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ORIGINAL ARTICLE

How do soil P tests, plant yield and P acquisition by *Lotus tenuis* plants reflect the availability of added P from different phosphate sources

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Abstract The relative effectiveness (RE) of each one of three different sources of P—P in solution (Psol), triple superphosphate (TSP) and phosphate rock (PR)—for reflecting the availability of P in a P-deficient soil were assessed by measuring in *Lotus tenuis* variables associated with growth, organ morphology, and plant tissue P-content together with the amounts of P extracts from soil by two of the currently used soil-P tests—Bray I and Olsen. A hyperbolic equation was used to fit the response curves of each one of those plant variables to added-P. The ratio between the shapes of paired response curves of any P-sources was used to compute the RE and substitution rate (*K*) of one source relative to the other. More P was needed from TSP and PR compared to Psol-100% soluble P-source. On the average P applications as TSP relative to Psol and PR relative to TSP were only 68 and 63% effective respectively for plant growth. Plant roots were more sensitive than soil-P tests to detect shifts in P-availability from different P-sources. Because soil tests are commonly used to estimate the current P status in soil in order to calculate the optimum application levels of fertilizer P for a crop or pasture, these results would have practical agronomical consequences if reproduced in other cultivated species because they show that the response curve of a plant species as a function of added P and soil test might differ among fertilizer types, measured plant variables, and the test used to measure P availability in the soil.

Keywords Bray I and Olsen · Effectiveness of added P · *Lotus tenuis* · P-sources · P uptake · Response curves fitting

Introduction

Regular applications of phosphate (P) fertilizers are currently required to obtain maximum forage yield from most of grassland soils over the World. This is the case of the natural grasslands in the Argentinean Pampas, which is the most productive area for beef and dairy cattle farms. These natural grasslands are dominated by perennial grasses, which production in terms of quantity and quality of forage is constrained by a marked deficiency of N and P in the soil (Ginzo et al. 1982; Escudero and Mendoza 2005; Garcia and Mendoza 2008).

Lotus tenuis is an important naturalized legume present in a variety of those grasslands. It is a species much appreciated by farmers because it produces highly nutritive forage for livestock on nutrient-deficient soils (Hidalgo and Cauhépé 1991; Mendoza 2001), and it significantly increases the forage quality of the grassland communities it is consociated with (Miñón et al. 1990). The winter forage production of a

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fescue–*Lotus* mixture almost tripled the daily growth rates of native grasslands without *Lotus*, which was close to 4–5 kg dry yield ha^{-1} day^{-1} (Hidalgo and Cauhépé 1991). In addition, it was reported that adding P fertilizers to P-deficient grassland soils increased the presence, cover and growth of *L. tenuis* (Ginzo et al. 1982; Mendoza 2001). As the prices of P fertilizers increase, farmers tend to apply less P than is required for maximum forage yield. Therefore the application of phosphate rock (PR) may be an economically attractive alternative to the use of more expensive soluble-P fertilizers such as triple superphosphate (TSP).

Phosphorus is the one of most immobile macronutrients in the soil; the availability of P for plant nutrition depends on the degree of accessibility of P-sources to plant roots. As soon as P is applied to the soil its concentration in the soil solution increases at a rate, which depends on the solubility of the P-source, soil water content, temperature and the amount of applied P (Barrow 1980). After the application of a P-source, a first rapid reaction between the soil and P in solution begins; P is adsorbed on soil clay and minerals, and its concentration in the soil solution decreases as a result of a balance between dissolved P from the source and adsorbed-P (Mendoza and Barrow 1987). In terms of time of application, after the first reaction a slow secondary reaction between the soil and adsorbed P takes place such that P continually diffuses into the soil solid phase (Barrow 1985). Both reactions decrease the offer of P to plant roots by decreasing P-availability in the soil solution, and ultimately decreasing plant yield.

The availability of a P-source to plants largely depends on its rate of dissolution. However, the influence of soil, plant and P-source management factors may alter the availability of P to plants (Rajan et al. 1996). The rate of PR dissolution in a given soil mainly depends of the chemical composition and the particle size of the PR used. Previous studies suggest that PR from North Caroline (USA), Gafsa (Tunisia) and Sechura (Peru) are the more reactive PRs available in the World (Rajan et al. 1996; Zapata and Roy 2007). In addition, the finer of the particle size in a given PR, the greater rate of dissolution (Kanabo and Gilkes 1988a).

Flexible mathematical models were proposed to describe response curves of pasture species at a range of added water-soluble P (Ozanne et al. 1969), and then used in different studies with appropriate modifications to improve curve fitting (Campbell and Keay 1970; Bolan et al. 1983; Barrow and Mendoza 1990). It is commonly observe that PR and WSP do not share the same maximum crop yield (Chien et al. 1990; Barrow and Bolland 1990; Rajan et al. 1996; Truong 2004). However, some reports have shown that PR and WSP can reach the same maximum yield (Bolland and Barrow 1991; Rajan et al. 1996). In these two cases, P rate added from PR is often higher than that from WSP to attain the same yield at or below the maximum yield.

Soil-tests are commonly used to estimate the current soil-P status in order to calculate the optimum application levels P-fertilizer for a crop or pasture. However, the relationship between plant yield and soil-test values may differ among fertilizer sources, year of application and plant species (Bolland and Gilkes 1992; Kumar et al. 1992; Covacevich et al. 2006). The agronomic effectiveness of P fertilizers to the soil depends on the chemical composition of the P-source, soil properties and plant species (Chien et al. 1990; Chien 2004).

In view of these interrelationships we hypothesized that the nature of the association between plant yield and either P-uptake or the fluctuation of some plant variable for a range of amounts of P added to the soil is determined by the soil test used, the characteristics of the P-source and the sensitivity of a plant variable in a given plant species to P. It is clear that there is not just one answer to the estimation of how much P in the soil is needed to attain the maximum yield in a particular plant species.

