. Soil Phosphatase Collection

Two 5 mL aliquots of soil suspension solution of rhizosphere soil were transferred into two centrifuge tubes for measurement of Rs–ACP and RS–ALP, respectively, using the spectrophotometric method based on the measuring of p-nitrophenol phosphate (PNP) absorbance at 405 nm [15,38,39]. The bulk soil was also air–dried and ground to pass through a 1 mm sieve for analysis of bulk soil acid phosphatase (Bs–ACP) and bulk soil alkaline phosphatase (BS–ALP). The reaction mixtures of BS–ACP consisted of 3.0 g soil, 0.15 mL toluene, and 20 mL 0.5% substrate (Shanghai Maclean Biochemical Technology Co., Shanghai, China) prepared with acetate buffer (5.0) solution. BS–ALP was measured in the same way as ACP, except that the substrate was prepared from a borate buffer (9.4) solution. All reaction mixtures were incubated at 25 °C for 2 h. After incubation, the phenol released from the substrate under the action of phosphatases was determined spectrophotometrically at 510 nm [18]. Phosphatase activities are expressed in μg PNP h^{-1} g^{-1} soil.

2.4. Calculations and Statistics

Some calculation formulas are as follows:

Proportion of fine roots in total root length (PFRL) = fine root length/total root length [14,36].

Root tissue density (RTD) (g cm^{-3}) = root volume/root biomass.

P use efficiency (PUE) (kg kg^{-1}) = plant total biomass/P fertilizer [3,40,41].

P acquisition per root length (PARL) (mg m^{-1}) = total amount of shoot P concentration/total root length [40].

The calculations for N and K were the same as for P.

Shoot and root biomass, root-to-shoot ratio, root morphological traits, and plant P, N, and K uptake were plotted for each treatment using the Origin 2017 94c statistical software. One-way analysis of variance for wheat growth, root morphology, soil phosphatase activity, nutrient uptake, and nutrient utilization was performed using Excel 2010 and SPSS 20.0 statistical software. Significant differences among means were assessed using the LSD multiple range analysis test ($p < 0.05$), n = 4.

3. Results

3.1. Plant Growth

With the delay of P application, the total biomass of wheat at P21 was 0.084 g plant^{-1} at harvest time, which was significantly higher than that at P0, P7, and NP ($p < 0.05$, Figure 1). The total biomass at NP was 0.064 g plant^{-1}, which was the minimum. Compared with P0 (0.075 g plant^{-1}), the total biomass at P21 increased by 12.4% ($p < 0.05$). With the delay of P application, the trend for the shoot biomass was similar to that for the total biomass (Figure 1). The shoot biomass at P21 was 0.066 g plant^{-1}, which was the maximum. The shoot biomass at NP was 0.048 g plant^{-1}, which was the minimum. Compared with P0, which was 0.059 g plant^{-1}, the shoot biomass at P21 increased by 11.8% ($p < 0.05$).

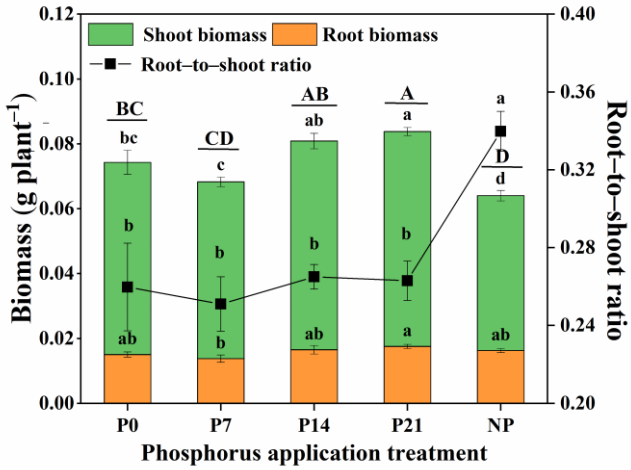

Figure 1. Shoot and root biomass and root-to-shoot ratio as a function of P application time. P0 means P application at planting; P7 means P application at 7 d after planting; P14 means P application at 14 d after planting; P21 means P application at 21 d after planting; and NP means no P application. Different capital letters mean significant differences in total biomass between treatments at $p < 0.05$, n = 4; different lowercase letters mean significant differences in either shoot biomass or root biomass among different treatments at $p < 0.05$, n = 4.

The root-to-shoot ratio of wheat in the P application treatments was maintained between 0.251 to 0.263, and the root-to-shoot ratio at NP was 0.340 (Figure 1). Compared with P0, the root-to-shoot ratio at NP increased significantly, by 30.8% ($p < 0.05$).

3.2. Root Morphology

With the delay of P application, the total root length (TRL) of wheat at P14 was the highest, which was 101 cm plant^{-1}, significantly higher than that at P0, P7, and P21 at harvest time ($p < 0.05$, Figure 2a). Compared with P0, which was 67.6 cm plant^{-1}, the TRL at P14 increased by 2.03% ($p < 0.05$), while the value at P21 increased by 1.61%.

With the delay of P application, the proportion of fine roots in total root length (PFRL) of wheat gradually increased (Figure 2b). The PFRL at NP was 99.9%, which was the maximum among all the treatments. The PFRL at P0 was 99.4%, which was the minimum among all the treatments. Compared with P0, the PFRL at P14 and P21 increased by 0.356% and 0.417%, respectively ($p < 0.05$). Compared with P0, the PFRL at NP increased by 0.505% ($p < 0.05$).

