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Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil

G. Feng^a, Y.C. Song^a, X.L. Li^a, P. Christie^{a,b,*}

^a Department of Plant Nutrition, College of Agricultural Resources and Environmental Sciences,
China Agricultural University, Beijing 100094, PR China

^b Agricultural and Environmental Science Department, Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK

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Abstract

A glasshouse pot experiment investigated the uptake by arbuscular mycorrhizal (AM) fungi associated with red clover of three organic sources of P added to a sterilized calcareous soil of low P availability. Each pot was separated into a central compartment for plant growth and two outer compartments for external mycelium using 30- μ m nylon mesh to restrict the roots but allow hyphal penetration. Plants in the central compartments were inoculated with the AM fungus *Glomus versiforme* and uninoculated controls were included. Plants were harvested on three occasions: 5, 7 and 10 weeks after sowing. Application of each of the three organic P sources (lecithin, RNA and sodium phytate) or inorganic P (KH_2PO_4) at 50 mg P kg⁻¹ to the outer compartments of mycorrhizal and uninoculated pots increased the yield, P concentration and total P uptake of red clover compared with pots to which no P was applied, with no differences among P sources in non-mycorrhizal plants but differences observed in mycorrhizal plants both 7 and 10 weeks after sowing suggesting differences in availability of the four P sources to AM mycelium. The contribution of external mycelium to plant uptake of applied P increased with time. The three organic P sources made smaller contributions to plant P nutrition than KH_2PO_4 at the first and second harvests. At the third harvest, the contribution from KH_2PO_4 was 23%, while those from lecithin, RNA and sodium phytate were 23, 17 and 31%, respectively. This suggests that with the mediation of AM fungi, soil organic P sources can make a contribution to host plant P nutrition comparable to that of soluble orthophosphate.

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Keywords: *Trifolium pratense*; *Glomus versiforme*; Lecithin; RNA; Sodium phytate; P uptake

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1. Introduction

Organic P comprises 30–80% of total soil P (Dalal, 1977) and is an important potential source of P for plants in most agricultural soils. The three principal groups of organic P compounds are the inositol phosphates (e.g. phytate), phospholipids (e.g. lecithin),

and nucleic acids (e.g. RNA) and their degradation products or derivatives (Stevenson and Cole, 1999). Some attention has been paid to the bioavailability and contribution of soil organic P sources to plant P nutrition in recent years (Li et al., 1997a,b; Hayes et al., 2000a,b). The various forms of organic P differ in their lability to phosphatase enzymes released by plant roots or microorganisms in soil, and this results in differences in their availability to plants with the declining order of: lecithin > RNA > phytate (Bowman and Cole, 1978; Stevenson and Cole, 1999).

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* Corresponding author. Tel.: +44-28-9025-5335;
fax: +44-28-9025-5005.
E-mail address: p.christie@qub.ac.uk (P. Christie).

Several studies have shown increased plant P uptake from inositol hexaphosphate following inoculation of growth substrates with bacteria, soil fungi and mixed populations of soil microorganisms (Richardson et al., 2000; Tarafdar and Marschner, 1994, 1995).

To become available for plants, organic P compounds must be hydrolyzed by a group of enzymes called phosphatases or phytases which may be of plant or microbial origin (Theodorou, 1971; Tarafdar and Claassen, 1988; Tarafdar and Marschner, 1994; Hayes et al., 1999; Li et al., 1997a,b; Richardson and Hadobas, 1997). Some investigations have confirmed that utilization of organic P is enhanced by arbuscular mycorrhiza. This enhancement effect could be due to both an increase in surface area and phosphatase activity of the extraradical hyphae (Li et al., 1997a; Tarafdar and Marschner, 1994). It is well established that arbuscular mycorrhiza can increase the P uptake of plants through the extraradical hyphae under certain conditions, but most research has dealt with inorganic P and few investigations have considered the utilization of organic P compounds by AM plants (Jayachandran et al., 1992; Joner and Jakobsen, 1994; Cade-Menun and Berch, 1997).

The relative contribution of soil organic P sources to plant P nutrition is therefore poorly understood. The role of AM fungi in the mineralization of organic P in soil and the contribution of P directly absorbed by AM fungal mycelium from soil organic P sources to plant P nutrition are thus unclear (Joner et al., 2000). The aims of this study were to identify the influence of AM fungi on the availability of three organic P sources to plants, follow changes in the hydrolysis of the organic P compounds in soil as affected by phosphatases released by AM fungi during plant growth, and estimate the contribution of P absorbed by AM external mycelium to plant total P uptake.