The greenhouse study reported here addressed the effect of P-availability on the relative effectiveness of the application by three different sources of P—which differed in the solubility of P—to a P-deficient soil collected from a field site currently populated with *Lotus tenuis*. We investigated how two of the commonly used soil-P tests (Bray I and Olsen), plant dry matter yield, some morphological features and P-nutrition inform on the amount of available P in the soil for a range of additions of P.

Materials and methods

Soil collection and experimental set up

Soil samples were collected from the upper 10-cm of the silt loamy top horizon of a Typic Natraquoll in early autumn. The sampled site was at that time under a natural grassland dominated by *Lotus tenuis* Waldst. & Kit., *Cynodon dactylon* (L.), *Stenotaphrum secundatum* (Walt.) O. K., and *Lolium multiflorum* Lam. The soil chemical characteristics before the experiment were taken from a late summer sampling of a previous field study (Garcia and Mendoza 2008), they were: pH (1:2.5 water), 6.2; total C, 1.9%, total N, 0.19%; available P (Bray I), 5.7 ppm P; electrical conductivity (EC), 0.22 dS/m, and exchangeable Na per cent (ESP), 25.1%. The soil was air dried and sieved through a 2 mm-mesh screen, spread over a flat surface as a 2-cm thick layer, solarized and thoroughly homogenized every 2–3 days to partly eliminate inocula of arbuscular mycorrhizal (AM) fungi, thus minimizing the likely overestimating effect of plant-AM symbiosis on P availability from a P-source.

We ran two experiments. In the first one, 1.6 l non-draining pots were filled with 740 g of air dry soil previously fertilized with a range of P rates (0–480 mg P per kg soil) to adequately draw plant-response curves. Phosphorus from three different sources was mixed homogenously and applied to the soil; those sources were KH_2PO_4 in solution (Psol), powdered triple superphosphate (TSP) (manufactured by Petrobras Energy; Campana, Buenos Aires, Argentina), and powdered phosphate rock (PR) (imported by M. Weissfeld & Assoc. Buenos Aires Argentina from Gafsa (Tunisia). Psol was applied diluted into 20 ml of distilled water, and TSP (20% P) and PR (13% P) were mixed with the soil and then 20 ml of distilled water were added as in Psol. In addition, a basal dose of nutrients except P was also applied in 20 ml of solution and mixed with the soil to ensure that P was the only limiting nutrient for plant growth (Mendoza 2001). This nutrient solution was previously used by Ozanne et al. (1969) and includes nitrogen. Seeds of *L. tenuis* were surface sterilised (alcohol 95% plus H_2O_2 in 100 vol.), inoculated with *Rhizobium* sp., pre-germinated in sterile conditions, and five seedlings were transplanted to each pot, which surface was covered with 1 cm of sterilized sand to minimize water evaporation. The pots were maintained near field capacity (31% w/w) by daily watering to constant weight. Plants were grown in a greenhouse for 40 days (mean day temperature 30 ± 4°C, mean night temperature 19 ± 3°C). The pots were randomised and daily rotated to minimize gradient effects of the glasshouse environment. The mean ambient relative humidity was 65–75% and the photoperiod length from 10 to 12 h during the experimental period.

In the second experiment we tested the variability of plant response for only one level of added-P by measuring plant yield at 50 mg P per kg soil applied as Psol, TSP and PR. This level was arbitrarily chosen in an attempt to obtain at about half the maximum shoot dry matter yield with respect to the maximum yield approached by the Psol treatment. Five replicates per P-source and a control were randomly laid out in a greenhouse, and pots were daily moved around as described for the first experiment.

Plant measurements and harvest

First experiment

At the end of 40 days of growth, shoots and roots were harvested and their fresh weights were determined. The length of main shoot and the length of the middle leaflet situated on the basal part of the sixth internode were measured before harvest.

Oven dried (70°C for 48 h) shoot tissues were weighted and digested in a nitric–perchloric acid mixture to determine P by the molybdovanadophosphoric acid method (Jackson 1958).

Second experiment

Shoots and roots were harvested 53 days after transplanting, and their respective fresh and dry weights were measured as in the first experiment. Fresh roots (2.5 g) were cleared in 10% KOH for 5 min at 90°C, and stained in 0.05% lactic acid–glycerol Trypan Blue (Phillips and Hayman 1970) to test for the presence of colonizing arbuscular mycorrhizae.

Soil analysis

At the end of the experiment, soil samples of 200 g were taken from the center of each pot and analyzed for pH (water, 1:2.5 w/w), and available P by the both the Bray I (Bray and Kurtz 1945) and the Olsen (Olsen et al. 1954) methods.

Analysis of data

A mathematical description of a response curve must not only provide a good statistical fit but must also describe the curvature and estimated accurately both the maximum and the minimum yield. Flexible techniques in describing mathematically a range of response curves of pasture species were proposed by Campbell and Keay (1970). A generalized hyperbola (1) derived from an equation given by Campbell and Keay (1970) was used to fit the response curve of *L. tenuis* to P applications from each source of P because it includes an extra coefficient (m) compared with the simplest Mitcherlitch equation, and allows for more accurate description:

$$Y = A - [B/(1 + mcx)^{1/m}] \quad (1)$$

where Y is the plant dry matter yield per pot for any rate x of added-P; A is the maximum value of Y when P does not limit growth; $A{-}B$ is the plant yield without added–P ($x = 0$); B represents the responsiveness of P applications and it is calculated from the difference between the maximum yield (A) and the yield actually obtained without added P and can be expressed as a fraction of A. Thus, B can range from 0 to 1 and depends on the status of the unfertilised soil. In addition, B value can also vary with phosphate buffering capacity of the soil. For a given dose of added P, soils with high sorption capacity will have a higher value of B with respect to soils of low sorption capacity. The m coefficient controls the rate of curvature of the response; and coefficient c is the curvature coefficient. Coefficient c is currently associated to the P buffering capacity of the soil, and differences in P availability are expected to change the value of c. When P does not limit growth, plants fertilized with either Psol, TSP or PR does not differ in growth so they realize the same maximum yield (A). The value of B is the same for Psol, TSP and PR sources because it depends on the availability of native P in soil. When each of A and B tend to attain a common value for all sources of P, Eq. (1), can be written as

$$Y = A - [B/(1 + (mc_1x_1 + mc_2x_2 + mc_3x_3)^{1/m}] \quad (2)$$

where x_1, x_2 and x_3 are the levels of added-P for Psol, TSP and PR; and c_1, c_2 and c_3 are the curvature coefficients for sources Psol, TSP and PR, respectively.

