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Bi-directional transfer of phosphorus between red clover and perennial ryegrass via arbuscular mycorrhizal hyphal links

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Abstract

Perennial ryegrass and red clover were grown in low-P soil in separate compartments within rhizoboxes. The compartments were separated by 30- μ m pore nylon mesh and a buffer compartment to prevent root-to-root contact but allow hyphal penetration. Both plant species were established as donor and receiver plants. Donors were inoculated with an arbuscular mycorrhizal (AM) fungus, *Glomus mosseae*, forming hyphal links between donors and receivers. Two rates of P (50 and 100 mg P kg⁻¹) were applied to the soil in the receiver compartments. All other compartments received 50 mg P kg⁻¹. In one experiment ³²P tracer was applied to the roots of the donors after 6 weeks' growth to detect P transfer from donors to receivers. In a second experiment, plants harvested 2 weeks later were examined for root AM colonisation, shoot yield and P uptake. Arbuscular mycorrhizal fungal inoculation of donor roots led to increased yield, P concentration and P uptake of receiver shoots. Shoot P concentrations were higher in non-mycorrhizal ryegrass than in non-mycorrhizal clover. The ³²P tracer study showed a lower apparent P transfer rate from ryegrass to clover than from clover to ryegrass despite a comparable amount of P transferred. Internal P requirements of the host plants may influence P transfer rates. The AM hyphae formed an important link between the plants but no appreciable net transfer of P was likely to occur.

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Keywords: Arbuscular mycorrhiza; Bi-directional transfer; Hyphal links; Pasture; ³²P Tracer

1. Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous and can colonise the roots of most terrestrial plants under a wide range of soil conditions [1]. The AM fungi colonising one plant may also make contact with the root systems of neighbouring plants in the field, and the hyphae connecting two or more plants are referred to as hyphal links. Hyphal links can be established between plants of the same species (for example, clover) [2], or between different species (for example, soybean and maize) [3], and are particularly likely to form

among the plant species growing in perennial plant communities [4]. It is a widely held belief that hyphal links are potentially important for the transfer of mineral nutrients and carbohydrates (C) among plants [5,6]. An experiment using ¹⁴C labelling techniques has indeed indicated that C transfer via AM hyphal links did occur among 19 of 20 associated plant species [6]. The grass *Festuca ovina* was fed with ¹⁴C and detection of the tracer after 72 h revealed that ¹⁴C was transferred to all the other plants of 18 species connected to *F. ovina* by hyphal links. The one exception that did not contain the tracer was a non-mycorrhizal plant species. Transfer of phosphorus (P) and nitrogen (N) between plants via hyphal links has also been reported for many plants in pot culture and in glasshouse conditions [7–10]. Most of the experiments were designed to investigate transfer in one direction

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Fig. 1. Diagram showing the design of the rhizoboxes.

despite the possible occurrence also of transfer in the opposite direction. Once attention was drawn to the occurrence of nutrient transfer by hyphal links, its potential ecological significance was gradually recognised, particularly in grassland ecosystems [11].

Grasses and legumes are often important components of grassland ecosystems. Fodder production in such systems may be increased by 13% by introducing legumes, and previous work has attributed the growth promotion to the fixation of atmospheric N_2 by legumes [12]. In China, clover and ryegrass are commonly grown together in mixtures for fodder production on soils of low P availability. The question of whether hyphal links may contribute to plant nutrition and yield in addition to the effects of N_2 fixation is therefore an interesting and promising area of study. This paper describes two experiments in which red clover and perennial ryegrass were grown together and studied as both donor and receiver plants with respect to applied P. The apparent transfer of P from donors to receivers was determined and compared in order to elucidate the bi-directional transfer of P via hyphal links.

2. Materials and methods

2.1. Rhizobox design

PVC boxes were used as plant growth containers, with each box divided into five compartments as shown in Fig. 1. Donor and receiver plants were grown in compartments D and R, respectively. Compartment S was set up to minimise the risk of free transfer of P between compartments D and R, while the two outermost compartments (B) were set up to buffer the water status in the plant growth compartments D and R. Length and height of the five compartments were 8 and 8 cm, respectively, while widths of compartments D, S, R and compartments B were 2 and 3 cm, respectively.

2.2. Plant growth

Red clover (*Trifolium pratense* L.) and perennial ryegrass (*Lolium perenne* L.) were selected as both donor and receiver

plants. Seeds of the two species were surface-sterilised with 10% v/v H_2O_2 for 30 min, placed in the dark at 25 °C until the stage of embryo emergence, and then sown in rhizoboxes. The AM fungal species used was *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe. Inoculum comprised a sandy soil containing spores and maize root fragments. The original inoculum was kindly provided by Professor Hong-gang Wang, Institute of Soils and Fertilisers, Chinese Academy of Agricultural Sciences, 30 Baishiqiao Road, Haidian District, Beijing 210008, China and propagated on maize plants grown in a sandy soil for 10 weeks. The fungus has not been lodged in an international culture collection but can be obtained directly from Professor Wang. Sandy loam soil was air dried and sieved through a 1-mm mesh. The soil had the following properties: pH (in H_2O) 7.8, Olsen-P 3.2 mg kg^{-1} , Kjeldahl-N 0.027% and organic matter 0.39%. Soil was autoclaved at 121 °C for 2 h to kill the native AM fungi. Seeds were sown in compartments D and R, which were separated from compartments S and B by nylon net of pore size 30 μm to prevent penetration by roots while allowing hyphal penetration.