2. Materials and methods

Plants were grown in acrylic (Plexiglas^{TM}) pots separated into three compartments by 30- μ m nylon mesh to allow the spatial separation of soil zones for root and mycorrhizal hyphal growth. The central compartment was used for root growth and the two outer compartments for growth of arbuscular mycorrhizal hyphae (Li et al., 1991). The calcareous loamy soil

was collected from field plots receiving 270 kg fertilizer N ha⁻¹ year⁻¹ at Changping Long-Term Fertilizer Station of China Agricultural University in Beijing. The soil had 1.24% organic C, 2.6 mg kg⁻¹ Olsen-P, 87.2 mg kg⁻¹ NaOH-extractable N, 100 mg kg⁻¹ ammonium acetate-exchangeable K, and a pH value (in CaCl₂) of 7.5. The soil was air dried, sieved (<2 mm), and steam-sterilized. The central compartment of each pot contained 100 g and each outer compartment contained 360 g of air-dried sterilized soil.

Glomus versiforme (Karsten) Berch was propagated on maize roots and inoculum consisted of spores, mycelium, fine root segments and soil. The fungus was kindly donated by Professor Honggang Wang, Institute of Soils and Fertilizers, Chinese Academy of Agricultural Sciences, 30 Baishiqiao Road, Haidian District, Beijing 210008, PR China. The fungus has not been lodged in an international culture collection but can be obtained directly from Professor Wang. Fifteen grams of inoculum were placed in the central root compartment of the mycorrhizal pots and 15 g of sterilized inoculum were added to the non-mycorrhizal pots. Ten milliliters of washings of the inoculum filtered to remove arbuscular mycorrhizal propagules were added to the central compartment of the non-mycorrhizal treatments to minimize differences in the rhizosphere microbial communities of mycorrhizal and non-mycorrhizal plants.

The experiment had five main treatments: 50 mg P kg⁻¹ soil applied as KH₂PO₄ (analytical grade, Beijing Chemical Co.), RNA (Beijing Chemical Reagents Co.), lecithin (Shanghai Organic Chemical Co.) or sodium phytate (phytic acid (inositol hexaphosphoric acid) sodium salt from corn, cat. no. P-8810, Sigma), and unfertilized controls. Within each main treatment, there were two sub-treatments: inoculation with *G. versiforme* and non-mycorrhizal. In addition, one extra type of mycorrhizal control ('AM control') was included in which the central compartment was separated from the two outer compartments by 0.45- μ m nylon mesh through which neither fungal hyphae nor roots could pass but which allowed solute diffusion to occur. There were four replicates of each treatment, giving 132 pots in total arranged fully randomly in the greenhouse and with the position of each pot re-randomized every week.

All pots received mineral nutrients which were added uniformly to each compartment at rates of

200 mg N (as NH_4NO_3), 150 mg K (as K_2SO_4), 50 mg Mg (as MgSO_4), 5 mg Zn (as ZnSO_4), 2 mg Cu (as CuSO_4) and 10 mg Ca (as CaSO_4) kg^{-1} soil. The pots receiving the different P sources had P applied to the two outer compartments at the rate of 50 mg P kg^{-1} .

Red clover (*Trifolium pratense* L.) was used as the host plant. Seeds were surface-sterilized in 10% (v/v) hydrogen peroxide for 10 min and rinsed at least five times in deionized water. Fifty seeds were sown in each pot and thinned to 20 after emergence. The seedlings grew in a glasshouse at a temperature range of 20–25 °C and with a photoperiod of 15 h.

The experiment was harvested three times: 35, 49 and 70 days after sowing. On each occasion 44 pots representing four randomly selected replicates of each treatment were carefully opened and the compartments were separated. The roots were removed from the central compartment and washed with running tap water. A sub-sample of soil was collected for determination of phosphatase activity. Shoots and roots were washed with deionized water. Roots were cut into 1-cm segments and thoroughly mixed. A sub-sample of 0.5 g was cleared with 10% (w/v) KOH at 90 °C for 2 h and stained with Trypan blue. Mycorrhizal colonization was assessed by the grid-line intersect method as described by Giovannetti and Mosse (1980). The shoots and the remainder of the roots were oven dried at 70 °C and the dry weights were recorded. The samples were ground and dry-ashed in a muffle furnace at 300 °C for 3 h and at 550 °C for 5 h. The ash was dissolved using 2% (v/v) nitric acid. Phosphorus was determined by the molybdovanadophosphate method (Kitson and Mellon, 1944).