In terms of efficiency of P applications for plant growth, holding constant the value of m the ratio between the shapes of the response curves c_i/c_j represents a measure of the relative effectiveness of phosphorus applied as a source i relative to a source j, $\mathrm{RE}_{i,j}$. Formally, Y can be expressed:

$$Y = A - \{B/1 + [mc_1(x_1 + \mathrm{RE}_{2,1}x_2 + \mathrm{RE}_{3,1}x_3)^{1/m}]\} \quad (3)$$

where $\mathrm{RE}_{i,j}$ is the relative effectiveness and its values range from 0 to 1 ($c_2 = \mathrm{RE}_{2,1}\ c_1$; $c_3 = \mathrm{RE}_{3,1}.c_1$; $c_3 = \mathrm{RE}_{3,2}.c_2$). The reciprocal of RE is the substitution rate K ($K_{j,i} = 1/\mathrm{RE}_{i,j}$) that represents e.g. the amount of source 2 (x_2) required to give the same effect on yield as a given amount of source 1 (x_1). Equation (3) was used to calculate the values of the coefficients to estimate the measures of c_1, c_2, c_3, $\mathrm{RE}_{i,j}$ and $K_{j,i}$.

The $\mathrm{RE}_{i,j}$ or $K_{j,i}$ value for one P-source with respect to any other source and for a plant variable can also be calculated for only one level of P applied. This measure of effectiveness is currently called Agronomic Relative Effectiveness—$\mathrm{ARE}_{i,j}$:

$$\mathrm{ARE}_{2,1} = (Y_2 - Y_c)/(Y_1 - Y_c) \quad (4)$$

where ARE is the agronomic relative effectiveness for one level of P application; Y_1 and Y_2 are the yields of source 1 and source 2, respectively; and Y_c is the yield measured without P applied.

The reciprocal of ARE is the substitution rate of one level of P-application ($\mathrm{AK}_{j,i}$).

Critical phosphorus concentration

The critical P-concentration in shoots to give 90% of maximum shoot growth from different phosphorus sources was calculated from a rescaled version of the Eqs. (1, 2, 3) previously used by Barrow and Mendoza (1990):

$$\ln y = a - \left[b/\left(1 + mcx^d\right)^{1/m}\right], \quad (5)$$

where y is shoots dry weight (g); x is the concentration of P in shoots (%); and a, b, c and d are coefficients. The value of a, b, m and c coefficients have a similar meaning to the coefficients A, B, m and c of Eq. (1), respectively, and coefficient d represents a sigmoid component. Increasing the value of d at a constant value of c and m, makes the curve increasingly sigmoid and also determines that the maximum shoot yield is approached more quickly. Sigmoid responses can be described by transforming the data to logarithms and then plotting the back transforming data. In this case we fitted the log-transformed data but also plotted on a logarithm scale to show the sigmoid response. The sigmoid response to added P was proposed by Bolan et al. (1983) to describe plant and soil factors, and mycorrhizal colonization causing this response form. Barrow and Mendoza (1990) used Eq. (5) to describe the sigmoid yield responses by legume plants to both, freshly and incubated P with the soil. In this work we compared the response to freshly P applied from different P-sources. A discussion on the flexibility of Eq. (5) to fit a response curve can be found in Barrow and Mendoza (1990) and Bolland and Barrow (1991).

Statistical analysis

Plant responses to P sources were compared by curves fitted to them. The statistical differences among curves were tested by a significant variation ($P < 0.05$) of the residual sum of squares of observed values. In the case that the equations to be compared differed in the number of coefficients, those with most coefficients were reduced to forms with fewer coefficients in so far the change brought about in the residual sum of squares was not statistically significant ($P < 0.05$). The simplex method of Nelder and Mead (1965) was used to bring forth the values of the coefficients that gave the smallest residual sum of squares. Data were log-transformed for the analysis, and back-transformed for their plotting.

Results

Lotus tenuis plants strongly responded to a wide range of P from the P-sources. The hyperbolic equation used adequately described the responses of the five plant variables: shoot fresh weight, root fresh weight, shoot:root ratio, shoot dry weight, shoot length and leaflet length; (Fig. 1a–f). All the plots in