With the delay of P application, the root tissue density (RTD) of wheat gradually increased till P21 at harvest time; the highest value was 0.454 g cm^{-3} at P21 (Figure 2c). The RTD at NP was 0.299 g cm^{-3}, which was the minimum. Compared with P0 (0.30 g cm^{-3}), the RTD at P21 increased significantly, by 51.4% ($p < 0.05$).

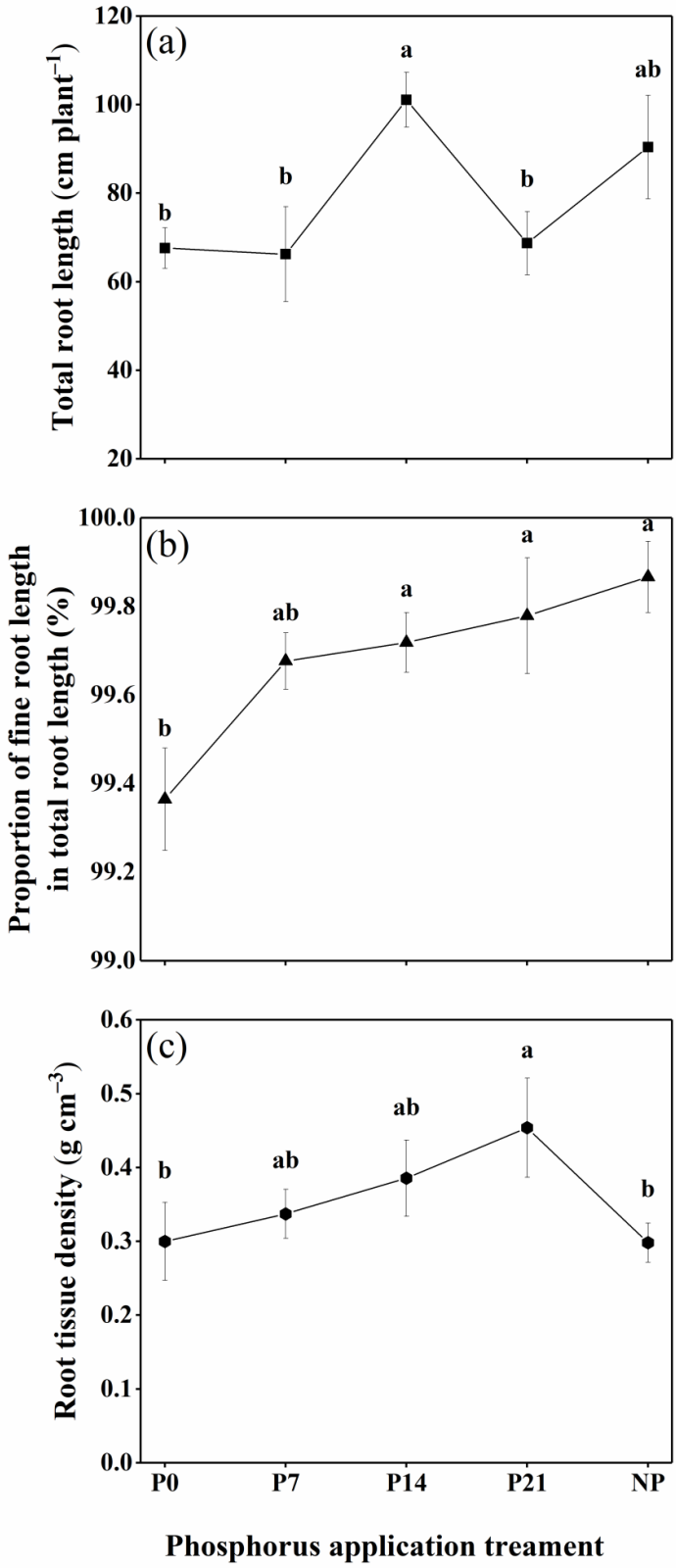

Figure 2. Root morphology as a function of P application time: (**a**) total root length (TRL); (**b**) the proportion of fine roots (diameter 0–2 mm) in total root length (PFRL), and (**c**) root tissue density (RTD). P0 means P application at planting; P7 means P application at 7 d after planting; P14 means P application at 14 d after planting; P21 means P application at 21 d after planting; and NP means no P application. Different lowercase letters mean significant differences in TRL, PFRL, and RTD among different treatments at $p < 0.05$, n = 4.

3.3. Soil Phosphatase Activity

With the delay of P application, the acid phosphatase activity in soil (RS–ACP) showed an increasing trend until P14 and then a decreasing trend at harvest time (Figure 3). The RS–ACP activity at P14 was 107 μg PNP h^{-1} g^{-1} soil, which was the maximum. The RS–ACP activity at NP was 29.4 μg PNP h^{-1} g^{-1} soil, which was the minimum among all the treatments. Compared with P0, which was 44.9 μg PNP h^{-1} g^{-1} soil, the RS–ACP activity at P14 increased significantly, by 140% ($p < 0.05$). The trend for the alkaline phosphatase activity in rhizosphere soil (RS–ALP) was similar to that of RS–ACP activity (Figure 3). The RS–ALP activity at P14 was 1414 μg PNP h^{-1} g^{-1} soil, which was the maximum. The RS–ALP activity at NP was 366 μg PNP h^{-1} g^{-1} soil, which was the minimum. Compared with P0 (581 μg PNP h^{-1} g^{-1} soil), the RS–ALP activity at P14 increased significantly, by 143% ($p < 0.05$). BS–ACP was maintained between 2.07–2.69 μg PNP h^{-1} g^{-1} soil, and BS–ALP was maintained between 3.29–3.75 μg PNP h^{-1} g^{-1} soil.

