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The relationship between available P and selected biological properties in the rhizosphere of ten crop species under glasshouse conditions

Relación entre el P disponible y algunas propiedades biológicas en la rizosfera de diez especies vegetales cultivadas en invernadero

Relação entre o P disponível e um conjunto de propriedades biológicas selecionadas na rizosfera de dez espécies vegetais cultivadas em estufa

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ABSTRACT

The aim of the present study was to evaluate the effect of cultivation of 10 agronomic plant species on selected biological activities and bioavailability of phosphorus (P) in different sampling zones. The lowest available P was measured in the planted soil with *Solanum lycopersicum*. *Helianthus annuus*, *Zea mays* and *Phasaeolous vulgaris* had a relatively larger effect on the decrease of available P in the rhizosphere soil and in the soil adhering to the root mats. *S. lycopersicum* and *P. vulgaris* had the highest P concentration (> 980 mg kg^{-1}) and *H. annuus* and *Z. mays* had the highest P uptake (17.62 mg pot^{-1} and 13.13 mg pot^{-1} respectively). The mean soil microbial biomass P (MBP) was significantly high in the rhizosphere soil and in the soil adhering to root mats of *T. aestivum*, *Z. mays*, *S. tuberosum* and *S. lycopersicum* (> 16 mg kg^{-1}). The mean spore numbers of glumales (SNG) were significantly high in the rhizosphere soil and in the soil adhering to root mats of *P. vulgaris*, *S. lycopersicum*, *T. aestivum* and *Z. mays* (> 167 N/10 g soil). The negative correlations of available P and soluble P with soil biological properties like SNG and alkaline phosphatase activity and the positive correlation with soil pH shows the importance of rhizomicroorganisms and glumales on P nutrition by plants in calcareous soil. Thus rhizosphere acidification of calcareous soil may not be as important as the improvement of biological properties in P uptake and acquisition by plants.

RESUMEN

El objetivo del presente trabajo fue evaluar el efecto del cultivo de 10 especies de plantas vegetales sobre determinadas actividades biológicas y sobre la biodisponibilidad de fósforo (P) en diferentes zonas de muestreo. El valor más bajo de P disponible se midió en el suelo con Solanum lycopersicum. Helianthus annuus, Zea mays y Phasaeolous vulgaris tuvieron un efecto relativamente mayor sobre la disminución de P disponible en la rizosfera del suelo y en el suelo adherido a las raíces. S. lycopersicum y P. vulgaris presentaron la concentración de P más elevada (> 980 mg kg^{-1}) y H. annuus y Z. mays fueron las especies que mostraron una mayor asimilación de P (17,62 mg $maceta^{-1}$ y 13,13 mg $maceta^{-1}$, respectivamente). El contenido medio de P en la biomasa microbiana del suelo (MBP) fue significativamente elevado en la rizosfera del suelo y en el suelo adherido a las raíces de T. aestivum, Z. mays, S. tuberosum y S. lycopersicum (> 16 mg kg^{-1}). La media del número de esporas de glomales (SNG) presentó valores elevados en la rizosfera del suelo y en el suelo adherido a las raíces de P. vulgaris, S. lycopersicum, T. aestivum y Z. mays (> 167 N/10 g suelo). Las correlaciones negativas entre el P disponible y soluble con propiedades biológicas

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del suelo, como el SNG y la actividad fosfatasa alcalina, así como la correlación positiva con el pH del suelo, pusieron de manifiesto la importancia de los microorganismos de la rizosfera y de los glomales sobre la nutrición fosfatada de especies vegetales en suelos calizos. Así, la acidificación de la rizosfera en suelos calizos podría no ser un factor tan importante en la asimilación y adquisición de P por las plantas como es la mejora de las propiedades biológicas.

KEYWORDS
Phosphorus, bioavailability, nonrhizosphere

PALABRAS CLAVE
Fósforo, biodisponibilidad, no rizosférico

PALAVRAS-CHAVE
Fósforo, biodisponibilidade, não rizosfera

RESUMO

O objectivo do presente estudo foi avaliar, em diferentes zonas de amostragem, o efeito do cultivo de 10 espécies vegetais em determinadas propriedades biológicas selecionadas bem como na disponibilidade de fósforo (P). A menor disponibilidade de P observou-se nos solos cultivados com Solanum lycopersicum. A Helianthus annuus, Zea mays e o Phasaeolous vulgaris foram as espécies que tiveram um efeito mais acentuado no decréscimo do P disponível na rizosfera do solo e nas zonas do solo aderentes às raízes. S. lycopersicum e o P. vulgaris foram as espécies que apresentaram a maior concentração em P (> 980 mg kg^{-1}) e a H. annuus e a Z. mays as que absorveram maior quantidade de P (17,62 mg $vaso^{-1}$ e13,13 mg $vaso^{-1}$ respectivamente). A média da biomassa microbiana de P (MBP) apresentou valores significativamente elevados na rizosfera do solo e no solo aderente às raízes de T. aestivum, Z. mays, S. tuberosum e S. lycopersicum (> 16 mg kg^{-1}). A média do número de glumelas dos esporos (SNG) foi significativamente elevada na rizosfera do solo e nas zonas do solo aderentes às raízes nas espécies P. vulgaris, S. lycopersicum, T. aestivum e Z. mays (> 167 N/10 g solo). As correlações negativas entre o P disponível e o P solúvel com as propriedades biológicas do solo nomeadamente o SNG e a actividade da fosfatase alcalina e a correlação positiva com os valores de pH do slo demonstram a importância dos rizomicrorganismos e das glumelas na nutrição fosfatada das plantas instaladas em solos calcários. Assim para a aborção de P pelas plantas a acidificação pela rizosfera de solos calcários pode não ser tão importante como a melhoria de determinadas propriedades biológicas.