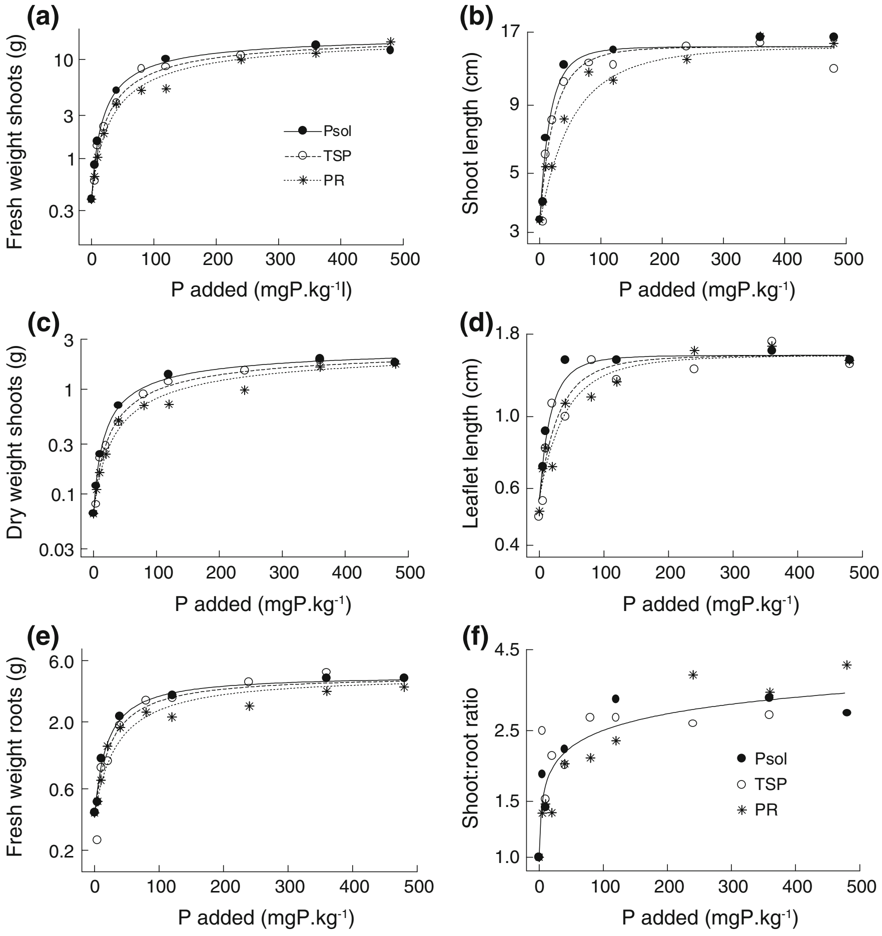

Fig. 1 Curves fitted by Eq. (1) or Eq. (2) to describe the response to three sources of added P (Psol, TSP and PR) by *Lotus tenuis*. Fresh weight shoots (**a**), shoot length (**b**), dry weight shoot (**c**), leaflet length (**d**), fresh weight roots (**e**) and shoot:root ratio (**f**). The values of coefficient of Eq. (1) or Eq. (2) are indicated in Table 2. Experiment 1. Figure 1**a**–**f** are presented in semi-log scale

Fig. 1 showed that the responses of plant variables by the three P-sources approached to a same maximum value supporting the model used to fit the data.

We firstly fitted the 4-coefficient Eq. (1) to each plant-variable data without discriminating among the sources of P. Secondly data were fitted with the 6-coefficient Eq. (2) to test whether the response-curves from different sources would differ among these and, in case they did, whether differences among P-sources would be reflected in the values of the *c*-coefficients of Eq. (2). Table 1 shows that in five out of six cases, fitting the data with Eq. (2) resulted in a statistically significant decrease in the residual sum of squares of deviations compared to Eq. (1). In just one case (S/R) Eq. (2) did not improve on the fitting of Eq. (1), so one curve was used to fit the data from the whole set of P-sources.

The initial slope of the response-curves of the plant-variables (root length included but not shown in Fig. 1)—i.e. the value of coefficient *c* in Eqs. (1, 2, 3)—was highest for Psol, lowest for PR and intermediate for TSP (Table 2). The value of $\mathrm{RE}_{\mathrm{TSP,\ Psol}}$ was always higher than the value of $\mathrm{RE}_{\mathrm{PR,\ Psol}}$ for all plant-variables (Table 3). For instance and with regard to shoot dry weight, $\mathrm{RE}_{\mathrm{TSP,\ Psol}} = 0.71$ means that P from TSP was 71% as effective as P derived from the soil solution for shoot (dry weight) growth. Similarly, added-P from PR was 76% effective compared to TSP (Table 3). RE averaged over all plant variables showed that P added as either TSP or PR were only 68 or 43% effective compared to P added as Psol, respectively, (Table 3).

For root fresh weight the value of $K_{\mathrm{TSP,\ Psol}}$ shows that it would be necessary to add 1.28 times more P as TSP as from soil (Psol) to get the same root weight. Similarly, 1.84 more P from PR would be needed to produce the same yield as P from Psol (Table 3). Figure 2 shows that one curve was used to fit the all data sets by replacing in Eq. (3) the values of $\mathrm{RE}_{i,j}$ by the substitution rates ($K_{i,j}$) shown in Table 3.

Table 1 Comparison of two equations fitted to the individual responses of some *Lotus tenuis* variables to three sources of phosphorus (experiment 1)

Plant variable	Source of variation	Sums of squares	Degrees of freedom	Variance	Variance ratio (*F*)[a]
Shoot fresh weight	Eq. (1)	35.313	4	8.828	
	Residual Eq. (1)	0.8951	21	0.0426	207.14***
	Eq. (2)	35.689	6	5.948	
	Residual Eq. (2)	0.5193	19	0.0273	217.62***
	Improvement of Eq. (2) over Eq. (1)	0.3758	2	0.1879	6.88**
Shoots dry weight	Residual Eq. (1)	0.9181	21	0.0437	
	Residual Eq. (2)	0.4697	19	0.0247	
	Improvement of Eq. (2) over Eq. (1)	0.4484	2	0.2242	9.07**
Root fresh weight	Residual Eq. (1)	0.7180	21	0.0342	
	Residual Eq. (2)	0.4756	19	0.0250	
	Improvement of Eq. (2) over Eq. (1)	0.2424	2	0.1212	4.85*
Shoot length	Residual Eq. (1)	1.4462	21	0.0689	
	Residual Eq. (2)	0.9751	19	0.0513	
	Improvement of Eq. (2) over Eq. (1)	0.4711	2	0.2356	4.59*
Leaflet length	Residual Eq. (1)	0.3539	21	0.0169	
	Residual Eq. (2)	0.2370	19	0.0125	
	Improvement of Eq. (2) over Eq. (1)	0.1169	2	0.0585	4.68*
Shoot:root ratio	Residual Eq. (1)	0.7324	21	0.0349	
	Residual Eq. (2)	0.6278	19	0.0330	
	Improvement of Eq. (2) over Eq. (1)	0.1046	2	0.0523	1.58 NS