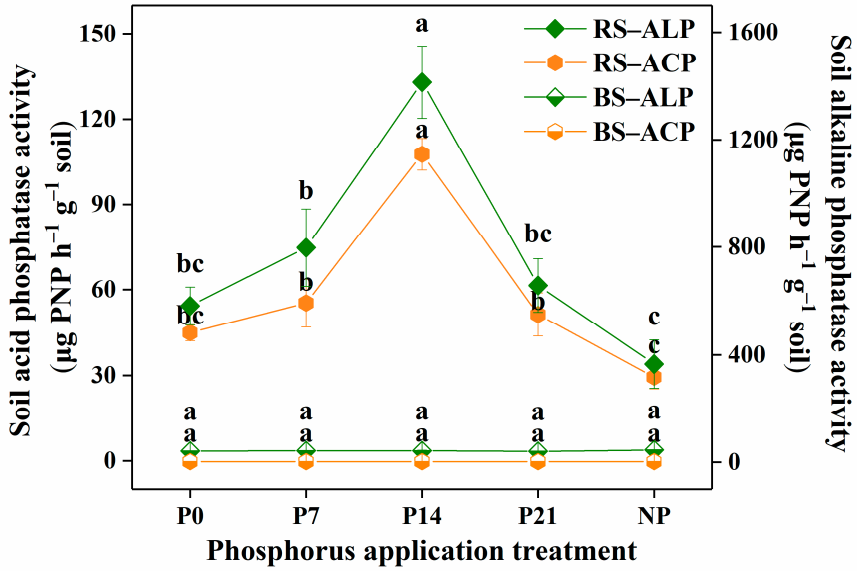

Figure 3. Phosphatase activity (acid phosphatase activity in rhizosphere soil (RS–ACP), alkaline phosphatase activity in rhizosphere soil (RS–ALP), acid phosphatase activity in bulk soil (BS–ACP), and alkaline phosphatase activity in bulk soil (BS–ALP)) as a function of P application time. P0 means P application at planting; P7 means P application at 7 d after planting; P14 means P application at 14 d after planting; P21 means P application at 21 d after planting; and NP means no P application. Different lowercase letters mean significant differences in RS–ACP, RS–ALP, BS–ACP, and BS–ALP among different treatments at $p < 0.05$, n = 4.

3.4. Nutrient Uptake

3.4.1. P Uptake

With the delayed P application, the shoot P concentration of wheat showed an increasing trend until P21 and a decreasing trend at the NP treatment at harvest time (Figure 4a). The P concentration in the shoot at P21 was 9.53 mg g^{-1}, which was the maximum. The shoot P concentration at NP was 3.33 mg g^{-1}, which was the minimum. Compared with P0 (6.13 mg g^{-1}), the shoot P concentrations at P7 to P21 increased significantly, by 41.1–55.6% ($p < 0.05$). Compared with P0, the shoot P concentration at NP decreased significantly, by 45.6% ($p < 0.05$). With the delayed P application, the root P concentration of wheat increased at P21 and then decreased at NP at harvest time (Figure 4a). The root P concentration at P21 was 1.87 mg g^{-1}, which was the maximum, The root P concentration at NP was 0.752 mg g^{-1}, which was the minimum. Compared with P0, which was 1.45 mg g^{-1}, the root P concentration at P21 increased significantly, by 28.8% ($p < 0.05$). Compared with P0, the root P concentration at NP decreased significantly, by 48.1% ($p < 0.05$).

With the delayed P application, the shoot P uptake of wheat gradually increased until P21 and then decreased at NP at harvest time (Figure 4b). The shoot P uptake at P21 was 0.544 mg plant^{-1}, which was the maximum. The shoot P uptake at NP was 0.132 mg plant^{-1}, which was the minimum. Compared with P0, which was 0.318 mg plant^{-1}, the shoot P uptake at P14 and P21 increased significantly, by 38.4% and 71.2% ($p < 0.05$). Compared with P0, the shoot P uptake at NP decreased significantly, by 58.7% ($p < 0.05$). The trend for the root P uptake was similar to that for the shoot P uptake (Figure 4b). The root P uptake at P21 was 0.099 mg plant^{-1}, which was the maximum. The root P uptake at NP was 0.043 mg plant^{-1}, which was the minimum among all treatments. Compared with P0 (0.069 mg plant^{-1}), the root P uptake at P21 increased significantly, by 44.6% ($p < 0.05$), while the root P uptake at NP decreased significantly, by 37.3% ($p < 0.05$).

