Characterisation of soil phosphorus forms in the soil-plant system using radioisotopic tracer method

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Abstract: Soil incubation and pot experiments were conducted to follow the sorption processes of added phosphorus (P) fertiliser using the radioisotope tracer technique. Increasing doses of P fertiliser (40, 80, 160, 320 mg P/kg soil) were added to Chernozem and Arenosol and incubated for 1, 3, and 13 weeks. After incubation, perennial ryegrass (*Lolium perenne* L.) was sown in one group of pots, and the experiment had been continuing for another 9 weeks. The yield, grass P uptake, isotopically exchangeable (P_{IE}), water-soluble (P_W), and ammonium lactate soluble phosphorus (P_{AL}) fractions of soils were measured. On Chernozem, plant P uptake, P_{IE}, P_W and P_{AL} were significantly less in the case of the longest incubation period compared to shorter incubations. This suggests a transformation of P into tightly sorbed form. On Arenosol, there were only small changes in the parameters as the incubation period increased, suggesting less intense P transformation to tightly sorbed form. The P_W/P_{IE} ratio enhanced with increasing P-doses, and the ratios were higher on Arenosol. On Arenosol, the higher P doses caused a greater increase of P_W than on Chernozem. The $P_{IE} + P_W$ showed a good correlation with plant P uptake proving this value can be a good indicator of plant-available phosphorus.

Keywords: heterogeneous isotope exchange; ^{32}P-labeled phosphate; plant nutrient; P cycling; adsorption; bioavailability

Phosphorus (P) is one of the most important plant nutrients (Mengel 2001, Goll et al. 2012).

The total P content of soil usually ranges from 0.02% to 0.15%. The major part of soil P is not available to plants and is therefore often a limiting nutrient in crop production (Fuhrman et al. 2005, Jakab 2020). Nowadays, increased environmental concerns and continuously decreasing P resources drive the study of P cycling processes in the soil-plant system (Helfenstein et al. 2018).

Phosphate anion, as the dominant chemical species of P, can be found dissolved in the soil solution (water-soluble P, P_W) and sorbed on the different soil components (minerals, Fe/Al oxides, hydroxides, calcium carbonate, organic matters (Hinsinger 2001, Pierzynski et al. 2005, Arai and Sparks 2007). Plants can take up dissolved phosphate species primarily in the forms of $H_2PO_4^-$ and HPO_4^{2-} ions, the distribution of these being governed by the pH of the solution and may be estimated from thermodynamic data (Schilling 2000).

The phosphate species sorbed on soil components can be classified as weakly (isotopically exchangeable phosphorus, P_{IE}) and tightly sorbed P species (organic forms, minerals, precipitates $P_{tightly}$) (Barrow and Shaw 1975, Mansell et al. 1977, Sparks 1989, Del Campillo

et al. 1999, Shuai et al. 2014). There is a continuous exchange between the weakly sorbed and dissolved phosphate. This exchange process is driven by the change of phosphate concentration of the soil solution. As the plant grows, the phosphate concentration of the soil solution decreases since the plant takes up some of the dissolved phosphates. The addition of water-soluble phosphate fertilisers increases, while the adsorption of phosphate on soil components and the transformation of weakly sorbed phosphate to tightly sorbed forms decreases the phosphate concentration of soil solution (Moody and Bolland 1999).

When the phosphate concentration of soil solution is less than what the plant requires over the growing season, the P in soil solution needs to be replenished from P pools, including weakly and tightly sorbed P species (Pierzynski et al. 2005). This replenishment process is affected by soil P species and their transformation processes (Sposito 2008). The bioavailability of P correlates with the P concentration of soil solution, which in turn, depends on the sorption-desorption, precipitation-dissolution, mobilisation-immobilisation processes of P in soil (Frossard et al. 1995, Hinsinger 2001, Barrow and Debnath 2014).