From the total P uptake of the plants, we calculated the amount of P derived from P applied to the hyphal compartments by subtracting the P uptake of unfertilized controls from that of treatments to which P was applied as follows:

$$\text{AM hyphal contribution to plant P uptake} = 1 - \frac{\text{total P uptake by non-AM plants} \times 100}{\text{total P uptake by AM plants}} \quad (\text{a})$$

In addition, the P absorbed by external mycelium from the P applied to the hyphal compartments was calculated by subtracting the amount of P derived from added sources by non-mycorrhizal plants from that

derived by mycorrhizal plants. The proportion of total P uptake taken up by the mycelium was then calculated as a percentage to compare the contribution of AM mycelium to the total plant uptake of P from the various sources of applied P as follows:

$$\text{AM hyphal contribution to plant uptake of applied P} = \frac{\text{P uptake by AM mycelium}}{\text{total P uptake}} \quad (\text{b})$$

The contribution of native soil P to plant P nutrition was calculated and the contribution of P uptake by hyphae from the soil was calculated by subtracting the total P uptake of non-mycorrhizal plants from the total P uptake of mycorrhizal plants as reported previously by Tarafdar and Marschner (1995):

$$\text{AM hyphal contribution to uptake of soil native P} = 100 - \frac{\text{P uptake by AM mycelium}}{\text{total P uptake}} \quad (\text{c})$$

Data were subjected to analysis of variance using the GENSTAT 5 statistical package (GENSTAT Committee, 1994) in a factorial design of five applied P sources (including no-P control) $\times$ two mycorrhizal colonization treatments + one ‘AM control’ (mycorrhizal, as described above) $\times$ three harvest dates $\times$ four replicates. A separate set of 44 pots representing four replicates of each treatment was randomly selected for each harvest and destructively harvested to recover shoots and roots. Thus, the data for the three harvests were obtained from different replicates and are statistically independent. Data presented are the means of four replicates with standard deviations. Estimates of P derived from applied and native soil P and from uptake by external mycelium were calculated from mean values because the experimental design was fully randomized and not include randomized blocks. These estimates could not therefore be calculated separately for individual replicates and were not amenable to statistical analysis.

3. Results

3.1. Mycorrhizal colonization

Root growth was dense in the central compartments and the roots were in close contact with the nylon net

Table 1

Mycorrhizal colonization (% of root length colonized) at different growth stages of red clover roots inoculated with *Glomus versiforme* and supplied with inorganic P or organic P sources. Values are means ($n = 4$) $\pm$ one standard deviation (S.D.)

Applied P source	Days after sowing		
	35 days	49 days	70 days
Control (no applied P)	46.7 $\pm$ 3.3	59.3 $\pm$ 13.1	58.5 $\pm$ 7.9
KH ₂ PO ₄	49.2 $\pm$ 2.8	55.6 $\pm$ 2.5	50.0 $\pm$ 2.0
Lecithin	50.1 $\pm$ 5.4	67.3 $\pm$ 3.6	60.3 $\pm$ 5.5
RNA	50.5 $\pm$ 2.4	68.0 $\pm$ 8.3	62.1 $\pm$ 6.6
Na phytate	47.8 $\pm$ 5.0	71.7 $\pm$ 6.7	65.7 $\pm$ 10.4
'AM control'	46.2 $\pm$ 4.0	62.1 $\pm$ 2.4	57.6 $\pm$ 1.8
Significance ^a due to:			
P source		NS	
Harvest time		***	
P source $\times$ harvest time		NS	

NS, not significant.

^a By analysis of variance.