[a] Eqs. 1 and 2

NS non-significant

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 2 Values of the coefficients of Eq. (1) or Eq. (2) fitted to the values of *Lotus tenuis* variables in response to the three sources of added P (experiment 1)

Plant variable	Coefficients of Eq. (1) or Eq. (2)[a]						R^2
	A	B	m	c_1	c_2	c_3	
FW shoots	2.706	3.671	1.061	0.0602	0.0451	0.0344	0.986
DW shoots	1.018	3.787	1.570	0.0589	0.0417	0.0311	0.987
FW roots	1.527	2.534	0.933	0.0524	0.0409	0.0285	0.976
Shoot length	2.707	1.539	0.262	0.0613	0.0438	0.0189	0.880
Leaflet length	0.459	0.131	0.664	0.0932	0.0501	0.0342	0.940
Soot:root ratio[b]	17.080	17.070	92.470	0.1668	–	–	0.821
Root length	9.749	3.396	6.145	0.0388	0.0226	0.0104	0.930

[a] Eqs. 1 and 2

[b] Shoot:root ratio was fitted by Eq. (1)

Table 3 Values of the effectiveness (RE) and substitution rates (K) of one source of phosphate relative to other source of phosphate for *Lotus tenuis* as measured by Eq. (2) (experiment 1)

Variable	Relative effectiveness ($RE_{i,j}$)			Substitution rate ($K_{j,i}$)		
	c_2/c_1	c_3/c_1	c_3/c_2	$K_{2,1}$	$K_{3,1}$	$K_{3,2}$
FW shoots	0.75	0.57	0.76	1.33	1.75	1.31
DW shoots	0.71	0.53	0.75	1.41	1.89	1.44
FW roots	0.78	0.54	0.70	1.28	1.84	1.44
Shoot length	0.71	0.31	0.43	1.40	3.23	2.32
Leaflet length	0.54	0.37	0.68	1.86	2.70	1.47
Root length	0.58	0.27	0.46	1.72	3.70	2.17
Mean	0.68	0.43	0.63	1.50	2.51	1.68

The concentration of P in shoots was influenced by the source of added-P. After little changes at low levels of addition, P in shoot (%) was high for Psol, intermediate for TSP and low for PR, but with a little difference between TSP and PR (Fig. 3a). The total P-content in shoots followed the same response pattern as for %P (Fig. 3b). However, the variation of shoot dry weight in terms of %P was represented by a sigmoid curve (Eq. 4) common to all sources of added-P (Fig. 3c); i.e. the individual fitted curves did not statistically differ among P-sources. We used Eq. (4) to find that the critical P concentration in shoots required to realize 90% of maximum dry matter yield (1.71 g/pot) was 0.23% for the three P-sources.

The amount of P in the soil as estimated with either the Bray or the Olsen tests did not match with the same dry matter yield for some levels of extracted-P (Fig. 4a). For shoot dry weight values

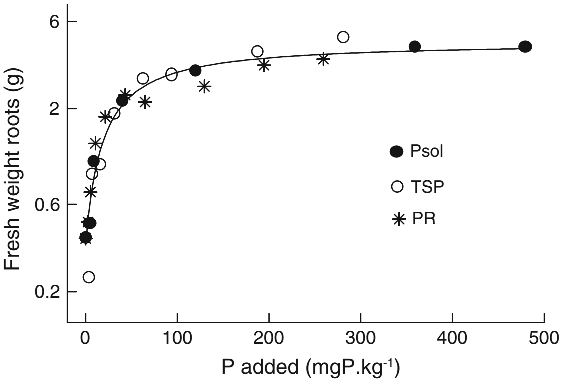

Fig. 2 Curve fitted by Eq. (2) to describe the response to the three sources of added P (Psol, TSP and PR) by *Lotu tenuis* with the levels of added P from TSP and PR divided by the relative effectiveness (RE) and thus expressed as Psol equivalents. Experiment 1. Figure is presented in a semi-log scale