The phosphate species available for plants are traditionally determined by extraction methods such as Olsen (Olsen et al. 1954), Mehlich 3 (Mehlich 1984), Bray (Bray and Kurtz 1945), Egnér (Egnér et al. 1960) methods (Daly et al. 2015, Audette et al. 2016, Duminda et al. 2017, Miller and Arai 2017, Yan et al. 2017). However, according to Frossard et al. (1994), these chemical methods determine the available and a significant part of non-available P together because the extractants can modify the equilibrium between the various P forms. They state that the isotopic exchange method for characterising the available P for plants is more accurate because the determination of isotopically exchangeable P (P_{IE}) does not modify the equilibrium of soil-solution phases. The P_{IE} is presumably an easily accessible P fraction for plants (Morel and Plenchette 1994). According to Hamon et al. (2002), the quantity of P available for the plant can be approximated by $P_W + P_{IE}$. Other researchers (Pote et al. 1996) state that the water-extractable P represents the readily available P fraction of soil.

The isotope exchange suggested by Frossard et al. (1994) is a good method for the study of the exchange of P between the soil solution and the weakly sorbed P species. In our previous works, we proposed a model (Kónya and Nagy 2015) for the evaluation of the isotope exchange of the radioactive ^{32}P isotope,

which provides a more accurate description of the kinetics of the isotope exchange. This model was used to study the equilibrium of the dissolved and weakly sorbed phosphate. Furthermore, we examined the effect of P doses and incubation periods. The quantity of weakly sorbed P as well as the exchange rate between the soil and soil solution were also described (Nagy et al. 2018, 2019).

In this work, we investigate the changes of phosphate fractions (P_W, P_{IE}, $P_{tightly}$, P_{AL}) in the soil in dependence on P supply, incubation time and the plant growing. In addition, we study the correlations between P fractions and P uptake by the plant.

A better understanding of P sorption processes in soil-plant systems will allow contributing to achieving nutrient management more effectively.

MATERIAL AND METHODS

Pot experiment. In order to measure the changes of different P forms with increasing P fertiliser rates in time and to evaluate the effect of plant presence on the P forms, a greenhouse pot experiment was set up on Chernozem and Arenosol. The soils were classified according to World Reference Base (WRB) for Soil Resources (European Soil Bureau Network European Commission 2005, IUSS Working Group 2014). The soil samples were collected from the upper 0–30 cm layer of arable areas in the eastern part of Hungary. The main properties of soils can be found in Table 1.

Chernozem (2.5 kg) and Arenosol (3.0 kg) were weighed into pots and were incubated at room temperature with an increasing rate of P containing fertiliser (0, 40, 80, 160, 320 mg P/kg soil), as KH_2PO_4, respectively. (Since P was added as KH_2PO_4, K supply was also improved, which is mainly important in the case of Arenosol with poor K supply.) All treatments were set up in three replications. The soils were incubated at room temperature (20 °C) with constant soil water content (60% of maximum water holding capacity of soil) for 1, 3, and 13 weeks, respectively.

After 1, 3, and 13 weeks of soil incubation, the pot experiment was continued as follows:

Pots containing incubated soils were divided into two groups. Perennial ryegrass (*Lolium perenne* L.) was sown into one group of pots (two replications), and no plants were grown into the other ones. All pots, with and without plants, were irrigated with deionised water to keep the soil at 60% of the water holding capacity.

Table 1. The main properties of experimental soils

	Arenosol	Chernozem
pH_{H_2O}	8.25	6.59
pH_{KCl}	7.4	5.57
Plasticity according to Arany*	< 30	40
Hydrolytic acidity	0.54	2.15
Organic carbon (g/kg)	4.7	13.8
Total nitrogen (g/kg)	0.465	1.36
Clay and silt (%)	5.15	44.26
$CaCO_3$ (%)	1.24	–
Ammonium-lactate extractable-P (mg/kg)	147	36.4
Ammonium-lactate extractable-K (mg/kg)	76.1	173.3
Total P (digestion with H_2SO_4) (mg/kg)	700	925

*plasticity index according to Arany: water quantity taken up by soil to reach plasticity capacity ($cm^3/100$ g)

The scheme of the experiment is presented in Table 2.

The total duration of the experiment was 10, 12 and 22 weeks (1, 3 and 13 weeks soil incubation + 9 weeks plant growing), respectively.

Analysis of plant samples. At the end of the experiment, ryegrass was harvested, dried at 60 °C, and the total dry biomass was measured. The total plant P was determined after the digestion with H_2SO_4-H_2O_2 by ammonium molybdate vanadate spectrophotometric method (EN ISO 6878, 2014, Thamm et al. 1968). The P uptake of the plant was calculated from the P concentration in the shoot and dry matter yield of the plant shoot and was expressed in mg P/kg soil.