*** $P < 0.001$.

separating the root and hyphal compartments. Mycorrhizal colonization rates determined 35, 49 and 70 days after sowing of red clover in inoculated pots are shown in Table 1. No mycorrhizal colonization of uninoculated plant roots was observed under the microscope. The plants inoculated with *G. versiforme* had mean colonization rates ranging from 46.2 to 50.5%, 55.6 to 71.7% and 50.0 to 65.7% 5, 7 and 10 weeks after sowing, respectively. There was a general trend of increasing colonization rate at the second harvest with a subsequent slight decrease by the third harvest, but there was considerable variation in the data. There was little difference among P sources in root colonization after 35 days, but at later stages of plant growth, the plants receiving organic P sources tended to have higher colonization than those receiving inorganic P. The 'AM control' results, in which hyphal growth was restricted to the root compartment, were similar to the conventional controls.

3.2. Effect of *G. versiforme* inoculation on growth of clover receiving different P sources

Mycorrhizal plants had higher dry matter (DM) yields than did non-mycorrhizal plants with all applied P sources and zero-P controls and at all three harvests (Table 2) and this mycorrhizal effect became more pronounced as plant growth proceeded. All added P sources had similar effects on shoot

yield, but root yields were slightly depressed when the added P source was Na-phytate or RNA. The 'AM control' had shoot and root yields that were intermediate between non-mycorrhizal and mycorrhizal controls. In general, different trends among P sources were mainly due to the fertilizer effect of all P sources compared with unamended controls rather than differences among added P sources.

3.3. Plant P concentrations

Inoculation with *G. versiforme* increased shoot and root P concentrations (Table 3) and this mycorrhizal effect became more pronounced as plant growth proceeded. By the tenth week of growth, mycorrhizal plants with added organic P sources tended to have higher shoot P concentrations than those receiving orthophosphate. There was no such effect of P source on root P concentration at the final harvest, when mycorrhizal plants receiving RNA had lower root P concentrations than mycorrhizal plants growing with added inorganic P.

3.4. Plant P uptake

Mycorrhizal plants took up more P than did non-mycorrhizal plants at all three harvests (Table 4) and this mycorrhizal effect became more pronounced as plant growth proceeded. By the third harvest, my-

Table 2

Shoot, root and total (shoot + root) dry matter yield of mycorrhizal and non-mycorrhizal red clover plants (g per pot) at different stages of plant growth with applied inorganic P or organic P

Applied P source	Mycorrhizal status	Shoot			Root			Total		
		35 days	49 days	70 days	35 days	49 days	70 days	35 days	49 days	70 days
Control (no P)	Non-mycorrhizal	0.35 ± 0.07	0.68 ± 0.04	1.06 ± 0.18	0.09 ± 0.01	0.25 ± 0.02	0.26 ± 0.04	0.44 ± 0.07	0.93 ± 0.04	1.32 ± 0.21
Control (no P)	Mycorrhizal	0.49 ± 0.07	1.09 ± 0.07	1.80 ± 0.19	0.11 ± 0.01	0.34 ± 0.05	0.42 ± 0.03	0.60 ± 0.07	1.43 ± 0.11	2.22 ± 0.18
KH ₂ PO ₄	Non-mycorrhizal	0.45 ± 0.08	0.74 ± 0.01	1.02 ± 0.06	0.15 ± 0.05	0.24 ± 0.01	0.30 ± 0.04	0.60 ± 0.11	0.98 ± 0.02	1.32 ± 0.03
KH ₂ PO ₄	Mycorrhizal	0.52 ± 0.12	1.17 ± 0.03	1.99 ± 0.24	0.17 ± 0.05	0.36 ± 0.02	0.42 ± 0.06	0.68 ± 0.17	1.52 ± 0.03	2.41 ± 0.30
Lecithin	Non-mycorrhizal	0.39 ± 0.10	0.72 ± 0.04	1.09 ± 0.11	0.11 ± 0.02	0.28 ± 0.02	0.29 ± 0.03	0.49 ± 0.12	1.00 ± 0.06	1.38 ± 0.12
Lecithin	Mycorrhizal	0.49 ± 0.07	1.18 ± 0.12	2.07 ± 0.22	0.14 ± 0.02	0.45 ± 0.03	0.44 ± 0.04	0.63 ± 0.09	1.63 ± 0.15	2.51 ± 0.25
RNA	Non-mycorrhizal	0.42 ± 0.04	0.77 ± 0.08	1.05 ± 0.01	0.12 ± 0.03	0.22 ± 0.03	0.25 ± 0.03	0.54 ± 0.04	0.99 ± 0.11	1.30 ± 0.03
RNA	Mycorrhizal	0.49 ± 0.09	1.21 ± 0.11	1.92 ± 0.25	0.12 ± 0.02	0.33 ± 0.06	0.43 ± 0.09	0.61 ± 0.08	1.54 ± 0.12	2.35 ± 0.33
Na-phytate	Non-mycorrhizal	0.40 ± 0.09	0.74 ± 0.03	1.19 ± 0.19	0.10 ± 0.02	0.24 ± 0.03	0.28 ± 0.05	0.50 ± 0.10	0.98 ± 0.05	1.47 ± 0.23
Na-phytate	Mycorrhizal	0.49 ± 0.07	1.06 ± 0.02	2.10 ± 0.10	0.13 ± 0.03	0.33 ± 0.03	0.44 ± 0.04	0.61 ± 0.09	1.39 ± 0.03	2.54 ± 0.14
'AM control'	Mycorrhizal	0.40 ± 0.04	0.93 ± 0.04	1.64 ± 0.12	0.10 ± 0.01	0.39 ± 0.06	0.43 ± 0.04	0.50 ± 0.04	1.31 ± 0.10	2.07 ± 0.15
Significance due to:										
Applied P source			NS		***				*	
Mycorrhizal status			***		***				***	
Harvest time			***		***				***	
P source × mycorrhizal status			NS		NS				NS	
P source × harvest time			NS		*				NS	
Mycorrhizal status × harvest time			***		***				***	
P source × mycorrhiza × harvest time			NS		NS				NS	