One hundred and sixty grams of soil were placed in each of compartments D, S and R, and 250 g in the outer compartments (B). Mycorrhizal treatments were established by mixing fresh inoculum with the soil in compartment D at a ratio of 1:15 by weight, and non-mycorrhizal controls were prepared in the same way using sterilised inoculum. All donor compartments also received 10 ml of an aqueous filtrate (0.25 μm pore size) of unsterilised soil. Phosphorus (as KH_2PO_4) was applied at either 50 (P1) or 100 (P2) mg P kg^{-1} to compartments R of both mycorrhizal and non-mycorrhizal pots. All other compartments received 50 mg P kg^{-1} . The aim was to supply enough P to this P-deficient soil to allow plant establishment but not enough to inhibit mycorrhiza development. Nitrogen (as NH_4NO_3) was applied at a rate of 200 mg kg^{-1} and potassium (as K_2SO_4) at 150 mg kg^{-1} to all five compartments. The various treatments are shown in Table 1 for clarity. The plants grew in a glasshouse at China Agricultural University in Beijing from August to October 2000, with temperature variation from 20 to 25 °C, and a light/dark photoperiod of 14/10 h was maintained using supplementary illumination. The water content of the boxes was adjusted regularly by weight. Water was added to the central separation compartment to minimise movement of P between the donor and receiver compartments or from the outer hyphal compartments to the root compartments by mass flow or diffusion.

Eight replicates of each treatment were established in a randomised block design for two experiments. The first experiment (the tracer experiment) was conducted using four replicates randomly selected for ^{32}P labelling. In the second experiment the remaining four replicates were prepared for determination of plant dry matter (DM) yield, mycorrhizal infection rate and plant nutrient uptake. Data were tested by analysis of variance using the GENSTAT package [13].

Table 1

Quantities of phosphorus applied to the growth compartments of the mycorrhizal and non-mycorrhizal donor and receiver plants

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass (mg P kg ⁻¹ soil)		Ryegrass to clover (mg P kg ⁻¹ soil)	
		Donor	Receiver	Donor	Receiver
50	Non-mycorrhizal	50	50	50	50
	Mycorrhizal	50	50	50	50
100	Non-mycorrhizal	50	100	50	100
	Mycorrhizal	50	100	50	100

Eight replicate pots of each treatment were established, giving a total of 64 pots.

2.3. Experiment 1: ³²P labelling

After the plants had grown for four weeks the rhizoboxes were placed in plastic trays containing distilled water and a 5-mm-diameter hole was opened in the base of compartment D. Part of the donor plant root system penetrated through the hole and grew into the distilled water in the plastic tray. This part of the root system was labelled with ³²P at a rate of 5.36×10^5 Bq pot⁻¹ as carrier-free orthophosphate. The radioactively labelled KH₂³²PO₄ with 14.5 µCi activity was applied to each tray after the roots in the distilled water had grown in the trays for 2 weeks. The KH₂³²PO₄ solutions were completely absorbed by the roots within 12 h and after a further period of 2 weeks the plants were harvested, giving a total growth period of 8 weeks.

2.4. Experiment 2: harvest and plant analysis

The plants were harvested after 8 weeks of growth. Shoots and roots were separated and their oven dry weights (70 °C for 48 h) were determined. Root subsamples were stained by the method of Phillips and Hayman [14] and mycorrhizal colonisation rate (the percentage of root length colonised) was determined using the grid-line intersection method of

Giovannetti and Mosse [15]. Samples of ground plant material were dry ashed at 560 °C, dissolved in HCl and analysed for a range of nutrient elements by inductively coupled plasma-atomic emission spectroscopy. Radioactivity of ³²P in the shoots from the labelling treatments was measured using a low background Model BH1216 scintillation counter.

3. Results

No AM colonisation was observed in any root subsamples from the non-mycorrhizal treatments. In the mycorrhizal treatments, both clover and ryegrass had higher colonisation rates as donors than as receivers, especially at the higher of the two P levels (Table 2). The colonisation rates of both clover and ryegrass were slightly (but not significantly) inhibited by the higher P level when they were receivers. Clover had a higher overall colonisation rate (range 25–37%) than did ryegrass (20–26%).