Analysis of soil sample, the study of heterogeneous isotope exchange. At the end of the pot experiment, from pots with and without plants, P_{IE}, P_W and P_{AL} fractions were measured.

P_W was determined by shaking 1:200 soil to water suspension for 2 h, and the P concentration of the extract was measured by ammonium phospho-molybdate blue photometric method (EN ISO 6878, 2004).

To determine the amount of exchangeable P, a heterogeneous isotope exchange examination was performed. Kónya and Nagy (2015) published a detailed description of the method. Here is given a brief overview: soil suspension with 1:200 soil to tri-distilled water ratio was prepared, then carrier-free ^{32}P (as KH_2PO_4) solution was added to the suspension. Aliquot samples were collected at different times, filtered, and the amount of ^{32}P isotope of the solution was measured by liquid scintillation. The heterogeneous isotope exchange equilibrium was approached in 2 h. The amount of exchangeable P was calculated from the steady-state data. The amounts of tightly sorbed P form with and without plant growing were calculated as follows:

$$P_{tightly} \text{ without plant} = P_{total} - P_{IE} - P_W$$

$$P_{tightly} \text{ with plant} = P_{total} - P_{IE} - P_W - P \text{ uptake of plant}$$

The ammonium-lactate – acetic acid-soluble P content (P_{AL}) was determined according to Egnér et al. (1960). In this method, 5.00 g soil was extracted for 120 min with 100 cm^3 ammonium lactate acetic acid (pH 3.7). After filtration, the phosphate concentration of the solution was measured colorimetrically.

Statistical analysis. One-way ANOVA analysis was carried out to evaluate the effect of phosphorus rates and incubation period separately on dry biomass, on plant P uptake and soil P fractions. The means of the studied parameters were compared by Tukey post hoc test at probability $P < 0.05$. Significantly different means were indicated by different letters. In the case of pots without plants, the above-mentioned soil and plant parameters were not analysed statistically, just presented in a graph to show the tendencies. In order to compare the amount of soil phosphorus fractions between the treatments with and without plants, paired-sample T-tests were carried out. The relationships between various phosphorus forms were developed by regression analysis. Data analysis was performed using SPSS 13.0 software package (Chicago, USA).

Table 2. The treatments of the experiment

Phosphorus application (mg P/kg soil)		Incubation periods		Plant growing		Soil
0						
40		1 week				Chernozem
80	×	3 weeks	×	no/yes	×	Arenosol
160		13 weeks				
320						

Table 3. Shoot dry biomass production (g/pot) as affected by phosphorus (P) application and incubation time

P rates (mg P/kg)	Chernozem			Arenosol		
	1 week	3 weeks	13 weeks	1 week	3 week	13 weeks
0	8.95[aA]	9.70[aA]	8.05[aA]	2.93[aA]	2.58[aA]	2.78[aA]
40	9.30[aA]	10.20[aA]	9.85[bA]	2.83[aA]	2.33[aA]	2.78[aA]
80	9.45[aA]	9.55[aA]	8.95[abA]	2.53[aA]	2.38[aA]	2.78[aA]
160	9.05[aA]	9.05[aA]	9.15[abA]	2.53[aA]	2.33[aA]	3.13[aA]
320	8.60[aA]	8.30[aA]	8.60[abA]	2.23[aA]	2.23[aA]	3.43[aA]

Different lowercase letters indicate significant differences between phosphorus rates within a column, different uppercase letters indicate significant differences between incubation periods, within a row at probability P = 0.05

RESULTS AND DISCUSSIONS

Dry biomass production and P uptake of ryegrass. The dry biomass production of the plant on two soils is shown in Table 3. As seen, the values of Chernozem were about three times larger than those on Arenosol. This is caused by the better N supply of Chernozem (Table 1). Similar shoot biomass ranges of ryegrass (1.73–14.1 g/pot) were measured in the experiment of Kremper et al. (2008), where 17 significantly different soil types with different fertility were compared.

In the case of Chernozem, the smallest P rate (40 mg P/kg soil) slightly increased the yield as expected from the poor P supply, but the highest P rate (320 mg P/kg) caused a slight decrease compared to the values of 40 mg P/kg treatment.

In the case of Arenosol, where the P supply of soil originally was good, the increasing P rates did not influence biomass production.