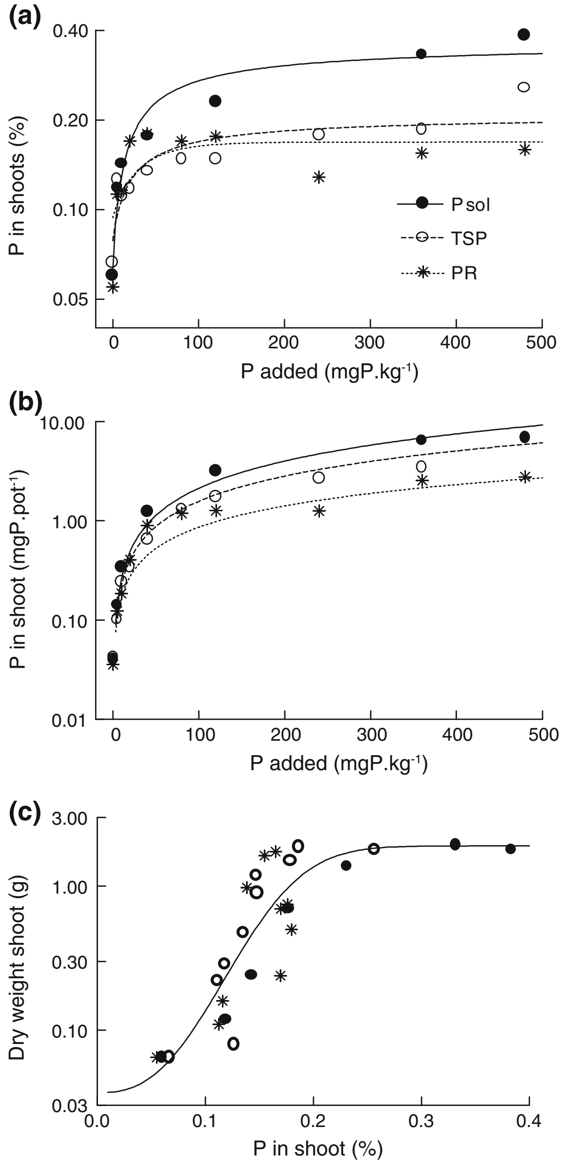

Fig. 3 Curves fitted by Eq. (2) to describe the response to the three sources of added P (Psol, TSP and PR) by the concentration of P in shoots (**a**), total P in shoots (**b**) and the relationship between dry weight of shoots as a function of the concentration of P in shoots (**c**) in *Lotus tenuis* plants. Experiment 1. Figure 3**a**–**c** are presented in semi-log scale

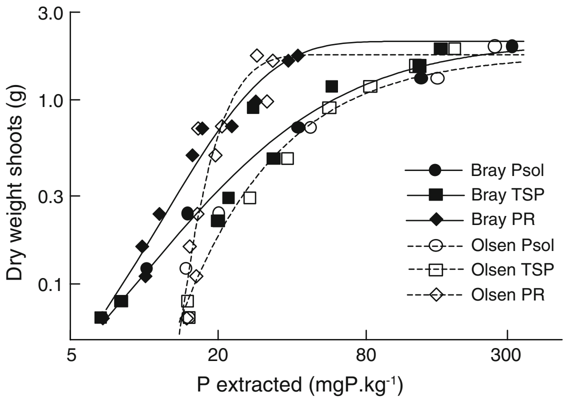

Fig. 4 Curves fitted by Eq. (2) to describe the response to the three sources of added P (Psol, TSP and PR) by dry yield of shoots in *Lotus tenuis* plants as a function of the extracted P from the soil by Bray I and Olsen soil tests. Experiment 1. Figure 4 is presented in a log–log scale

well below maximum—1.71 g/pot—BR extracted less P from the soil than Olsen (Fig. 4b). Close to maximum fitted dry weight values were much less influenced by soil test than by P-source. More P was extracted from both Psol and TSP sources than from PR by both soil tests (Fig. 4a), but Olsen produced extracted-P readings larger than Bray corresponding to same shoot dry weight values lower than 0.3 g (Fig. 4b). Both soil tests showed that approximately 40 ppm of P were required from soil fertilized with PR to realize 90% of maximum dry matter yield, and more than 200 ppm P were required from the soil fertilized with either TSP or Psol (Fig. 4b).

In the second experiment the addition of 50 ppm P to the soil from any of the three P sources resulted in *Lotus tenuis* producing higher dry matter yield of shoots (Fig. 5a) and roots (Fig. 5b) with Psol than with either TSP or PR after 53 days of growth. The value of the agronomic relative effectiveness—ARE—was highest (79%) for TSP/Psol, lowest (29%) for PR/Psol, and intermediate (36%) for PR/TSP (Table 4).

In Figs. 1, 2, 3 and 4, a log scale for the *Y* values was used because the precision of each estimate with increasing added-P seemed to be dependent of its magnitude. However, in Fig. 5a linear scale was used because the precision of each estimate was independent of its magnitude.

Testing roots for AM colonization showed that the percentage of root length colonized was quite similar among P sources—10.1, 11.4 and 9.6% for PR, TSP and Psol respectively—and control roots—12% colonized.

Before planting and after adding the nutrient solution soil pH at no-added P was 5.8, and at the end of the experiment it varied 0.5 units among sources and levels of added-P; ranges were 5.3–5.8, 5.3–5.5 and 5.2–5.4 for Psol, TSP and PR, respectively.

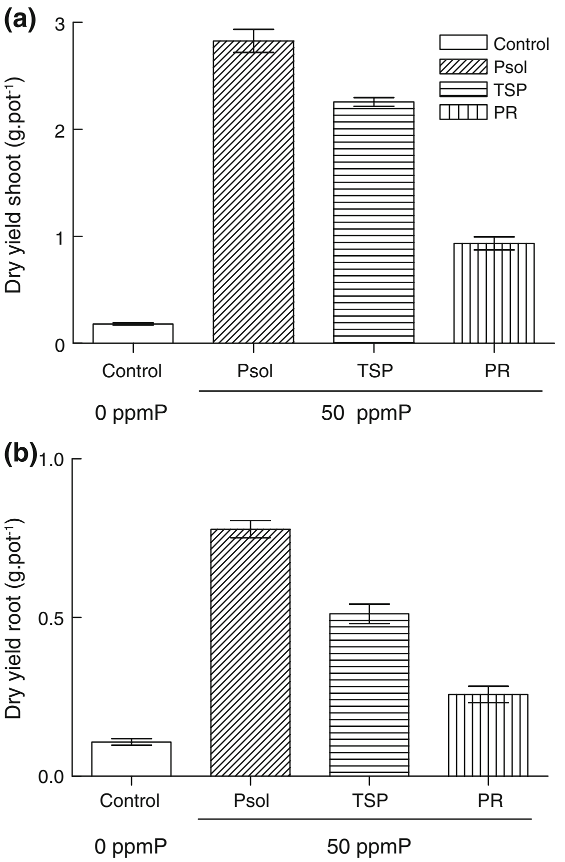

Fig. 5 Response of dry yield of shoot (**a**) and root (**b**) of *Lotus tenuis* plants to an addition of 50 ppm P to soil as Psol, TSP and PR. Experiment 2