1. Introduction

Phosphorus deficiency is one of the major limiting nutrition problems for plants, particularly in both acidic and calcareous soils where P retention and precipitation is greatest (Marschner 1995; Hinsinger 2001). Plant phosphorus absorption depends on its concentration gradient and diffusivity in the soil near the roots. Under such conditions, root-soil interactions in the rhizosphere noticeably affect the availability of P to plants (Marschner 1995).

Plant species have evolved various adaptations in order to obtain soil P (Hinsinger 2001; Vance et al. 2003; Raghothama and Karthikeyan 2005), such as rhizosphere acidification and H^+ secretion (Neumann and Römheld 1999; Hinsinger et al. 2003; Shen et al. 2004), exudation of carboxylates (Neumann and Römheld 1999; Shen et al. 2001; Wang et al. 2007), and secretion of acid phosphatase (Tadano et al. 1993; Neumann et al. 2000).

Guo et al. (2000) reported that continuous cropping of maize (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr) on different tropical soils loaded with high dose of fertilizer P highly depleted the labile and moderately labile inorganic P (Pi) fractions. These authors also stated that the organic P (Po) and stable P fractions remained almost constant at the end of 14 crop harvests except the labile Po in a Mollisol that was highly depleted. Similarly, it was reported that growing alfalfa (*Medicago sativa* L.) for 66 days highly depleted the native labile Pi in the rhizosphere of different soil orders without affecting the Po fractions (Crews 1996).

A study by Safari and Rashidi (2011) on inorganic P fractions in soil revealed that plant species significantly decreased all the inorganic P fractions in the soil adhering to the root mats and in rhizosphere soil compared to those in nonrhizosphere soil. However these decreases were not equal for each fraction, and the percentage of apatite-P increased in rhizosphere soil. In general the mean total P, soluble P, dicalcium phosphate P (DCP-P), octacalcium phosphate P (OCP-P), Al-oxide and -hydroxide bonded P (Al-P) and Fe-oxide and -hydroxide bonded P (Fe-P) were lower in soil adhering to root mat compared to those in rhizosphere soil (Safari and Rashidi 2011).

Different techniques for studying chemical changes in the rhizosphere have been used for annual crops, grasses and legumes (Hedley et al. 1994). Some of these studies assumed that soil particles adhering to the roots were representative of rhizosphere soil and that the soil distant from the roots was bulk soil and not influenced by plant roots (Ohno 1989; Riley and Barber 1971). The practical problem of taking soil samples at known distances from the root surface is a significant obstacle in this approach. In other studies this problem was overcome by growing plants in soil in a cropping device based on the early work of Kuchenbuch and Jungk (1982), where a planar mat of roots was physically separated from the soil by a polyester mesh (Zoysa et al. 1997). The rhizobox of Kuchenbuch and Jungk (1982) consists of a soil-root compartment separated by a membrane, which is located on top of a soil compartment. In this design, the root plane displays horizontal orientation as a root mat is formed on the membrane at the bottom of the soil-root compartment. Thin sections of soils at various distances from the mesh (rhizoplane) were sliced and chemically analysed to determine root induced chemical changes (Gahoonia and Nielsen 1991; Hedley et al. 1994; Wang et al. 1995; Youssef and Chino 1989). However the differences and precision of the above methods for sampling the rhizosphere soil need to be further studied and evaluated, and in particular whether there are any significant differences in the results of the above methods for rhizosphere study.

The objective of this paper was to examine P bioavailability and relevant biological soil properties in the soil adhering to the root mat as a representative of rhizosphere soil, compared to the rhizosphere soil sampled by the modified Kuchenbuch and Jungk (1982) technique. In order to determine which mechanism was more important in P acquisition by plants in calcareous soils, we also examined the correlations between available P and soil pH, microbial biomass P (MBP), alkaline and acid phosphatase activities and spore numbers of glumales in the rhizospheric and nonrhizospheric soil of 10 different plant species under glasshouse condition for a better understanding of the importance of different mechanisms in P acquisition by plants with the use of thin slicing technique.

2. Materials and Methods

• Soil sampling and analysis

A surface soil (0-30 cm deep) was sampled from the Hydare fields in the Hamadan province, northwest of Iran with a semi-arid climate (annual rainfall of 300 mm; annual average temperature 13 °C). The methods applied for soil biological, physical and chemical analysis and the results were reported previously (Safari and Rashidi 2011). Selected soil properties were determined according to standard methods. Particle-size was measured using the hydrometer method. Equivalent calcium carbonate (ECC) was measured by back titration. Soil pH and electrical conductivity (EC) were measured in a 1:5 soil:water extract after shaking for 30 min. Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate. Total nitrogen in all samples was determined by the Kjeldahl method. Cation-exchange capacity (CEC) and available K were measured by the method of sodium saturation. Available phosphorus was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid. These soil characteristics were measured according to methods of soil analysis published by SSSA (Klute 1986; Page et al. 1992).

Fresh soil samples were stored at 4 °C for microbiological analyses. We determined microbial biomass P (MBP) in each sample using $CHCl_3$ as a biocide, and bicarbonate as an extractant (Brookes et al. 1982; Hedley and Stewart 1982). Spores of VAM fungi were counted and reported for 10 g of soil (Sylvia 1994). Basal respiration was measured as CO_2 evolved in 5 days. Substrate induced respiration was determined over 72 hours (Alef and Nannipieri 1995). Soil acid and alkaline phosphatases were analyzed according to the methods of Eivazi and Tabatabai (1977).