P uptake of ryegrass increased on Chernozem as the P rates increased; however, the values belonging to the same P rates were smaller as the incubation periods were longer (Figure 1). At 13 weeks of incuba-
tion, the P uptake of fertilised plants on Chernozem became significantly lower than that of appropriate values at 1 and 3 weeks of incubations.

On Arenosol, the P uptake of ryegrass was much less than that on Chernozem due to the considerably lower biomass production. The addition of P and the increase of incubation time did not change the plant P uptake to such an extent as in Chernozem.

These results will be discussed later, considering the P fractions of soils.

P fractions of the soil. The values of P_{IE}, P_W, P_{AL} and $P_{tightly}$ with and without plant are shown in Figure 2.

Figure 2A shows that after plant growing, the P_{IE} values on Chernozem ranged between 5.0–67.9 mg/kg, while on Arenosol, these values were from 3.9 to 27.8 mg/kg. The P_{IE} results were almost similar to the results obtained by Cabeza et al. (2013) on acidic sandy soil and neutral loamy soil (26.1–63.2 mg/kg). The P_{IE} values of Chernozem were much higher than those on Arenosol. This can be explained by the higher organic matter and clay content of Chernozem compared to Arenosol. This assumption has also supported a statement by Cabeza et al. (2019), namely,

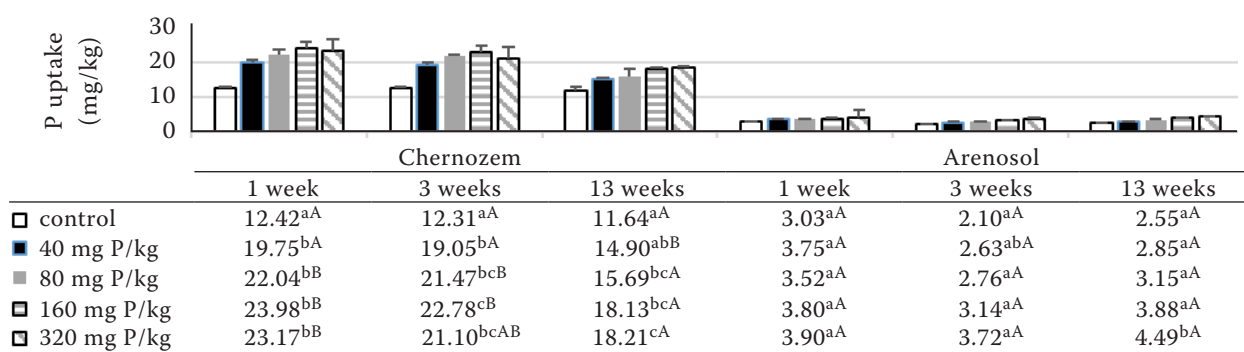|	1 week	3 weeks	13 weeks	1 week	3 weeks	13 weeks
	Chernozem			Arenosol		
☐ control	12.42[aA]	12.31[aA]	11.64[aA]	3.03[aA]	2.10[aA]	2.55[aA]
■ 40 mg P/kg	19.75[bA]	19.05[bA]	14.90[abB]	3.75[aA]	2.63[abA]	2.85[aA]
▩ 80 mg P/kg	22.04[bB]	21.47[bcB]	15.69[bcA]	3.52[aA]	2.76[aA]	3.15[aA]
☐ 160 mg P/kg	23.98[bB]	22.78[cB]	18.13[bcA]	3.80[aA]	3.14[aA]	3.88[aA]
☐ 320 mg P/kg	23.17[bB]	21.10[bcAB]	18.21[cA]	3.90[aA]	3.72[aA]	4.49[bA]

Figure 1. The phosphorus (P) uptake of the plant grown on Chernozem and Arenosol. Different lowercase letters indicate significant differences between phosphorus rates within a column, different uppercase letters indicate significant differences between incubation periods, within a row at probability P = 0.05

that the P_{IE} value is influenced by the mineralogy and chemical properties of soil. With increasing P rates, the P_{IE} values of pots with plants significantly enhanced on both soil types, but the relative increase was higher on Chernozem: at 1 week of incubation, 12.5–18.9% and 5.2–6.6% of added P (40–320 mg P/kg) was present as P_{IE} on Chernozem and Arenosol, respectively.