Values are means ($n = 4$) ± S.D.

Table 3

Shoot and root P concentration in mycorrhizal or non-mycorrhizal red clover plants (mg P g^{-1}) at different stages of plant growth with applied inorganic-P or organic-P

Applied P source	Mycorrhizal status	Shoot			Root		
		35 days	49 days	70 days	35 days	49 days	70 days
Control (no added P)	Non-mycorrhizal	0.88 $\pm$ 0.12	0.71 $\pm$ 0.05	1.36 $\pm$ 0.15	1.84 $\pm$ 0.29	2.18 $\pm$ 0.16	1.03 $\pm$ 0.21
Control (no added P)	Mycorrhizal	2.02 $\pm$ 0.10	1.39 $\pm$ 0.10	1.70 $\pm$ 0.21	2.06 $\pm$ 0.84	1.92 $\pm$ 0.55	1.41 $\pm$ 0.06
KH ₂ PO ₄	Non-mycorrhizal	0.73 $\pm$ 0.13	0.82 $\pm$ 0.09	1.58 $\pm$ 0.08	2.07 $\pm$ 0.44	1.71 $\pm$ 0.39	0.99 $\pm$ 0.07
KH ₂ PO ₄	Mycorrhizal	1.95 $\pm$ 0.12	1.80 $\pm$ 0.12	1.73 $\pm$ 0.06	2.92 $\pm$ 0.59	2.71 $\pm$ 0.19	1.62 $\pm$ 0.11
Lecithin	Non-mycorrhizal	0.90 $\pm$ 0.14	0.73 $\pm$ 0.10	1.63 $\pm$ 0.54	1.58 $\pm$ 0.55	2.11 $\pm$ 0.28	0.76 $\pm$ 0.12
Lecithin	Mycorrhizal	2.01 $\pm$ 0.10	1.65 $\pm$ 0.04	2.09 $\pm$ 0.15	2.80 $\pm$ 0.46	2.27 $\pm$ 0.09	1.74 $\pm$ 0.05
RNA	Non-mycorrhizal	0.98 $\pm$ 0.20	0.78 $\pm$ 0.10	1.70 $\pm$ 0.22	1.23 $\pm$ 0.27	1.82 $\pm$ 0.16	0.70 $\pm$ 0.03
RNA	Mycorrhizal	2.10 $\pm$ 0.17	1.54 $\pm$ 0.26	2.08 $\pm$ 0.13	3.07 $\pm$ 0.84	2.22 $\pm$ 0.14	1.40 $\pm$ 0.11
Na-phytate	Non-mycorrhizal	1.04 $\pm$ 0.28	0.66 $\pm$ 0.05	1.65 $\pm$ 0.21	1.74 $\pm$ 0.58	1.82 $\pm$ 0.15	0.76 $\pm$ 0.10
Na-phytate	Mycorrhizal	2.08 $\pm$ 0.08	1.76 $\pm$ 0.24	2.36 $\pm$ 0.23	2.79 $\pm$ 0.19	2.58 $\pm$ 0.13	1.71 $\pm$ 0.14
‘AM control’	Mycorrhizal	1.84 $\pm$ 0.15	2.11 $\pm$ 0.11	1.25 $\pm$ 0.09	2.36 $\pm$ 0.31	1.50 $\pm$ 0.13	1.51 $\pm$ 0.25
Significance due to:							
Applied P source		***			*		
Mycorrhizal status		***			***		
Harvest time		***			***		
P source $\times$ mycorrhizal status		NS			***		
P source $\times$ harvest time		**			NS		
Mycorrhizal status $\times$ harvest time		***			***		
P source $\times$ mycorrhiza $\times$ harvest time		NS			**		