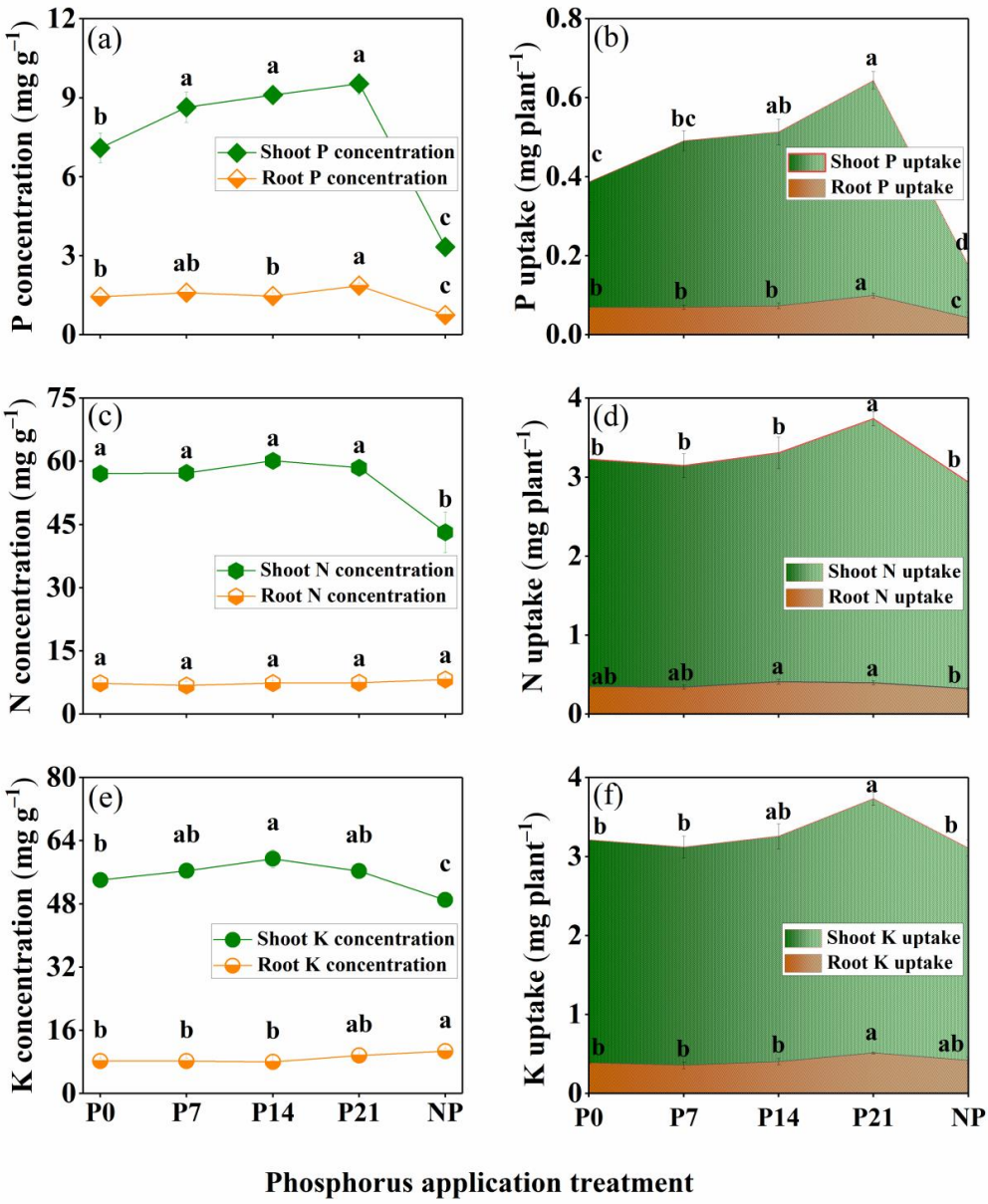

Figure 4. P, N, and K concentration and uptake as a function of P application time: (**a**) P concentration; (**b**) P uptake; (**c**) N concentration; (**d**) N uptake; (**e**) K concentration; (**f**) K uptake. P0 means P application at planting; P7 means P application at 7 d after planting; P14 means P application at 14 d after planting; P21 means P application at 21 d after planting; and NP means no P application. Different lowercase letters mean significant differences in P, N, and K concentrations and uptake among different treatments at $p < 0.05$, n = 4.

3.4.2. N Uptake

With the delay of P application, the shoot N concentration at P0 to P21 was basically maintained between 57.1–60.1 mg g^{-1} (Figure 4c). The shoot N concentration at P14 was 60.1 mg g^{-1}, which was the maximum. The shoot N concentration at NP was 43.2 mg g^{-1}, which was the minimum. Compared with P0, the shoot N concentration at NP decreased significantly, by 24.4% ($p < 0.05$). The root N concentration at P0 to NP was basically maintained between 6.79–8.22 mg g^{-1} (Figure 4c).

With the delay of P application, the shoot N uptake at P21 increased and then decreased at NP (Figure 4d). The shoot N uptake at P21 was 3.34 mg $plant^{-1}$, which was the maximum. The shoot N uptake at NP was 2.61 mg $plant^{-1}$, which was the minimum. Compared with P0 (2.88 mg $plant^{-1}$), the shoot N uptake at P21 increased by 16.0% ($p < 0.05$). With the delay of P application, the root N uptake at P0 to P21 was basically maintained between 0.343–0.411 mg $plant^{-1}$ (Figure 4d). The root N uptake at NP was 0.321 mg $plant^{-1}$, which was the minimum.