• Plant culture and rhizosphere study technique

The experiment was set out in a glasshouse as a completely randomised design (Safari and Rashidi 2011). The experiment comprised 3 replicates of 11 treatments (unplanted (control) and 10 plant species from five families). Three seeds of *Triticum aestivum* cv. Alvand, *Zea mays* cv. Ksc 500, *Solanum tuberosum* cv. Sante, *Solanum lycopersicum* cv. Primoerly, *Helianthus annuus* cv.; 5 seeds of *Lathyrus sativus* cv. IFLS 170 SEL. 439, *Phasaeolous vulgaris* cv. Dehghan, *Brassica napus* cv. Okapi, *Carthamus tinctorius* cv. Padede, or up to 20 seeds of *Lepidium sativum* were sown per pot. These plant species are commonly cultured in the region. The thin slicing technique described by Kuchenbuch and Jungk (1982) and Zoysa et al. (1997) was adopted from Chen et al. (2002). It was modified and used to sample rhizosphere soil in this study. The experimental setup has been described in the previous paper (Safari and Rashidi 2011). **Figure 1** shows the schematic representation of the plant growth container. Each plant growth container (PGC) comprised a two-compartment PVC cylinder, the compartments separated by 25 µm galvanized-iron mesh. The upper compartment was 20 cm high with an internal diameter of 20 cm and had 3 kg soil. While the lower compartment had 1.8 kg soil and comprised one PVC cylinder of the same diameter (20 cm) with heights of 50 mm with 100 µm nylon mesh in the bottom which separates it from silicon powder base. Soil sampled from the top 10 mm of the lower compartment was taken as rhizosphere soil (R) and that sampled from bottom 40 mm was taken as nonrhizosphere soil (S). Soil adhering to the root mat (SAD) was gathered and analyzed separately for comparing with rhizosphere soil in this thin slicing technique.

Seeds of each species were directly planted into the upper compartment and thinned to a suitable number of seedlings per pot after germination. Soil moisture was maintained near field capacity (FC) for the duration of the experiment by daily watering. Water was added to the 5 cm silicon powder base to saturate this fine sandy layer by daily watering. Excess water was gathered by an automatic drainage system. Soil wetting was therefore done by upward movement of water.

When root mats had developed after 60 days growth, plants were harvested. Some of the plants were at flowering stage by this time. After washing, plant shoots and roots were air-

dried and oven-dried at 70 °C. The dry weight of shoots and roots of plants were measured. The plant materials were dry-ashed at 550 °C and thereafter the ash was taken up in 2 M HCl solution and analysed for P spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid. Phosphorus concentration in shoots and roots of plants were determined and P uptake was calculated:

P uptake (mg pot^{-1}) = P concentration (mg kg^{-1} DW) in plant x plant dry weight (kg pot^{-1})

Soil adhering to the root mat, rhizosphere soil and nonrhizosphere soil was sampled separately and analyzed for available P, MBP, the activities of alkaline and acid phosphatases and the number of AM fungi spores.

• Statistical analyses

The experiment was considered a completely randomized factorial design in three replicates. In this study the factors were plant species (10 different species and control) and soil sampling zone (soil adhering to plant root, rhizosphere soil and nonrhizospheric soil). Data were statistically analyzed for standard deviation, means were calculated, and Duncan's new multiple range tests were performed to assess the effect of plant species and sampling zone on available P, MBP, alkaline and acid phosphatase activities and spore numbers of glumales. The computer programs used for data analysis were Ms-Excel and SAS 6.12 for Windows.

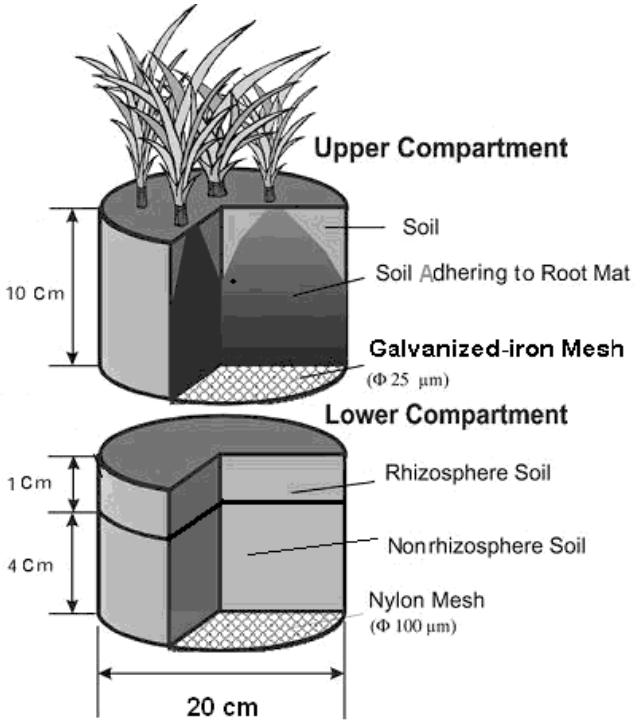

Figure 1. Schematic representation of the plant growth container.

3. Results and Discussion

The chemical, physical and biological soil properties are reported in **Table 1**. The studied plant species had significantly different dry weights and P uptakes (**Table 2**). The dry weights of *Z. mays* and *H. annuus* were markedly higher than the dry weights of the other plant species. They were 17.78 and 14.53 mg pot^{-1}, respectively. They were significantly different. The lowest plant dry weight belonged to *L. sativus* (6.19 mg pot^{-1}). There were no significant differences between the dry weight means of *T. aestivum, S. tuberosum, S. lycopersicum, P. vulgaris*, and *B. napus*.