The amount of P_{IE} was significantly less at 13 weeks incubation period than at one week of incubation on Chernozem, but a smaller decrease was observed on Arenosol. At 13 weeks of incubation, about 6.6–9.5% of added P (40–320 mg P/kg) were measurable as P_{IE} on Chernozem, while on Arenosol, 0.8–4.0% of added P were present as P_{IE}. The direction of the changes in P_{IE} contents without plants (Figure 2B) as a function of P rates and incubation periods were similar to the direction obtained after plant growing. According to the paired *T*-test probe results (at $P < 0.05$), the P_{IE} values without plant were higher in both soil types than those obtained after plant growing, which can be explained by the P uptake of plant. These results agree with Cabeza et al. (2013), who also found that the P_{IE} values were higher without plant than values with plants because of the plant P uptake.

The P_W values after plant growing (Figure 2C) in all treatments were higher than P_{IE} values. The P_W values on Chernozem ranged between 9.0–222.0 mg/kg, while on Arenosol varied between higher values: 16.0–297.0 mg/kg. The high P_W values on Arenosol call attention to the risk of environmental pollution of this soil type: the fertiliser P remains in the soil solution for a long time and can get into water bodies causing eutrophication instead of plant uptake.

The addition of water-soluble phosphorus fertiliser significantly increased the P_W contents in both soils, but the increase was greater on Arenosol, showing the higher P sorption ability of Chernozem. The incubation period, however, decreases the P_W values on both soil types. This decrease was much higher on Chernozem: at 13 weeks of incubation, the P_W values decreased by half to a third compared to the P_W values at 1 and 3 incubation periods. At the same time, the $P_{tightly}$ values increased (Figure 2G, 2H), suggesting the transformation of isotopically exchangeable/weakly sorbed phosphate to tightly sorbed phosphate species (Nagy et al. 2019). On Arenosol, the P_W values much less decreased with increasing incubation period than on Chernozem. At the same time, the $P_{tightly}$ values on Arenosol slightly changed, the degree of isotopically exchangeable/

weakly sorbed to tightly sorbed P transformation was much less, showing that the number of P binding sites (e.g., humus content, clay content, Table 1) is less on Arenosol than Chernozem. The direction of the changes in P_W without plants (Figure 2D), including the effects of P rates and incubation periods, were similar to the changes in the presence of the plants. The P_W values of Chernozem with and without plants did not differ, while on Arenosol, the P_W values after plant were higher (at $P < 0.1$). These results suggest that the root-induced organic acids decreased the soil pH and caused P desorption and P dissolution from calcium phosphate precipitate on Arenosol. Marschner (2012) also observed that root-induced acidification in the case of white lupine decreased the pH of the rhizosphere by 2–3 units resulting in the increased availability of phosphorus.

The P_{AL} on Chernozem ranged between 31–305 mg/kg (Figure 2E). The values, similarly to P_{IE} and P_W, significantly decreased after 13 weeks of incubation, also suggesting the increasing ratio of tightly sorbed P at long incubation time.

On Arenosol, similarly to P_W values, the P_{AL} values were much higher than on Chernozem, namely ranged between 182–439 mg/kg (Figure 2E). The values did not change with increasing incubation periods. By analysing the changes of P_{AL} values, it can be stated that the 50% of 40 mg and 80% of 320 mg of added P was measurable in the AL soil extract at 1 and 3 weeks of incubation periods in both soil types. At 13 weeks of the incubation period, a large difference is observed in the P_{AL} values of the two soil types at the same added P quantities. On Chernozem, only about 9–27%, on Arenosol about 80% of 40 mg/kg and 320 mg/kg of added P was measurable in the AL extract. The higher recovery values of added P in the case of Arenosol can be explained by the greater dissolving effect of acidic extractant, suggesting the formation of calcium phosphate precipitate in Arenosol.

The $P_{tightly}$ values on Chernozem ranged from 895–1 125 mg P/kg soil; on Arenosol, these values changed between 677–746 mg P/kg soil (Figure 2E, 2F). By increasing the incubation period, the $P_{tightly}$ values significantly increased on Chernozem and slightly increased on Arenosol as mentioned and interpreted previously in this section.