3.4.3. K Uptake

With the delay of P application, the shoot K concentration of wheat increased until P14 and decreased afterward at harvest time (Figure 4e). The shoot K concentration at P14 was 59.4 mg g^{-1}, which was the maximum. The shoot K concentration at NP was 49.0 mg $plant^{-1}$, which was the minimum. Compared with P0, which was 54.0 mg g^{-1}, the shoot K concentration at P14 increased by 9.97% ($p < 0.05$). Compared with P0, the shoot K concentration at NP decreased by 9.30% ($p < 0.05$). The root K concentration at P0 to P21 ranged from 8.00 mg g^{-1} to 9.64 mg g^{-1} at harvest time (Figure 4e). Compared with P0, the root K concentration at NP increased significantly, by 30.0% ($p < 0.05$).

With the delay of P application, the shoot K uptake at P21 increased and then decreased at NP at harvest time (Figure 4f). The shoot K uptake at P21 was 3.22 mg $plant^{-1}$, which was the maximum. The shoot K uptake at NP was 2.69 mg $plant^{-1}$, which was the minimum. Compared with P0, which was 2.82 mg $plant^{-1}$, the shoot K uptake at P21 increased by 14.1% ($p < 0.05$). The trend for the root K uptake was similar to that for the shoot K uptake at harvest time (Figure 4f). The root K uptake at P21 was 0.515 mg $plant^{-1}$, which was the maximum. The root K uptake at P7 was 0.359 mg $plant^{-1}$, which was the minimum. Compared with P0, which was 0.391 mg $plant^{-1}$, the root K uptake at P21 increased significantly, by 32.0% ($p < 0.05$).

3.5. Nutrient Utilization

With the delay of P application, the P use efficiency (PUE) of wheat decreased at P7 and increased until P21 at harvest time (Figure 5a). The PUE at P21 was 2.51 kg kg^{-1}, which was the maximum. The PUE at P7 was 2.05 kg kg^{-1}, which was the minimum. Compared with P0, which was 2.16 kg kg^{-1}, the PUE at P21 increased by 16.4% ($p < 0.05$). With the delayed P application, the trend for N use efficiency (NUE) was similar to that for the PUE at harvest time (Figure 5a). The NUE at P21 was 1.73 kg kg^{-1}, which was the maximum. The NUE at NP was 1.33 kg kg^{-1}, which was the minimum. Compared with P0 (1.54 kg kg^{-1}), the NUE at P21 increased by 12.4% ($p < 0.05$), while the NUE at NP decreased by 13.8% ($p < 0.05$). With the delayed P application, the trend for K use efficiency (KUE) was also similar to that for the PUE at harvest time (Figure 5a). The KUE at P21 was 2.51 kg kg^{-1}, which was the maximum. The KUE at NP was 1.93 kg kg^{-1}, which was the minimum. Compared with P0 (2.23 kg kg^{-1}), the KUE at P21 increased by 12.4% ($p < 0.05$), while the KUE at NP decreased by 13.8% ($p < 0.05$).

The P acquisition per root length (PARL) of wheat increased at P21 and then decreased at NP at harvest time (Figure 5b). The PARL at P21 was 0.807 mg m^{-1}, which was the maximum. The PARL at NP was 0.162 mg m^{-1}, which was the minimum. Compared with P0 (0.434 mg m^{-1}), the PARL at P21 increased significantly, by 86.1% ($p < 0.05$), while the PARL at NP decreased significantly, by 62.6% ($p < 0.05$). With the delay of P application, the N acquisition per root length (NARL) kept relatively constant, and NARL at NP decreased

at harvest time (Figure 5b). The NARL at P21 was 0.050 mg m^{-1}, which was the maximum among all treatments. The NARL at NP was 0.020 mg m^{-1}, which was the minimum among all treatments. Compared with P0, the NARL at P21 increased by 25.8%, while the NARL at NP decreased significantly, by 49.1% ($p < 0.05$). The KARL at P0 to P21 was basically maintained between 0.036–0.048 mg m^{-1} at harvest time (Figure 5b).