The P uptake from soil was significantly different between plant species (**Table 2**). The highest and the lowest P uptakes were by *Z. mays* (17.62 mg pot^{-1}) and *L. sativus* (5.58 mg

Table 1. Soil characteristics used in this study (dry weight basis)

Soil property	Unit	Amount	Soil property	Unit	Amount
Texture	-	Loam	Alkaline phosphatase act	µmol P.N.P g^{-1} soil h^{-1}	5.15
Sand	%	38	Acid phosphatase act	µmol P.N.P g^{-1} soil h^{-1}	1.71
Silt	%	37.4	Glumales spores	N/10 g soil	107
Clay	%	24.6	SIR*	mg CO_2 kg^{-1} soil day^{-1}	790.78
ECC*	%	6.82	Basal respiration	mg CO_2 kg^{-1} soil day^{-1}	32.79
pH	-	7.9	Microbial Biomass P	mg kg^{-1}	9.05
CEC	$cmol_c$ kg^{-1}	20			
EC	dS m^{-1}	0.18			
Organic C	%	1			
Total N	%	0.12			
Available K	mg kg^{-1}	368			
Available P	mg kg^{-1}	37.08			

* ECC- Equivalent $CaCO_3$. SIR- Substrate induced respiration.

Table 2. Plant dry weight, P concentration and P uptake by plant species. Data given as mean values #

Plant species	*Dry weight (g pot^{-1})*	*P concentration (mg kg^{-1})*	*P uptake (mg pot^{-1})*
T. aestivum	12.72 c	936 b	11.91 bc
Z. mays	17.78 a	991 a	17.62 a
S. tuberosum	12.83 c	866 d	11.12 c
S. lycopersicum	13.16 c	936 b	12.33 bc
L. sativus	6.19 f	901 c	5.58 f
P. vulgaris	11.98 c	992 a	11.89 bc
L. sativum	12.71 d	718 e	9.13 d
B. napus	12.38 c	944 b	11.69 bc
C. tinctorius	8.13 e	939 b	7.64 e
H. annuus	14.53 b	903 c	13.13 b

Means followed by the same letter in each column are not significantly different (P < 0.05).

pot^{-1}), respectively. The difference between the mean P uptake by *T. aestivum*, *S. tuberosum, S. lycopersicum, P. vulgaris*, and *B. napus* was not significant at 0.05 levels.

Analysis of variance of the effects of plant species and sampling zone on soil P bioavailability revealed that the effects of these treatments and their interaction on soil pH, available P, microbial biomass P (MBP), acid and alkaline phosphatase activities and spore numbers of glumales (SNG) after plant harvest were significant at 0.01 levels (**Table 3**).

Table 3. Analysis of variance of the effect of plant species (PS) and soil sampling zone (SSZ) on soil pH, available P (AP), microbial biomass P (MBP), acid and alkaline phosphatase activities (acid Ph. Act. and alkaline Ph. Act.) and spore numbers of glumales (SNG) after plant harvest

		pH	*AP*	*MBP*	*Acid Ph. Act.*	*Alkaline Ph. Act.*	*SNG*
Source	DF	MS	MS	MS	MS	MS	MS
PS	10	560.82**	114.28**	146.26**	12.72**	64.44**	2948.92**
SSZ	2	3182.28**	976.76**	415.63**	65.37**	912.21**	15372.64**
PS*SSZ	20	91.56**	14.42 **	30.32**	3.02**	16.03**	526.41**
Error	66	0.0005	0.43	1.01	0.003	0.01	18.05

* Mean square (MS) of the treatment is significant at the 0.05 level. ** Mean square of the treatment is significant at the 0.01 level.

Table 4 shows Duncan's new multiple range tests of means of soil pH, available P and MBP as affected by plant species and soil sampling zone. All plant species showed decreased pH in soils sampled from rhizosphere and soils adhering to root the mat. The highest pH was measured in the unplanted (control) soil (> 7.90). Non-rhizosphere soil had also a relatively high pH compared to those in the rhizosphere soil and in the soil adhering to root the mat of each species, due to soil acidification by plants and rhizo-microorganisms. In each soil sampling zone, soil pH was significantly low in the soil planted with *S. lycopersicum* (< 7.12) followed by the soil planted with *T. aestivum, Z. mays* and *S. tuberosum*.

The highest available P was measured in the unplanted (control) soil (**Table 4**), near 34 µg g^{-1}, with a decreased P availability for the plantings of all species. Nonrhizosphere soil had relatively high available P compared to the rhizosphere soil and in the soil adhering to the root mat of each species, due to P uptake by plants. Available P in nonrhizosphere soils was near 30 µg g^{-1}.

In each soil sampling zone, soil available P was significantly higher in the soil planted with *S. tuberosum*, followed by the soil planted with *L. sativum*. The mean available P in the rhizosphere soil and in the soil adhering to the root mat of *S. tuberosum* was 27.20 and 26.97 µg g^{-1} respectively, and for *L. sativum* 25.21 and 23.68 µg g^{-1}, respectively. This may be related to the low uptake and/or positive effect of the planting of these species on P availability in soil. These plant species had relatively high dry weight and P uptake values (**Table 2**). It can be concluded that these plant species had a positive effect on P availability in soil. The lowest available P was measured in the planted soil with *S. lycopersicum*. The mean available P in the rhizosphere soil and in the soil adhering to the root mat of *S. lycopersicum* was 16.54 and 17.44 µg g^{-1} respectively. *H. annuus, Z. mays* and *P. vulgaris* had relatively greater effects on the decrease of available P in the rhizosphere soil and in the soil adhering to the root mat. *Z. mays* and *P. vulgaris* had the highest P concentration and *H. annuus* and *Z. mays* had the highest P uptake.