The P_W/P_{IE} ratios with and without plant growing treatments (Figure 3) increased as a function of P doses. The P_W/P_{IE} ratio was much lower on Chernozem (1.1–3.0) than that on Arenosol (2.5–14.6). After 1 week of incubation, a higher ratio was
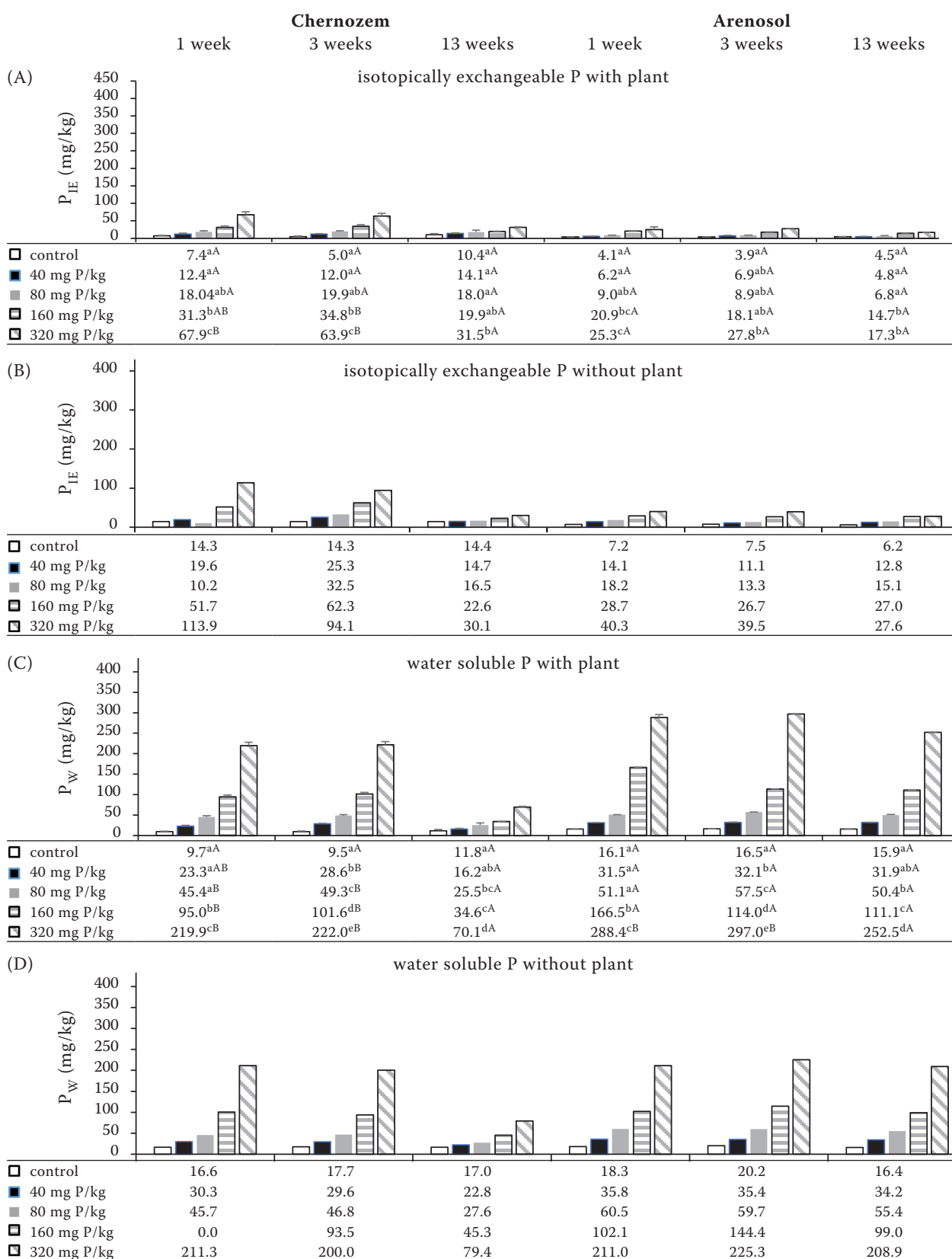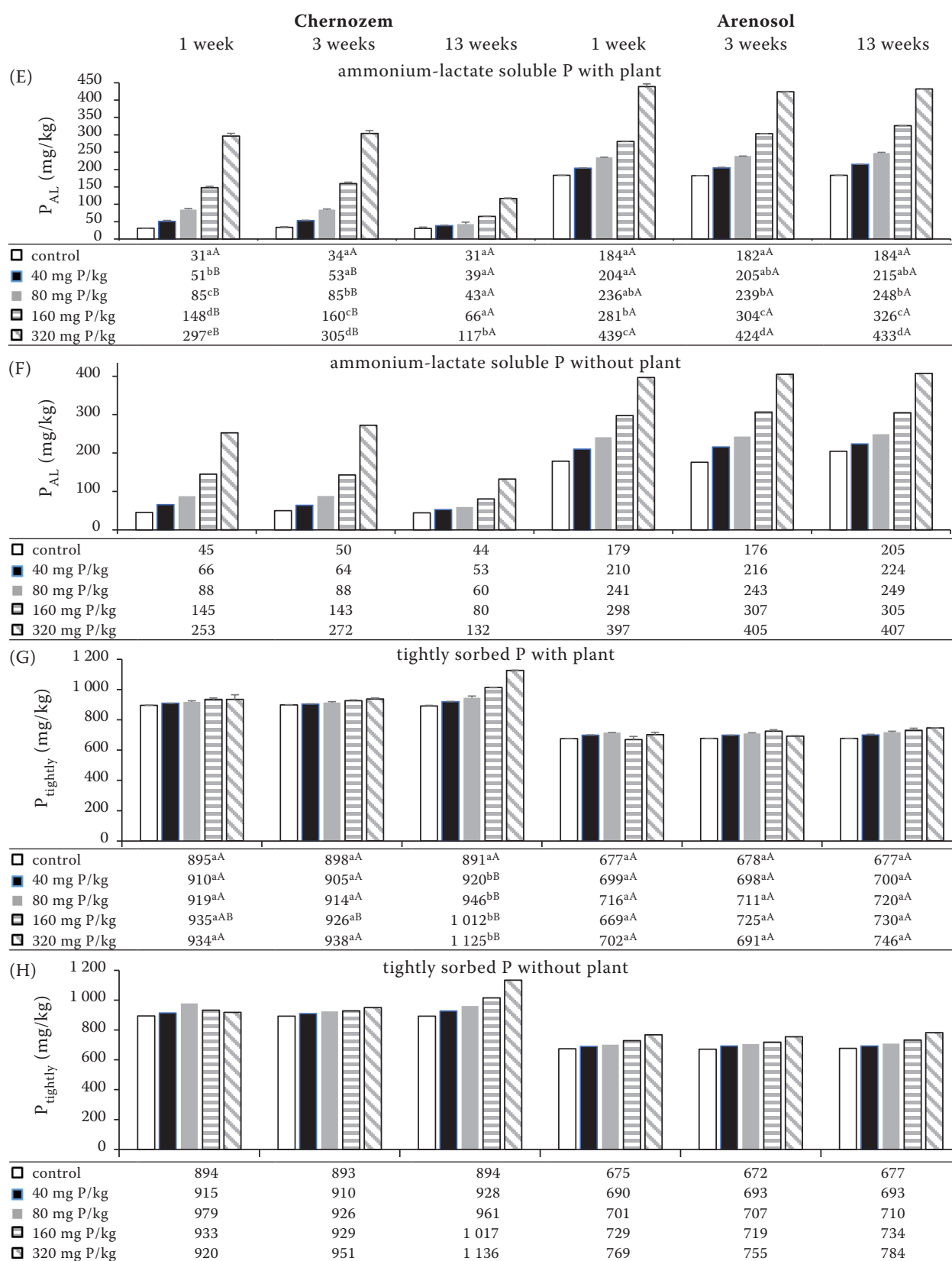
Figure 2. (A, B) The isotopically exchangeable phosporus (P_{IE}) and (C, D) water-soluble phosphorus (P_w) with and without plant growing. Different small letters indicate significant differences between phosphorus (P) rates, different capital letters indicate significant differences between incubation periods, at $P = 0.05$