Table 4 Values of the effectiveness (ARE) and substitution rates (AK) of one source of phosphate relative to other source at one level of P-addition (50 ppm P) for *Lotus tenuis* as estimated from Eq. (4) (experiment 2)

Equation	$ARE_{i,j}$			$AK_{j,i}$		
	TSP/ Psol	PR/ Psol	PR/ TSP	TSP/ Psol	PR/ Psol	PR/ TSP
Eq. (4)	0.79	0.29	0.36	1.27	3.45	2.78

Discussion

Lotus tenuis plants strongly responded to the range of P amounts added to a P-deficient soil from a field site where this plant species currently grows, no matter the source of P. Although *L. tenuis* is adapted to grow on P-deficient soils (Escudero and Mendoza 2005; Garcia and Mendoza 2008), the current investigation confirmed that it also responds well to added-P by markedly increasing both the quality and the quantity of forage it produces (Ginzo et al. 1982; Hidalgo and Cauhépé 1991; Mendoza 2001). At levels of added P lower than those needed for maximum dry matter, more P was always required from either TSP or PR than from Psol to obtain the same plant response, were it in terms of plant yield, plant morphological features or P concentration in plant tissue. This difference among the sources of available P, were adequately described by particular response curves of each plant variable to those sources. Phosphorus applied in solution (Psol) was used as a control P source treatment having 100% soluble P compared to the other sources, TSP and PR. TSP is a highly soluble P-source (87% w/w) and rapidly—i.e. in a few days after its application—becomes dissolved in the soil solution (Kumar et al. 1992). *Lotus tenuis* plants were apparently able to detect small differences in P-solubility between the Psol and TSP soluble sources and reflected these differences in the plant growth variables.

It is well reported that PR and WSP applications could not share the same plant yield due to the PR dissolution decrease with increasing the level of addition (Chien et al. 1990; Barrow and Bolland 1990; Rajan et al. 1996; Truong 2004). The main reason of this finding is due to the maximum growth requires a higher P concentration in soil solution than the solubility product of PR can permit (Chien and Black 1976; Khasawneh and Doll 1978; Rajan et al. 1996). The solution P of PR is fixed at a maximum value for each PR regardless how much of PR is added to the soil. This soil solution P concentration is much lower than that of WSP and explains often why PR and WSP so not share the same maximum yield even at high PR rate. However, we found that the PR from Gafsa and the two WSP sources approach the same maximum plant yield but more PR and TSP than Psol was required for yield below the maximum. The comparative performance of PR is strongly influenced by the chemical composition and particle size of the PR, soil properties, plant species and other factors. We have some explanations that may justify the results found in our experiment. The PR from Gafsa used in this work is one of the more reactive PR existing in the World (Zapata and Roy 2007); and in some soils it showed to have a same relative agronomic efficiency compared to TSP when both P-sources were used in glasshouse experiments (Truong 2004). The PR used here was powdered before applying to soil and, reducing particle size increases the PR dissolution (Kanabo and Gilkes 1988a). The PR was homogeneously mixed with the soil, and increasing the area contact of PR with soil enhanced PR dissolution (Kanabo and Gilkes 1988b). We have observed a reduction of soil pH compared to initial value at the field site, and it is accepted that with increasing soil acidity it is expected a greater dissolution of PR (Rajan et al. 1996). This change in soil pH was attributed mainly to the chemical composition of the nutrient solution applied to soil rather than changes in soil pH because of the P-sources applications. In addition, legume plants are able to release more protons to the soil solution than grasses and may also contribute to decrease soil pH (Rajan et al. 1996). However, the soil reaction was quite similar among P-sources; its increment in just 0.5 pH units by the addition of P in solution (Psol) is indicative of little influence of soil pH on P-availability during the experiment, specially from the less soluble PR, which is known to increase its solubility with increasing acidity in the soil solution (Kumar et al. 1992). The soil used was relative high in organic matter (3.9%), total carbon (1.9%), labile carbon (0.29%), total nitrogen (0.19%), labile nitrogen (0.03%), but low in P Bray I (5.7 ppm P), in comparison with other soils from the same region (Garcia and Mendoza 2008). It was reported a positive effect of organic matter on PR dissolution (Chien et al. 1990). Finally, the present experiment in non-draining pots avoids leaching of applied P from PR that is one of the causes because the residual value of P applications decreases with the level of addition at field conditions (Bolland and Barrow 1991). All of these findings showed the relative high levels of PR dissolution in soil and this may contribute to explain why we found that the PR from Gafsa and the two WSP sources approach the same maximum plant yield at high level of P applications.

In addition, arbuscular mycorrhizal colonization was below 12% in all cases, suggesting little influence of the symbiont in improving the access of plant roots to P.

The values of the *c* coefficient in Eq. (2) were higher for Psol than for either TSP or PR, very likely because the RE values obtained from the application of a wide range of P additions were always higher for TSP than for PR compared with the more soluble P control source (Psol). The substitution rate of PR relative to TSP–$K_{\mathrm{PR,TSP}}$—ranged from 1.31 to 2.32, with a mean value near 1.7 depending on the plant variable considered. This means that about 1.7 times more P should be added from PR to achieve the same growth of *L. tenuis* as under TSP-fertilization, or alternatively P added as PR was about 60% effective for stimulating growth in that plant species.

The results obtained in this work also show that plant morphological features—the lengths of shoots, roots and leaflets—differed from biomass measures—shoot and root weights—as relative efficiency indicators because the values of RE computed for the morphological features ranged from 0.43 to 0.68 whereas those for the biomass measures ranged 0.70–0.76 for all P-sources. In terms of substitution (*K*) values, and specifically for the effect of a P-source on *L. tenuis* roots, 1.44 more P as PR would be needed to attain the same root fresh weight as for P added as TSP (Table 3) but 2.17 more P from PR would be needed to attain the same root length as for P added as TSP, or 2.32 more P from PR in the case of shoot length. These results point to the existence of an interaction between a plant variable and applied P-sources in regard to the strength of P-source efficiency (substitution) as manifested by RE (*K*) values. The implication of this important fact is that different rates of added-P are needed to produce a certain increase in the value of a specific plant biomass or morphological feature.