Table 4. Duncan's new multiple range tests of means of soil pH, available P and microbial biomass P (MBP) as affected by plant species and soil sampling zone

Plant species	Soil sampling zone	pH	-	Available P	mg kg^{-1}	MBP	mg kg^{-1}
		Mean	SD	Mean	SD	Mean	SD
T. aestivum	SAD##	7.21 p	0.01	22.07 ij	0.68	27.56 a	0.69
	R	7.25 p	0.02	22.3 1i	0.51	28.94 a	2.8
	S	7.74 h	0.01	30.88 ed	0.49	10.60 fgh	0.66
Z. mays	SAD	7.23 p	0.03	19.02 m	0.68	23.24 b	0.57
	R	7.24 p	0.03	21.16 ijk	0.77	21.27 c	1.08
	S	7.85 cd	0.01	30.98 ed	0.29	10.11 gh	1.36
S. tuberosum	SAD	7.30 o	0.01	26.97 f	0.29	20.00 c	0.69
	R	7.30 o	0.01	27.20 f	0.36	21.06 c	2.12
	S	7.86 bc	0.02	33.71 ab	0.25	10.25 gh	0.03
S. lycopersicum	SAD	7.08 r	0.02	17.44 n	0.43	16.57 d	0.71
	R	7.12 q	0.02	16.54 n	0.33	16.79 d	1.43
	S	7.75 h	0.02	30.20 e	0.5	9.90 ghi	0.65
L. sativus	SAD	7.42 m	0.04	21.80 ij	0.65	8.08 i	0
	R	7.44 lm	0.05	21.76 ij	1.36	8.12 i	0.02
	S	7.82 def	0.03	34.23 a	1.55	9.18 hi	0.05
P. vulgaris	SAD	7.37 n	0.03	20.41 kl	1.22	14.52 e	1.13
	R	7.41 m	0.01	19.25 m	0.67	14.44 e	0.69
	S	7.81 fg	0.02	30.78 ed	0.19	9.90 ghi	1.29
L. sativum	SAD	7.58 k	0.03	23.68 h	0.53	11.52 fg	0.67
	R	7.61 jk	0.03	25.21 g	1.02	12.23 f	0.63
	S	7.82 def	0.01	32.75 bc	0.48	9.50 hi	0.63
B. napus	SAD	7.61 jk	0.04	20.94 jkl	0.44	14.68 e	0.65
	R	7.66 j	0.02	21.85 ij	0.42	15.34 de	1.12
	S	7.84 cdef	0.02	32.32 c	0.44	9.76 ghi	0.66
C. tinctorius	SAD	7.71 i	0.01	21.83 ij	0.65	14.00 e	0.64
	R	7.72 hi	0.01	21.86 ij	0.69	14.47 e	0.64
	S	7.86 bc	0.01	31.16 ed	0.43	10.08 gh	1.1
H. annuus	SAD	7.47 l	0.02	19.04 m	0.37	15.05 de	1.67
	R	7.48 l	0.02	19.81 lm	0.52	15.49 de	0.59
	S	7.78 g	0.02	31.69 cd	0.8	9.87 ghi	0.62
Control	SAD	7.90 ab	0.01	34.74 a	0.43	9.46 hi	0.66
	R	7.90 ab	0.02	34.37 a	0.41	9.06 hi	0.68
	S	7.91 a	0.02	34.55 a	0.29	9.22 hi	0.01

Means followed by the same letter in each column are not significantly different ($P < 0.05$).
SD- standard deviation, SAD- Soil adhering to the root mat, R- rhizosphere soil and S- nonrhizosphere soil.

Root-associated factors such as root morphology, architecture, root hair density, rate of nutrient absorption, ability to modify the rhizosphere and mycorrhizal symbiosis can strongly influence Pi acquisition (López-Bucio et al. 2003; Lynch and Brown 2006; Ramaekers et al. 2010). Root exudations such as amino acids, organic acids (Hinsinger 2001; Lambers et al. 2006; Richardson et al. 2009), H^+ (Wang et al. 2006; George et al. 2002; Hinsinger et al. 2003; Li et al. 2008) and acid phosphatase (Tadano et al. 1993; Asmar et al. 1995; Li et al. 2008) can influence Pi acquisition. In our previous report it was revealed that total P content decreased significantly in rhizosphere soil and in the soil adhering to the root mat. The greatest depletion of soil P was associated *S. tuberosum, S. lycopersicum, P. vulgaris* and *L. sativus*. These species belong to Solanaceae and Leguminose families (Safari and Rashidi 2011).

Hinsinger and Gilkes (1996, 1997) showed that different plant species are more or less efficient at acquiring P from Ca-bound P (apatite phosphate rock), depending only partly on their ability to acidify their rhizosphere. Rape (*Brassica napus* L.) and maize (*Zea mays* L.) also had different behaviors in mobilizing Ca-bound and Fe-bound P (Bertrand et al. 1999). **Table 4** shows the effects of plant species on pH of the studied calcareous soil in different sampling zones. All plants had a significant effect on decreasing soil pH in the vicinity of their roots. The lowest pH was measured in the rhizosphere soil and in the soil adhering to root mats of *T. aestivum, Z. mays, S. tuberosum* and *S. lycopersicum*. These species belong to Gramineae and Solanaceae families.

The results are in accordance with previous work (Safari and Sharifi 2007a), which showed that available P decreased significantly in the calcareous soil due to both plant uptake and immobilization in the rhizosphere for all studied species. In that study, available P was significantly higher in the control soil (39 μg g^{-1}) and decreased from 34.9 μg g^{-1} in the rhizosphere of *Anetum graveolens* to 15 μg g^{-1} in the rhizosphere of *S. lycopercicum*. It was also higher than 30 μg g^{-1} soil in the rhizosphere of *S. tuberosum, Ocimum basilicum* and *L. sativum*, and lower than 20 μg g^{-1} soil in the rhizosphere of *Allium cepa, Allium sativum* and *Petroselinum hotanse*.