Continued Figure 2. (E, F) Ammonium lactate soluble phosphorus (P_{AL}) and (G, H) $P_{tightly}$ with and without plant growing. Different small letters indicate significant differences between phosphorus (P) rates, different capital letters indicate significant differences between incubation periods, at $P = 0.05$
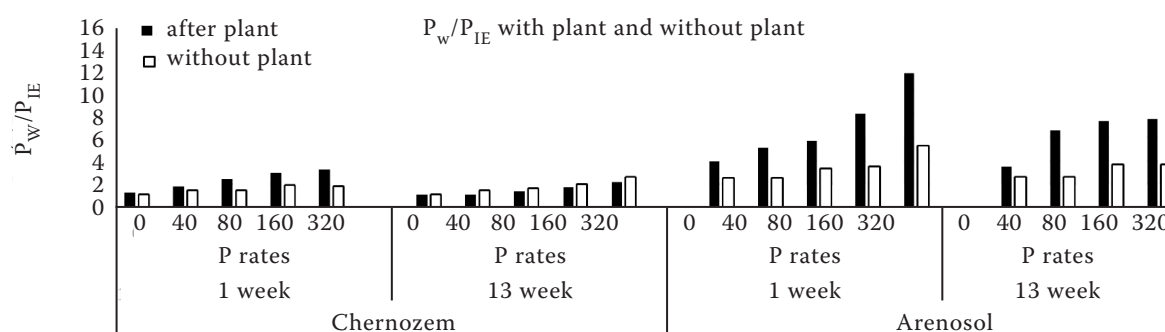
Figure 3. The ratio of water-soluble phosphorus (P_W) to isotopically exchangeable phosphorus (P_{IE}) with and without plants