Values are means ($n = 4$) $\pm$ S.D.

Table 4

Shoot, root and total (root + shoot) P uptake by mycorrhizal and non-mycorrhizal red clover plants (mg per pot) at different stages of plant growth with applied inorganic-P or organic-P

Applied P source	Mycorrhizal status	Shoot			Root			Total		
		35 days	49 days	70 days	35 days	49 days	70 days	35 days	49 days	70 days
Control (no P)	Non-mycorrhizal	0.31 ± 0.08	0.48 ± 0.06	1.45 ± 0.39	0.16 ± 0.03	0.55 ± 0.06	0.26 ± 0.06	0.47 ± 0.06	1.03 ± 0.10	1.73 ± 0.45
Control (no P)	Mycorrhizal	1.00 ± 0.13	1.53 ± 0.19	3.05 ± 0.34	0.23 ± 0.07	0.66 ± 0.27	0.59 ± 0.03	1.23 ± 0.11	2.19 ± 0.44	3.64 ± 0.32
KH ₂ PO ₄	Non-mycorrhizal	0.33 ± 0.09	0.60 ± 0.07	1.61 ± 0.07	0.32 ± 0.17	0.42 ± 0.12	0.30 ± 0.04	0.65 ± 0.20	1.03 ± 0.18	1.91 ± 0.07
KH ₂ PO ₄	Mycorrhizal	1.00 ± 0.22	2.10 ± 0.13	3.43 ± 0.31	0.49 ± 0.18	0.96 ± 0.08	0.68 ± 0.07	1.49 ± 0.37	3.06 ± 0.15	4.11 ± 0.35
Lecithin	Non-mycorrhizal	0.34 ± 0.07	0.52 ± 0.08	1.77 ± 0.55	0.16 ± 0.02	0.59 ± 0.11	0.23 ± 0.06	0.50 ± 0.06	1.11 ± 0.16	1.99 ± 0.53
Lecithin	Mycorrhizal	0.99 ± 0.17	1.94 ± 0.18	4.29 ± 0.18	0.39 ± 0.09	1.02 ± 0.06	0.77 ± 0.07	1.38 ± 0.26	2.95 ± 0.21	5.06 ± 0.24
RNA	Non-mycorrhizal	0.42 ± 0.12	0.61 ± 0.13	1.79 ± 0.24	0.14 ± 0.05	0.40 ± 0.04	0.17 ± 0.01	0.56 ± 0.14	1.01 ± 0.16	1.95 ± 0.25
RNA	Mycorrhizal	1.02 ± 0.15	1.86 ± 0.33	4.01 ± 0.75	0.35 ± 0.08	0.73 ± 0.17	0.59 ± 0.08	1.37 ± 0.14	2.59 ± 0.49	4.60 ± 0.82
Na-phytate	Non-mycorrhizal	0.40 ± 0.09	0.49 ± 0.06	1.93 ± 0.17	0.17 ± 0.06	0.44 ± 0.05	0.22 ± 0.06	0.57 ± 0.10	0.93 ± 0.11	2.14 ± 0.19
Na-phytate	Mycorrhizal	1.01 ± 0.16	1.86 ± 0.27	4.95 ± 0.41	0.36 ± 0.08	0.85 ± 0.09	0.75 ± 0.09	1.37 ± 0.21	2.71 ± 0.34	5.69 ± 0.49
'AM control'	Mycorrhizal	0.74 ± 0.10	1.96 ± 0.13	2.06 ± 0.17	0.23 ± 0.03	0.58 ± 0.12	0.65 ± 0.15	0.97 ± 0.10	2.54 ± 0.23	2.71 ± 0.28
Significance due to:										
Applied P source		***			***			***		
Mycorrhizal status		***			***			***		
Harvest time		***			***			***		
P source × mycorrhizal status		**			***			***		
P source × harvest time		***			*			***		
Mycorrhizal status × harvest time		***			***			***		
P source × mycorrhiza × harvest time		***			NS			**		