Planting reduced the bioavailability of P in soil due to its uptake. This effect was more intense in the rhizosphere soil of *S. lycopersicum, L. sativus* and *P. vulgaris*. The rhizosphere soil of these plants had lower available P compared to soil adhering to the root mats. The difference between available P in soil adhering to the root mat and available P in the rhizosphere of *P. vulgaris* was significant. This shows the importance of rhizomicroorganisms in P nutrition of these plants. However, P depletion was more intense in the soil adhering to the root mats of *T. aestivum, Z. mays, S. tuberosum, L. sativum, B. napus, C. tinctorius* and *H. annuus* compared to the rhizosphere soil of these plants. The differences between available P in the soil adhering to the root mat and available P in the rhizosphere of *Z. mays* and *L. sativum* were statistically significant ($p < 0.05$).

For each species, nonrhizosphere soil had the lowest MBP compared to the rhizosphere soil and the soil adhering to the root mat, due to plant rhizodepositions and rhizosphere effects. MBP in nonrhizosphere soils was near 9.50 μg g^{-1} (**Table 4**).

In general, the plant species belonging to the Gramineae and Solanaceae families had greater effects on the soil pH (**Table 4**) and biological properties compared to those belonging to families of Cruciferae and Compositae. The highest MBP, near 28 μg g^{-1}, was measured in the rhizosphere soil and in the soil adhering to root mat of *T. aestivum* (**Table 4**). The mean soil MBP was also significantly higher in the rhizosphere soil and in the soil adhering to the root mats of *Z. mays, S. tuberosum* and *S. lycopersicum*. In the rhizosphere soil and in the soil adhering to the root mats of *Z. mays, S. tuberosum* and *S. lycopersicum*, MBP was near 22.2, 20.5 and 16.6 μg g^{-1} respectively. In contrast, the MBP was significantly lower in the planted soil with *L. sativus* and *L. sativum*. The mean MBP in the rhizosphere soil and in the soil adhering to the root mat of *L. sativus* was 8.08 and 8.12 μg g^{-1} respectively.

In general the mean MBP in the rhizosphere soil was higher than in the soil adhering to the root mat, but this difference was not statistically significant. An exception was the mean MBP in the soil adhering to root mat of *Z. mays* (23.24 µg g^{-1}) which was significantly higher than in the rhizosphere soil of this plant species (21.27 µg g^{-1}).

Table 5 shows Duncan's new multiple range tests of mean acid and alkaline phosphatase activities and spore numbers of glumales (SNG) as affected by plant species and soil sampling zone. In planting of each species, nonrhizosphere soil had the lowest acid phosphatase activity compared to those in the rhizosphere soil and in the soil adhering to the root mat, due to plant rhizosphere effects. Acid phosphatase activity in nonrhizosphere soils was near 1.80 µmol P.N.P g^{-1} soil h^{-1} (**Table 5**). The highest acid phosphatase activity was measured in the rhizosphere soil and in the soil adhering to the root mat of *T. aestivum* and *S. tuberosum*. It was near 7 µmol P.N.P g^{-1} soil h^{-1}. The mean acid phosphatase activity was also significantly higher in the rhizosphere soil and in the soil adhering to root mats of *P. vulgaris, L. sativum* and *H. annuus*. In contrast, the acid phosphatase activities were significantly lower in the soil planted with *B. napus* and *C. tinctorius*. The mean acid phosphatase activity in the rhizosphere soil and in the soil adhering to root mats of these plants was near 2.80 µmol P.N.P g^{-1} soil h^{-1}.

In general the mean acid phosphatase activity in the rhizosphere soil was higher than that in the soil adhering to the root mat, but this difference was not statistically significant. However the mean acid phosphatase activity in the rhizosphere soil of *T. aestivum* and *C. tinctorius* was significantly higher than in the soil adhering to the root mats of the respective plant species.

In planting of each species, nonrhizosphere soil had the lowest alkaline phosphatase activity compared to those in the rhizosphere soil and in the soil adhering to the root mats, due to plant rhizosphere effects. Alkaline phosphatase activity in nonrhizosphere soils was near 5.5 µmol P.N.P g^{-1} soil h^{-1} (**Table 5**). The highest alkaline phosphatase activity was measured in the rhizosphere soil and in the soil adhering to root mats of *P. vulgaris* and *L. sativum*. It was higher than 18 µmol P.N.P g^{-1} soil h^{-1}. The mean alkaline phosphatase activity was also significantly higher in the rhizosphere soil and in the soil adhering to the root mats of *T. aestivum, S. tuberosum, H. annuus* and *C. tinctorius*. In contrast, the alkaline phosphatase activity was significantly lower in the planted soil with *B. napus* and *S. lycopersicum*. The mean alkaline phosphatase activity in the rhizosphere soil and in the soil adhering to root mats of these plants was near 11 µmol P.N.P g^{-1} soil h^{-1}.

In general the mean alkaline phosphatase activity in the rhizosphere soil was higher than in the soil adhering to root the mat, and this difference was statistically significant in planting of *T. aestivum, Z. mays, S. tuberosum, P. vulgaris, L. sativum, C. tinctorius* and *H. annuus*. This shows the importance of rhizomicroorganisms in alkaline phosphatase activity near the root mat of the plants.

Planting can significantly increase phosphatase activity in the soil adhering to root mat of plant species. Safari and Sharifi (2007a) reported that alkaline phosphatase activity increased 102-325% and acid phosphatase activity increased 205-455% in the soil adhering to the root mat compared to the nonrhizosphere soil. This suggests that agronomy species actively promote rhizosphere phosphatase activity either directly by secretion or indirectly by stimulation of microbial activity and/or by depletion of Pi.