observed in pots with plants in both soil types. At 13 weeks of incubation, the P_W/P_{IE} ratio decreased on Chernozem, and after plant growing, the ratio became lower than without plants. This may be attributed to two processes: the plants take up the decreased water-soluble P, and at the same time, most of P is transformed to tightly sorbed species. On Arenosol, the P_W/P_{IE} ratio did not change with the incubation period. The values were higher with the plant than without the plant. This could be explained by the fact that the root-induced organic acids dissolved the calcium phosphate precipitate and increase the P concentration in the soil solution.

Table 4 shows that the P_W was highly correlated with P_{AL} on both soils. Highly significant relationships were also found between $P_W + P_{IE}$ and P_{AL} values. Our results show that the sum of $P_W + P_{IE}$ values is approximately equivalent to the P_{AL} fraction on Chernozem, while on Arenosol, the P_{AL} is much larger than the sum of $P_W + P_{IE}$. This is likely due

to the acidic extraction: a part of tightly sorbed P is dissolved (e.g., from calcium phosphate).

A less tight correlation was found between P_{IE} and P uptake of plant, but a closer relationship was established between the sum of $P_{IE} + P_W$ and P uptake of plant. As was mentioned earlier here and in Kónya and Nagy (2015), P_W and P_{IE} are in equilibrium with each other. Thus, the sum of $P_{IE} + P_W$ is a better indicator of plant-available P fraction than P_{IE}. This is in good agreement with the results by Hamon et al. (2002), who stated that soluble phosphorus together with P_{IE} is the most likely available P source for plant uptake. Both P_W and P_{IE} can be measured accurately by using heterogeneous isotope exchange examination with ^{32}P tracer.

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Table 4. Relationship between different phosphorus (P) forms

		R^2
Chernozem (*n* = 30)		
$P_W - P_{AL}$	$P(P_{AL}) = 1.206\ P_W + 27.5$	0.981
$(P_W + P_{IE}) - P_{AL}$	$P(P_{AL}) = 0.866\ (P_W + P_{IE}) + 22.8$	0.956
$P_{IE} - P_{uptake}$	$P(P_{uptake}) = 6.05\ \ln(P_{IE}) + 1.58$	0.608
$(P_W + P_{IE}) - P_{uptake}$	$P(P_{uptake}) = 5.66\ \ln(P_W + P_{IE}) - 3.49$	0.848
Arenosol (*n* = 30)		
$P_W - P_{AL}$	$P(P_{AL}) = 0.942\ P_W + 183.24$	0.942
$(P_W + P_{IE}) - P_{AL}$	$P(P_{AL}) = 0.863\ (P_W + P_{IE}) + 177.1$	0.950
$P_{IE} - P_{uptake}$	$P(P_{uptake}) = 0.720\ \ln(P_{IE}) + 1.63$	0.502
$(P_W + P_{IE}) - P_{uptake}$	$P(P_{uptake}) = 0.545\ \ln(P_W + P_{IE}) - 0.924$	0.604

P_W – water-soluble phosphorus; P_{AL} – ammonium lactate soluble phosphorus; P_{IE} – isotopically exchangeable phosphorus

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