Values are means ($n = 4$) ± S.D.

Table 5

Calculated P derived from four applied P sources before sowing by mycorrhizal and non-mycorrhizal red clover at different stages of plant growth (mg per pot) and the contribution to plant P uptake by the external mycelium from the P sources applied to the outer compartments (mg per pot)

Applied P source	Mycorrhizal status	P derived from applied P			P uptake by mycelium		
		35 days	49 days	70 days	35 days	49 days	70 days
KH ₂ PO ₄	Non-mycorrhizal	0.10	0.00	0.43	NA ^a	NA	NA
KH ₂ PO ₄	Mycorrhizal	0.26	0.87	1.66	0.16	0.87	1.23
Lecithin	Non-mycorrhizal	0.03	0.08	0.24	NA	NA	NA
Lecithin	Mycorrhizal	0.15	0.76	1.41	0.08	0.66	1.17
RNA	Non-mycorrhizal	0.09	−0.02	0.28	NA	NA	NA
RNA	Mycorrhizal	0.14	0.40	1.11	0.05	0.4	0.83
Na-phytate	Non-mycorrhizal	0.10	−0.10	0.22	NA	NA	NA
Na-phytate	Mycorrhizal	0.14	0.52	2.02	0.04	0.52	1.80

Values calculated from mean of four replicates (see text for details).

^a NA, not applicable to non-mycorrhizal treatments.

Table 6

Calculated AM mycorrhizal hyphal contribution to (a) plant total P uptake, (b) plant uptake of applied P and (c) plant uptake of native soil P (all expressed as % of plant uptake) at different stages of growth of mycorrhizal red clover plants

Applied P source	(a) Total P			(b) Applied P			(c) Native soil P		
	35 days	49 days	70 days	35 days	49 days	70 days	35 days	49 days	70 days
Control (no added P)	62	53	53	0	0	0	100	100	100
KH ₂ PO ₄	62	66	60	11	28	23	89	72	77
Lecithin	64	62	61	6	22	23	94	78	77
RNA	59	61	58	4	15	17	96	85	83
Na-phytate	58	66	66	3	19	31	97	81	69

Values calculated from means of four replicates (see text for details).

corrhizal plants receiving organic sources of P (with the possible exception of RNA) had taken up more total P than plants growing with added KH₂PO₄.

3.5. Contribution of hyphal P uptake from the P sources applied to the outer compartments

In our experimental design, there were two main sources of P: native soil P and applied P, with only native soil P available to the controls. The calculated plant P uptake derived from applied P sources and the P taken up by mycelium from the hyphal compartments are shown in Table 5. In turn, these estimates were used to calculate the hyphal contribution to P absorption from different sources and the results are presented in Table 6.

The hyphal contribution to uptake of different organic P sources by red clover increased with time. In the fifth and the seventh weeks, the hyphal

contribution to uptake of the three organic P sources was lower than that of KH₂PO₄, with sodium phytate showing the smallest contribution. At the final harvest, the highest hyphal contribution to P uptake was 31% from sodium phytate. The value for both KH₂PO₄ and lecithin was 23% and for RNA was 17% (Table 6).