In planting of each species, nonrhizosphere soil had the lowest SNG compared to those in the rhizosphere soil and in the soil adhering to the root mat. SNG in nonrhizosphere soils was near 115 spores in 10 g soil (**Table 5**). The highest SNG was measured in the rhizosphere soil and in the soil adhering to root mats of *P. vulgaris* and *S. lycopersicum*. It was higher than 180 spores in 10 g soil. The mean SNG was also significantly high in the rhizosphere soil and in the soil adhering to the root mats of *T. aestivum* and *Z. mays,* higher than 150 spores in 10 g soil. In contrast, the SNG was significantly low in the planted soil with *L. sativum*. The mean SNG in

Table 5. Duncan's new multiple range tests of means of acid and alkaline phosphatase activities (acid Ph. Act. and alkaline Ph. Act.) and spore numbers of glumales (SNG) as affected by plant species and soil sampling zone

Plant species	Soil sampling zone	Acid Ph. Act. µmol P.N.P g^{-1} soil h^{-1}		Alkaline Ph. Act. µmol P.N.P g^{-1} soil h^{-1}		SNG N/10 g soil	
		Mean	SD	Mean	SD	Mean	SD
T. aestivum	SAD##	7.19 b	0.07	15.93 h	0.03	172.70 cd	4.53
	R	7.64 a	0.09	17.25 e	0.12	175.32 c	4.53
	S	1.92 k	0.04	5.54 p	0.05	122.98 lmn	4.53
Z. mays	SAD	3.12 h	0.05	12.80 m	0.07	167.47 ed	4.53
	R	3.18 h	0.05	13.14 l	0.04	170.08 cd	4.53
	S	1.89 kl	0.04	5.35 q	0.03	120.37 lmno	4.53
S. tuberosum	SAD	7.21 b	0.05	17.26 e	0.08	143.92 ij	4.53
	R	7.24 b	0.05	17.96 d	0.08	138.68 jk	4.53
	S	1.87 klm	0.04	5.33 q	0.11	117.75 mnop	4.53
S. lycopersicum	SAD	3.90 g	0.06	11.97 n	0.06	183.17 b	4.53
	R	3.97 g	0.04	12.03 n	0.03	201.48 a	4.53
	S	1.91 k	0.06	5.54 p	0.04	122.98 lmn	4.53
L. sativus	SAD	4.21 e	0.07	14.56 k	0.08	162.23 ef	4.53
	R	4.16 ef	0.05	14.63 k	0.09	159.62 f	4.53
	S	1.83 klmn	0.05	5.37 q	0.01	112.52 op	4.53
P. vulgaris	SAD	5.13 c	0.07	18.27 c	0.18	185.78 b	4.53
	R	5.18 c	0.1	19.41 a	0.03	198.87 a	4.53
	S	1.89 kl	0.03	5.53 p	0.06	122.98 lmn	4.53
L. sativum	SAD	4.66 d	0.06	18.31 c	0.08	125.60 lm	4.53
	R	4.66 d	0.02	18.58 b	0.08	128.22 l	4.53
	S	1.91 k	0.06	5.33 q	0.07	115.13 nop	4.53
B. napus	SAD	2.78 j	0.04	10.84 o	0.14	151.77 gh	4.53
	R	2.85 ij	0.05	10.87 o	0.11	149.15 hi	4.53
	S	1.83 klmn	0.04	5.33 q	0.07	115.13 nop	4.53
C. tinctorius	SAD	2.78 j	0.04	15.36 j	0.06	157.00 fg	4.53
	R	2.93 i	0.02	16.48 g	0.08	151.77 gh	4.53
	S	1.79 mn	0.06	5.36 q	0.05	122.98 lmn	4.53
H. annuus	SAD	4.10 f	0.02	15.49 i	0.06	136.07 k	4.53
	R	4.14 ef	0.01	17.04 f	0.01	136.07 k	4.53
	S	1.89 kl	0.05	5.39 q	0.07	117.75 mnop	4.53
Control	SAD	1.80 lmn	0.03	5.25 q	0.06	115.13 nop	4.53
	R	1.78 mn	0.04	5.37 q	0.05	112.52 op	4.53
	S	1.76 n	0.03	5.31 q	0.02	109.90 p	4.53

Means followed by the same letter in each column are not significantly different ($P < 0.05$).
SD-standard deviation, SAD-Soil adhering to the root mat, R-rhizosphere soil and S- nonrhizosphere soil.

the rhizosphere soil and in the soil adhering to the root mat of this plant were 128.22 and 125.6 spores, respectively, in 10 g soil.

In the plantings of *T. aestivum, Z. mays, P. vulgaris*, *S. lycopersicum* and *L. sativum*, the mean SNG in the rhizosphere soils was higher than that in the soils adhering to the root mat, but the differences were only significant in planting of *P. vulgaris* and *S. lycopersicum*. The mean SNG in the rhizosphere soil of the other plants was lower than those in the soil adhering to the root mat of these plants, but the differences were not statistically significant.

Safari and Sharifi (2007b) reported that the mean SNG in the soil adhering to the root mat of some plant species at the middle and end of vegetative growth was significantly different. At the end of vegetative growth the number of spores increased in planting of *Triticum aestium, Zea mays, Trifolium repens, Solanum tuberosum, Satureja hortensis*, and *Allium cepa*. In this stage of plant growth the number of spores in the soil planted with *R. sativa* was the lowest (126 spores in 10 g dry soil) and *A. cepa* the highest (453 spores in 10 g dry soil).