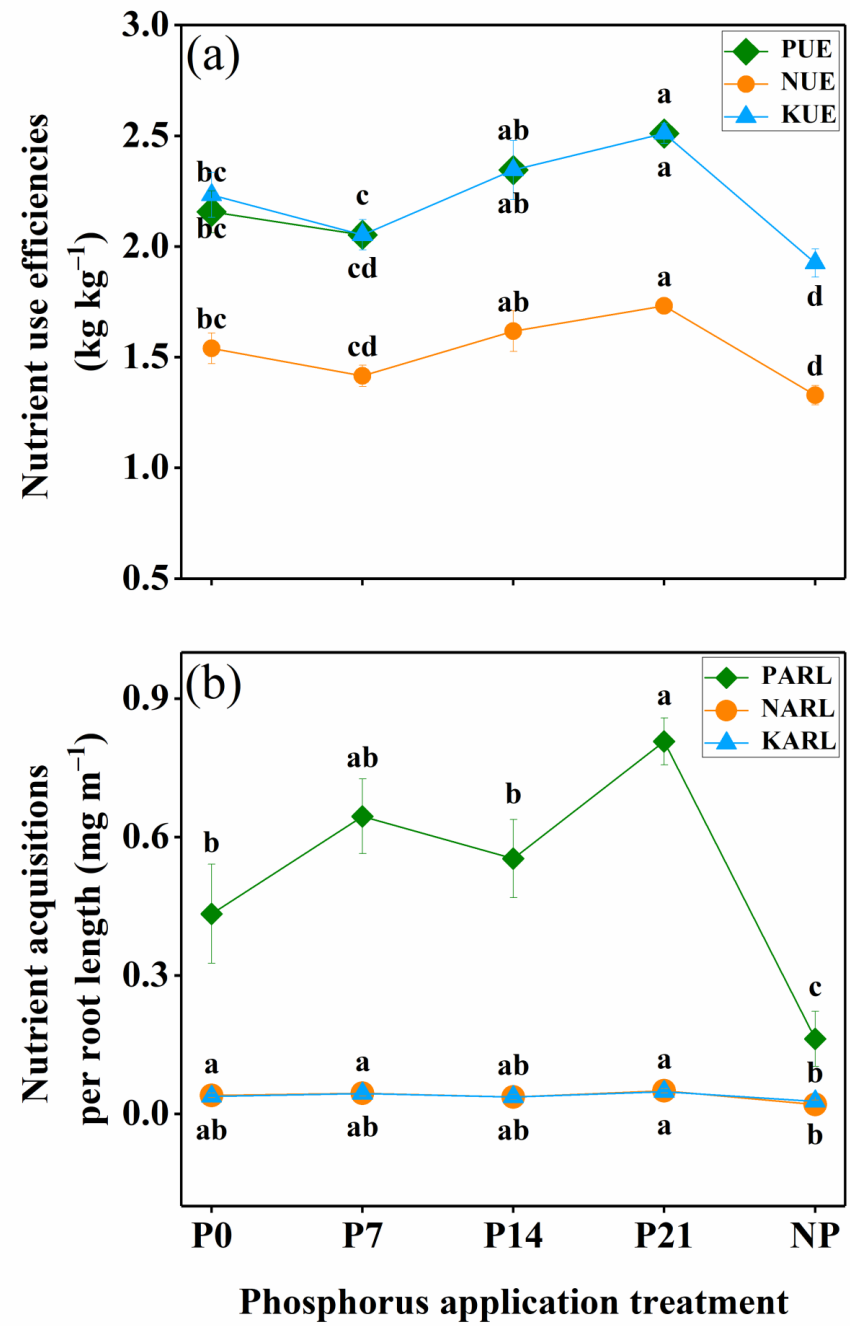

Figure 5. Nutrient use efficiencies and nutrient acquisitions per root length as a function of P application time: (**a**) Nutrient use efficiency (P use efficiency (PUE), N use efficiency (NUE), and K use efficiency (KUE) and (**b**) nutrient acquisition per root length (P acquisition per root length (PARL), N acquisition per root length (NARL) and K acquisition per root length (KARL). P0 means P application at planting; P7 means P application at 7 d after planting; P14 means P application at 14 d after planting; P21 means P application at 21 d after planting; and NP means no P application. Different lowercase letters mean significant differences in nutrient use efficiencies and nutrient acquisitions per root length among different treatments at $p < 0.05$, n = 4.

4. Discussion

4.1. Plant Growth

4.1.1. Plant Biomass and the Root-to-Shoot Ratio

Plant growth is limited under low P conditions. It was reported that P deficiency could cause decreased yield in wheat, barley, and other crops [42,43]. Hou et al. [44] found that 46.2% of the 652 P–addition field experiments worldwide showed P deficiency in aboveground plants. It is well–known that P application can increase the growth of most plants. The limitation of plant growth can be alleviated by P application afterward at a proper time. In our study, at 0–7 d after wheat seed germination, wheat utilizes nutrients partly from seeds, and the rest are taken up by roots from soils. Nadeem et al. [32] found that maize preferred utilizing P in seeds rather than absorbing P from the soil within 0–7 d after planting, and the consumption accounted for 60% of the total P content in seeds. Therefore, delayed P application for 7 d in the study had no effect on the shoot biomass of wheat. According to Liu's study [11], severe P deficiency inhibited root growth, but slight P deficiency promoted root growth. In this experiment, the root biomass of wheat did not decrease with delayed P application and without P application. Plants in a low P environment would allocate more assimilates to roots, to promote root growth and root activity, and increase root foraging ability for nutrients [9,45], so that more nutrients could be absorbed after P application. Our results indicate that delayed P application for 21 d can promote not only root growth but also shoot growth. Usually, plants distribute assimilates to the shoot when nutrients are sufficient [44]. However, it is the opposite when a specific nutrient is deficient. It was reported that more carbohydrates of maize and faba bean were transported to the root system in a P–deficient environment [9]. This explains why the root biomass did not decrease while the shoot biomass was the lowest without P application in our experiment. In addition, the shoot growth increased more than root growth by P application in a P fertilization experiment with maize [45]. It is the change of P concentration that leads to the difference in shoot and root biomass of plants. This explains the largest root-to-shoot ratio without P application in our study. Furthermore, due to the plasticity of roots and the principle of carbon allocation in plants, root growth is inhibited when plants are under abiotic stress for a long time rather than for a short time [11,46]. Thus, the shoot biomass showed earlier differences compared with root growth. Mollier and Pellerin [46] reported that reduced leaf area caused by P deficiency lowered the capacity of the leaf to intercept light so that root growth was finally reduced. As for the aspect of P deficiency, the observed root-to-shoot ratio in the experiment is a result of assimilate distribution to ensure crop survival and growth, where shoot biomass is susceptible to being restricted by P deficiency.