4. Discussion

Although organic P typically represents a major fraction of soil P and some organic P compounds (e.g. phytin, lecithin and glycerophosphate) are very important in the P nutrition of plants (Tarafdar and Claassen, 1988), the contribution of soil organic compounds to plant P nutrition is still poorly understood. Moreover, we are not aware of any published investigations on the contribution of AM external mycelium to host plant P uptake from organic sources. The present study has

allowed some quantitative evaluation of the contribution of some principal forms of organic P to plants with or without AM fungal colonization. The results show that when the roots of red clover were confined to the central compartment of the rhizoboxes the extraradical mycelium made a contribution of 53–65% of plant P uptake from the soil, 17–31% of which was derived from organic P. This implies that the contribution of AM hyphae to plant P nutrition by absorbing from the organic P pool in soil can be considerable (Table 6) and indicates that AM fungi may play an important role in cycling of organic P in the soil. The fully randomized experimental design used was not optimal for this work because it did not permit the calculation of independent replicate estimates of the hyphal contribution to plant P uptake that would have been possible using a randomized block design. Nevertheless, it did permit the calculation of mean values for the hyphal contribution to P uptake from different organic P compounds.

To evaluate the uptake of P from phosphates applied in hyphal compartments by AM hyphae the rhizosphere effect of roots and native soil P should be taken into account and subtracted from the total P uptake of mycorrhizal plants. According to the experimental design, significantly higher yields and P uptake in mycorrhizal plants compared with non-mycorrhizal plants at a given P status implies that the extraradical mycelium made a substantial contribution by absorbing P, including native soil P and applied P, from the hyphal compartments. The observed higher yields, shoot and root P concentrations and higher P uptake values in mycorrhizal plants with applied phytate, lecithin and RNA compared with mycorrhizal zero-P controls indicate that AM extraradical mycelium can increase the bioavailability of these three principal forms of soil organic P. The higher yields, plant P concentrations and P uptake sometimes observed in non-mycorrhizal plants that received applied phytate, lecithin and RNA compared with mycorrhizal controls demonstrates a marked rhizosphere effect of plant roots on uptake of organic and inorganic P. The rhizosphere effect not only increased P uptake from both inorganic and organic P sources, but also promoted plant growth and this indicates that plant roots may be able to utilize phytate, lecithin and RNA directly. These effects may have been due to the release of acid phosphatase and phytase from the plant roots (Li et al., 1997a,b; Shand and Smith, 1997; Hayes et al., 1999).

The abundance of the three principal forms of soil organic P follows the order inositol phosphate $\gg$ phospholipids $>$ nucleic acid. However, the same types of organic P compounds are found in plants in the reverse order of abundance (Stevenson and Cole, 1999). The accumulation of phytate in soils indicates that it is resistant to rapid mineralization (Anderson, 1980). In contrast, organic P compounds such as glucose 1-phosphate, nucleic acid and phospholipids may be mineralized more readily (Dalal, 1977). The results of the present study indicate that when organic P sources such as phytate and lecithin are present at the same concentration as KH_2PO_4 in soil, they may make a similar contribution to plant growth and P nutrition compared with soluble inorganic phosphorus.

The P in organic compounds can become available to plants after hydrolysis by phosphatase enzymes. It has been suggested that AM fungi are able to produce acid phosphatase that is released into the soil (Joner and Johansen, 2000; Saito, 1995) and enhance the soil acid phosphatase activity (Tarafdar and Marschner, 1995; Feng et al., 2002; Song et al., 2000). A recent report showed that the extraradical hyphae of *Glomus intraradices* can hydrolyze 5-bromo-4-chloro-3-indolyl phosphate, phenolphthalein diphosphate and phytate under axenic culture conditions (Koide and Kabir, 2000). In this study, unlike the inorganic phosphate (KH_2PO_4), the bioavailability of the organic P sources (phytate, lecithin and RNA) increased with the increasing growth stage of plant and AM fungus (Tables 2 and 4). This may be attributed to an increase in the activity of acid and alkaline phosphatases in soil by the extraradical hyphae of AM fungi (unpublished data). Numerous studies have demonstrated that extraradical hyphae can increase the acid or alkaline phosphatase activities of soil (Feng et al., 2002; Song et al., 2000; Tarafdar and Marschner, 1994, 1995).

The role of phytase released by plant roots in decomposition of phytate in soil has received much interest in recent years (Hayes et al., 1999, 2000a; Richardson et al., 2000; Li et al., 1997a,b; Shand and Smith, 1997). Phytase is believed to lack specificity and can hydrolyze bonds in a range of substrates including monoesters, a complex diester (RNA), pyrophosphate, a linear polyphosphate and a cyclic polyphosphate (Shand and Smith, 1997). However, whether or not AM hyphae can produce and release

phytase is still unknown and further studies are required to elucidate this.

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