Table 6 shows the correlation coefficients between available P, MBP, acid and alkaline phosphatase activities, SNG, soluble P and soil pH measured in different sampling zones. After plant harvest, the correlation coefficient between soil available P and MBP was not significant in each sampling zone. The correlation coefficients between soil available P and acid phosphatase activity in both the rhizosphere soil and in the soil adhering to the root mat were not significant, but the correlation coefficient in nonrhizosphere soil was negative and significant. There were negative and significant correlations between available P and alkaline phosphatase activity in all of the sampling zones. Available P had a negative and significant correlation with SNG but a positive and significant correlation with soluble P and pH in each sampling zone.

Chen et al. (2002) reported that the greater extension of depletion zones of BPi (sodium bicarbonate extracted inorganic P) and NPi (ammonium chloride extracted inorganic P) in soils under *Pinus radiata* may be ascribed to the effects of ectomycorrhizae (Marschner and Dell 1994), which could be clearly seen in soil in the bottom of the lower compartment although they were not quantified. In this study, the negative correlations of available P and soluble P with soil biological properties like SNG and alkaline phosphatase activity and the positive correlation with soil pH shows the importance of rhizomicroorganisms and glumales on P nutrition. Soil pH had negative and significant correlation with acid phosphatase activity and SNG in different sampling zones. These negative correlation coefficients were higher in the rhizosphere soil than those in other zones. The negative correlations between soil pH and MBP were only significant in the biologically active zones (rhizosphere soil and soil adhering to the root mat).

Table 6. The correlation coefficients between available P (AP), microbial biomass P (MBP), acid and alkaline phosphatase activities (acid Ph. Act. and alkaline Ph. Act.), spore numbers of glumales (SNG), soluble P and soil pH measured in different sampling zones

	AP	*MBP*	*Acid Ph. Act.*	*Alkaline Ph. Act.*	*SNG*
	Soil adhering to the root mat				
MBP	ns	1			
Acid Ph. Act.	ns	0.55**	1		
Alkaline Ph. Act.	-0.45**	ns	0.70**	1	
SNG	-0.68**	0.42**	ns	ns	1
Soluble P	0.59**	ns	ns	-0.42**	-0.40*
pH	0.61**	-0.64**	-0.59**	ns	-0.66**
Plant P uptake	-0.41*	0.65**	ns	ns	0.71**
Shoot P con.	-0.49**	ns	ns	ns	0.63**
Root P con.	ns	0.54**	ns	-0.38*	0.35*
	Rhizosphere soil				
MBP	ns	1			
Acid Ph. Act.	ns	0.65**	1		
Alkaline Ph. Act.	-0.44**	ns	0.69**	1	
SNG	-0.46**	ns	ns	ns	1
Soluble P	0.56**	ns	ns	-0.43**	-0.32*
pH	0.65**	-0.62**	-0.66**	ns	-0.73**
Plant P uptake	-0.41*	0.57*	ns	ns	0.68**
Shoot P con.	ns	ns	ns	ns	0.71 **
Root P con.	ns	0.50*	-0.38*	ns	0.38*
	Nonrhizosphere soil				
MBP	ns	1			
Acid Ph. Act.	-0.47**	ns	1		
Alkaline Ph. Act.	-0.54**	ns	0.46**	1	
SNG	-0.64**	0.41*	ns	0.58**	1
Soluble P	0.58**	ns	ns	ns	-0.43*
pH	0.65**	ns	-0.60**	ns	-0.48**
Plant P uptake	ns	ns	ns	ns	ns
Shoot P con.	ns	ns	ns	ns	ns
Root P con.	ns	ns	ns	ns	ns

*- The correlation coefficient is significant at the 0.01 level. **- The correlation coefficient is significant at the 0.01 level. ns- The correlation coefficient is not significant.

4. Conclusions

Analysis of variance of the effects of plant species and soil sampling sites on the soil available P revealed that the effects of these treatments and their interaction on soil pH, available P, microbial biomass P (MBP), acid and alkaline phosphatase activities and spore numbers of glumales (SNG) after plant harvest were significant.

In general, the plant species belonging to the Gramineae, Solanaceae and Leguminoseae families had higher effects on soil available P and biological properties than those belonging to Cruciferae and Compositae.

The mean soil MBP was significantly higher in the rhizosphere soil and in the soil adhering to root mats of *T. aestivum, Z. mays, S. tuberosum* and *S. lycopersicum*. The highest alkaline phosphatase activity was measured in the rhizosphere soil and in the soil adhering to root mats of *P. vulgaris* and *L. sativum*. The highest acid phosphatase activity was measured in the rhizosphere soil and in the soil adhering to root mats of *T. aestivum* and *S. tuberosum*. The mean acid phosphatase activity was significantly higher in the rhizosphere soils and in the soil adhering to root mats of *P. vulgaris, L. sativum* and *H. annuus*. The highest SNG was measured in the rhizosphere soil and in the soil adhering to root mats of *P. vulgaris* and *S. lycopersicum*. The mean SNG was also significantly higher in the rhizosphere soil and in the soil adhering to root mats of *T. aestivum* and *Z. mays*. Thus plant species with higher rhizodeposition and rhizomicrobial activity significantly lowered the available P. This shows the importance of rhizomicrobial activity on P dynamics in rhizospheric soil. The negative correlation of available P and soluble P with soil biological properties like spore numbers of glumales (SNG) and alkaline phosphatase activity and the positive correlation with soil pH show the importance of rhizomicroorganisms and glumales on P nutrition by plants in calcareous soil, and therefore the rhizosphere acidification of calcareous soils may not be as important as the improvement of biological properties in soil P uptake and acquisition by plants.

There were no significant differences, however, in most properties of soils sampled from rhizosphere and adhering to root mats, although the application of tine slicing techniques in pot cultures showed that some soil properties were significantly different in those sampling sites.

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