4.1.2. Root Morphology

Roots usually respond to P deficiency in different ways. In this study, wheat showed different types of responses to delayed P application, initially in root morphology and root exudates. All these functional characteristics reflect the plasticity of roots. Liu [11] supposed that plant root morphology changed to relieve P deficiency under the appropriate low P conditions. Compared with P application at planting, the total root length (TRL) showed no difference with delayed P application for 7 d in our experiment. This is probably because wheat preferred to use P stored in seeds rather than that taken up from the soil by roots at this stage, which is consistent with Nadeem's research on maize [32]. A study on maize with P deficiency suggested that root growth can be slightly promoted at a short–term low P supply level; then it can be significantly decreased [46]. Our results suggested that supplementation of P fertilizer 14 d after planting provided sufficient P for wheat growth in our experiment, thus obtaining higher TRL while avoiding root growth limitation due to long-term P deficiency. Duijnen et al. [34] found that compared with traditional P application, the root length of plants increased over time under delayed P application. In our study, the delayed P application for 21 d had no effect on TRL. We assume that after the P application, there would be a temporary excess of P, decreasing

the investment by root exploration for nutrients [34]. Higher specific root length (SRL) indicated that plants produce more root length and less root biomass, resulting in less investment in root foraging [8]. The SRL of wheat was not affected by the P application time in this experiment (Figure S1a). This showed that the changes in root biomass and root length were consistent. A delay of 14–21 d increased the proportion of fine root length in total root length (PFRL). The higher PFRL means more fine roots, which can absorb more nutrients.

Fine roots are crucial for nutrient uptake, and they are influenced by P availability in soil [14,47]. Wheat produces more fine roots with a larger root surface area utilizing less carbon in low P soil [8,48]. Severe P deficiency usually inhibits root growth. It was also reported that fine root (0–0.2 mm in diameter) production was promoted by P deficiency when the shoot P concentration of wheat was measured to be >3 mg g^{-1} [8]. With all the shoot P concentrations being >3 mg g^{-1}, the PFRL and fine root length (FRL) increased under the delayed P application for 14–21 d (Figure 2b and Figure S1b), demonstrating that properly delayed P application promoted the production of fine roots in our study. Generally, root tissue density (RTD) is negatively related to soil fertility, and RTD is the most consistent root trait that reflects the adaptation of plants to infertile soil [49]. High RTD indicates investment of higher carbon and the ability of the root to resist pressure [16]. The RTD of wheat increased with a 21–d delay in P application at the seedling stage and then promoted nutrient uptake in the study. Therefore, delayed P application at 21 d can cause wheat root morphology shifts in response to a temporal P-deficient environment.

4.1.3. Soil Phosphatase Activity

The production and secretion of phosphatase can promote the transformation of soil organic P (P_o) to inorganic P (P_i) and enhance the availability of P_i in soil, resulting in an increase in P uptake by plant roots [50]. There was a significant correlation between P_o consumption and phosphatase activity in rhizosphere soil [51,52]. Not only root morphology but also root exudation is plastic. Phosphorus-limited plants rely more on hydrolyzable P degraded by a one-step enzymatic reaction (monolipid P, pyrophosphate, and phytate) as a P source. Plant growth promotes rhizosphere acid phosphatase (RS–ACP) secretion, therefore hydrolyzing P_o in the rhizosphere, which is an important adaptive strategy for plants growing in soils with low P availability [14,53]. Rhizosphere alkaline phosphatase (RS–ALP) secretion by microorganisms is promoted under low P levels [18]. In our experiment, the increment of RS–ACP RS–ALP with delayed P application for 14 d was more intense under this treatment. It indicated that the response of wheat roots and rhizosphere microorganisms to a low P environment was more intense under this treatment. The RS–ACP with delayed P application for 14 d was 40 times as high as that in the bulk soil. Tarafdar and Jungk [51] supposed that the ACP activity of P–deficient plants in the rhizosphere was up to eight times as high as in the bulk soil, which was much smaller than our results. It is reported that phosphatase activity of the root mostly increases the mobilization of P in the rhizosphere [54]. Usually, the phosphatase activity in rhizosphere soil is higher than in the bulk soil, and the enzyme activity decreases with the increase of distance from the root surface [52,55]. The maximum distance for the ACP activity increment from the root surface was observed to be 2.0–3.1 mm, while the distance for the ALP activity ranged from 1.2 to 1.6 mm [51]. The high physiological plasticity of wheat Rs–ACP and Rs–ALP can mobilize soil P, improve P availability, and provide sufficient P for plant growth [28]. Phosphorus availability modified wheat root growth and thus changed the uptake and utilization of nutrients. Therefore, the procedure of proper P application delay can maximumly promote microorganism/plant root response to search for more P from soils.