The agronomic relative effectiveness (ARE) of any two P-sources for one level of added-P with regard to not-added-P was computed with Eq. (4); its values showed a similar trend as to P-sources but a different magnitude with respect to RE valued calculated with Eq. (3). The ARE of PR relative to TSP for shoot growth was only 36% compared to 76% for the range of added-P in the first experiment (Table 3). Although the two experiments differed as to the duration of plant growth and the way used to calculate the relative effectiveness of adding P from one source relative to other—so they are not strictly comparable—the values of the relative effectiveness as estimated with RE and ARE differed between the two experiments, those estimations are consistent with each other in showing that TSP is more effective than PR with respect to Psol.

Both substitution rate (*K*) and agronomic relative effectiveness (ARE) at one rate of P applied can vary with the PR application rate (Rajan et al. 1996). In the present case where PR and WSP response curves approach to the maximum yield, *K* remains constant but it is known that the ARE increases with increasing the rate of P applied (Rajan et al. 1996). Hence, the comparison of the relative effectiveness between the first (76%) and second (36%) experiment may change if a different rate of PR is applied.

The relationship between the concentration of P in shoots and shoot yield was similar for all three P-sources and adequately described by Eq. (5); this implies that differences in growth are more likely to be associated with differences in P-uptake rather than differences in P-utilization or other plant or soil variable for each P-source. However, the ability of plants to transfer the absorbed P in growth differed with increasing the concentration of P in shoot tissue determining a sigmoid response. The flexibility of Eq. (5) permitted to describe the sigmoid response between the concentration of P in shoots and shoot yield. Even when we do not have too many points at the lower end of P in shoot (%) in Fig. 3, the inclusion of the sigmoid coefficient (*d*) of Eq. (5) resulted in a significant fit improvement with respect to the equation which did not include the *d* coefficient. The sigmoid form was proposed by Bolan et al. (1983) to describe the plant response to added P. However, a sigmoid form was also used by Mendoza (2001) to find the critical value of P in shoot required to give 90% of maximum shoot yield in *Lotus*. In that work, a rescaled version of the Mitscherlich equation described adequately the sigmoid responses of *L. tenuis* and *L. corniculatus* shoot yield with increasing its P% in tissue. The critical P-concentration in shoots of *L. tenuis* in the present work was 0.23%; quite close to 0.28% as observed by Mendoza (2001) in the previous work.

The amount of P extracted from the soil that was required to obtain a plant yield lower than the maximum yield differed between soil tests (Bray I and Olsen) and P-sources. The change of plant variables such as yield, morphological features or tissue-P elicited by P-sources was described by different response curves. However, the amounts of P extracted by the Bray and the Olsen tests and needed to obtain the same plant yield were different between PR and the other two soluble sources (Psol and TSP). This is consistent with Covacevich et al. (2006), they found that from soils fertilized with PR the optimum level of P extracted by Bray I that allowed reaching the maximum plant yield was about 2.4 times lower than those fertilized by TSP. Chien (2004) showed that Bray I and Olsen may underestimate available P from PR with respect to TSP because a higher dry-matter yield was obtained with PR than TSP at the same level of extractable P by the two soil tests. Rajan et al. (1996) showed similar results. The conclusion is that the extracted P from Bray I or Olsen underestimates P availability from soils treated with reactive PR relative to test values obtained from soils treated by WSP. This partly because in soils fertilized with PR, two sources of P provide available P for plant growth, P from the PR itself and P from the reaction products, whereas only P from the reaction products provide P for plant growth in soils fertilized with WSP. In short-term periods of growth P from the PR is more important than from the reaction products and consequently available P is underestimated. In addition, Bray I and Olsen tests were performed after cropping whereas PR supplies available P during plant growth.

When comparing the two soluble P-sources (Psol and TSP), they were similar to each other because they were fitted by the same response curve for each one of the soil tests. This might have been due to the Bray and Olsen tests either displacing more P from the soil fertilized by TSP or less P from the soil fertilized by Psol, with the net result that the response curve was the same for each WSP-source. However, the curves for either yield or P taken up for growth as a function of the added P differed among P-sources; i.e. plant roots and soil tests removed from the soil different relative amounts of P with respect to the three P-sources characteristic. Plant roots were more sensitive to the nature of WSP-sources than soil tests to detect shifts in available-P. Theoretically, any plant can more sensibly detect the differences in P solubility and reflect this in plant growth comparing with any chemical solution, especially when plants grow shorter than longer periods of time as in the present work. This may be also part of the reasons why *Lotus tenuis* plants were able to detect small differences in P solubility between the Psol and TSP sources in terms of plant growth because of short-term plant growth (40 days).

In addition, the current study also showed differences between plant yield and the value of extracted-P as measured by acidity of the extracting solutions—acid (Bray) or alkaline (Olsen). The extracted-P required for low levels of plant yield (Fig. 3b)—was lower as measured by Bray than by Olsen; this difference became blurred at larger yields. This is because the two soil tests are different in soil:solution ratio, chemical solution, and time of reaction with the soil.

Because soil tests are commonly used to estimate the current P status in soil in order to calculate the optimum amount of P-fertilizer application levels for the next crop or pasture, the results of this work are quite relevant for the assessment of the reliability of those tests because they show that the response curve of a plant species as a function of added-P and soil-P may differ among fertilizer type, plant variable measured and the soil test used to measure P-availability in the soil.

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