4.2. Nutrient Uptake as Affected by P Application Time

It is common sense that P stress during the early growing season will restrict crop growth, thus reducing the final crop yield. Defects caused by P deficiency at the early

growth stage usually have a greater negative impact on crop productivity than P restrictions imposed at the late growth stage [30]. Therefore, early P application is considered important in most cases. Boeye et al. [56] emphasized that applying P fertilizer during the early season stimulates growth and increases shoot P concentration, while applying phosphate fertilizers during the late season does not stimulate growth but strongly increases the shoot concentration. In our results, delayed P application for 14–21 d increased P concentration and P uptake by wheat at the seedling stage. On the one hand, delayed P application for 14–21 d increased the PFRL of wheat, indicating that wheat had more fine roots and a larger root contact area with soil to take up nutrients. On the other hand, wheat lived in a P-deficient environment before P application, and higher ALP and ACP increased P availability to help plants to use some of the (P_o) in soils. Römer and Schilling [29] changed the P supply at different stages of hydroponic conditions until maturity and found that compared with 30 d later, wheat could produce more grain by applying the same concentration of P fertilizer within 30 d. This was similar to our conclusion. Zavišić et al. [57] believed that fertilized beech from P-deficient soil showed a strong increase in leaf P concentration from P deficiency to P-rich conditions. Therefore, the shoot and root P uptake of wheat increased by the application of P fertilizer within 14–21 d after planting.

Phosphorus deficiency causes a decrease in N and K concentrations and uptake by plants [58,59]. However, P application can increase the uptake of N, K, and other nutrients [60]. We found that delayed P application for 14–21 d also promoted the absorption of N and K in wheat. This was because when the P content in the plant increased, the N and K contents were at relatively low levels. In order to maintain the physiological needs of the plant, wheat increased N and K uptake. P deficiency reduced nitrate uptake by plants, while plants with sufficient P promoted ammonium uptake by 20% of the total N [61]. Similarly, P supplementation significantly enhanced the P, N, and K uptake in shoots, roots, pods, and the whole soybean plant [62]. The difference in nutrient content between the shoot and root in our study was related to the distribution of nutrients under different P conditions. It was generally reported that P deficiency decreased P and N concentrations in leaves and stems of *Catolaccus grandis*, decreased the P, N, and K concentrations in roots, while it increased the K concentration in leaves and stems [63]. Therefore, P, N, and K uptake in wheat increased with the proper P application delay.

4.3. Nutrient Utilization as Affected by P Application Time

Crops only absorb 15–30% of the seasonal P fertilizer, and the remaining amount (about 80%) becomes part of the soil phosphorus pool [64]. Therefore, increasing P use efficiency is very important as the P resource is finite. Many researchers made attempts to improve P fertilizer utilization in cultivation [65–67]. Cultivating P-efficient crops is also an effective method to improve P use efficiency, but P-efficient crops are not always associated with good quality of the grains, disease-resistant, or pest-resistant. As for P application time, it was reported that applying P early in crop growth can improve P uptake compared with late application [29]. According to our results, delaying P application for 21 d can significantly increase P, N, and K use efficiencies when the soil available P value is low. We can assume that a longer period of P application delay would be needed when soil available P is high. Further studies need to be carried out to investigate the relationship between soil available P level and the delay time for P application in crops.

The ability of plant roots to explore nutrients is an important factor determining nutrient uptake by plants except for P availability in soils. Phosphorus acquisition per plant is usually determined by two main components: root morphological characteristics and root physiological characteristics [40], which are RS–ACP and RS–ALP in the study. Plants with more fine roots have better foraging ability for nutrients and better P acquisition ability in low P soils [68]. Due to the low diffusion rate and chemical fixation of P_i in the soil, roots explore only about 20% of the topsoil during the period of vegetation, so enhancing topsoil foraging by roots is an important way to increase P_i uptake [3]. It is reported that annual plants usually delay flowering and maturation due to low P stress,

which leads to a longer duration of root foraging and increased P acquisition [68]. In our experiment, we increased the foraging ability of wheat roots for nutrient uptake by altering root morphology induced by a low P supply environment. When the soil is low in available P, delaying P application for 14–21 d can effectively improve the foraging ability for P by wheat roots. Therefore, controlling P application time can be an effective way to increase P acquisition ability by wheat.

5. Conclusions

As wheat is a vital food crop and the utilization rate of P fertilizer is low, improving P utilization rate and reducing P waste are of great significance for the sustainable management of P and precision agriculture. In this study, we found that the strategy of proper delayed P application at the early stage not only can increase P, N, and K uptake of wheat by changing root morphology and phosphatase activity but can also reduce the fertilizer input to some extent. With the development of fertigation, the strategy of delayed P fertilizer application will be increasingly used in agricultural production. In addition, the results are closely correlated to soil P conditions and soil properties associated with P transformation in soils. Therefore, soil P conditions and the time of P delay application need to be validated in the whole cycle field study before this strategy is widely used in the future. With the development of controlled release fertilization and fertigation, the strategy of proper delayed P fertilizer application will be utilized in agricultural production.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture13040884/s1, Figure S1: Wheat root morphological traits in response to P application time: (a) specific root length and (b) total fine root length. P0 means P application at planting; P7 means P application at 7 d after planting; P14 means P application at 14 d after planting; P21 means P application at 21 d after planting and NP means no P application. Different lower–case letters mean significant difference in specific root length and total fine root length among different treatments at $p < 0.05$, n = 4.

Author Contributions: H.Y. and Y.Z. contributed to the conception and design of the study. H.Y., S.H. and Y.L. conducted the measurements. H.Y. and Z.X. analyzed the data. H.Y. wrote the first draft of the manuscript. Y.Z., M.D. and X.W. revised the draft. All authors contributed to the manuscript revision and read and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

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