Shoot DM yield of all plants was always increased by AM inoculation (Table 3). The shoot yield of non-mycorrhizal clover was below 1 mg DM pot⁻¹ except when clover was the receiver and 100 mg P kg⁻¹ was applied. In contrast, all mycorrhizal clover shoot yields were above 1 mg DM pot⁻¹

Table 2

Mean AM colonization rate of mycorrhizal donor and receiver plant roots (*n* = 4)

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass (% of root length colonized)		Ryegrass to clover (% of root length colonized)	
		Donor	Receiver	Donor	Receiver
50	Non-mycorrhizal	0.0	0.0	0.0	0.0
	Mycorrhizal	35.8	22.7	26.4	25.3
100	Non-mycorrhizal	0.0	0.0	0.0	0.0
	Mycorrhizal	37.1	19.6	23.7	27.1
Standard error		1.76			

Significance^a due to:

Direction

*

P addition level to receiver soil

NS

Plant species

Direction × P addition level

NS

Direction × plant species

P addition level × plant species

NS

NS, not significant.

^a By analysis of variance.* *P* < 0.05.*** *P* < 0.001.

Table 3

Shoot yield of donor and receiver plants with and without AM hyphal links

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass (mg DM pot ⁻¹)		Ryegrass to clover (mg DM pot ⁻¹)	
		Donor	Receiver	Donor	Receiver
50	Non-mycorrhizal	0.95	3.60	3.60	0.95
	Mycorrhizal	1.23	3.76	3.85	1.03
100	Non-mycorrhizal	0.98	3.81	3.50	1.06
	Mycorrhizal	1.18	4.04	3.71	1.24
Standard error				0.056	

Significance^a due to:

Mycorrhizal colonization

Direction

*

P addition level to receiver soil

*

Plant species

Mycorrhiza × plant species

NS

Mycorrhiza × P level

NS

P level × plant species

NS

Direction × mycorrhiza

NS

Direction × P level

NS

Direction × plant species

*

NS, not significant.

^a By analysis of variance.* $P < 0.05$.*** $P < 0.001$.

(Table 3). The increase in clover shoot yield due to AM inoculation was significant except when clover was the receiver with the lower P addition level of 50 mg kg⁻¹. Ryegrass had much higher shoot yields than did clover.

Inoculation with the AM fungus significantly increased shoot P concentration in all treatments except ryegrass as receiver with 100 mg kg⁻¹ of applied P (Table 4). In general,

the increases in P concentration in clover shoots were higher than those in ryegrass. Inoculation greatly increased shoot P uptake, with a higher increase in clover shoots than that in ryegrass (Table 5).

When the differences in P uptake by mycorrhizal and non-mycorrhizal receiver shoots were used to calculate the contribution of mycorrhiza to P uptake by receiver shoots,

Table 4

Phosphorus concentration in donor and receiver shoots with and without hyphal links

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass (mg kg ⁻¹)		Ryegrass to clover (mg kg ⁻¹)	
		Donor	Receiver	Donor	Receiver
50	Non-mycorrhizal	0.90	1.39	1.40	0.88
	Mycorrhizal	1.58	1.57	1.55	1.37
100	Non-mycorrhizal	0.98	1.73	1.31	1.07
	Mycorrhizal	1.87	1.73	1.79	1.96
Standard error				0.096	

Significance^a due to:

Mycorrhizal colonization

Direction

NS

P addition level to receiver soil

Plant species

Mycorrhiza × plant species

Mycorrhiza × P level

NS

P level × plant species

NS

Direction × mycorrhiza

NS

Direction × P level

NS

Direction × plant species

NS

NS, not significant.

^a By analysis of variance.*** $P < 0.001$.

Table 6
Contribution of AM hyphae to phosphorus uptake by mycorrhizal receiver shoots

Receiver P level (mg kg ⁻¹)	Ryegrass as receiver (%)	Clover as receiver (%)
50	14.4	40.5
100	5.1	50.9
Standard error	3.65	5.29
Significance ^a due to:		
P addition level	NS	NS

NS, not significant.

^a By analysis of variance.

the contribution was found to be different in clover and ryegrass (Table 6). When ryegrass was receiver, the hyphal contributions were 14% and 5% with application rates of 50 and 100 mg P kg⁻¹ to the receiver compartment, respectively. The corresponding values when clover was receiver were 40% and 51%, much higher than when ryegrass was receiver, and there was no significant difference between the two rates of P applied to the receiver compartment.

Owing to root absorption of KH₂³²P₃O₄ from the plastic trays in the tracer experiment, radioactivity was detected in the donor plant shoots. Much higher radioactivity was detected in ryegrass shoots than in clover (Table 7). ³²P was transferred from donor plants to receiver plants in the mycorrhizal treatments via the external hyphae. The levels of ³²P radioactivity in the shoots were comparable in receivers of both plant species at both added P levels, ranging from 0.3 × 10⁴ to 0.5 × 10⁴ cpm pot⁻¹ (Table 7). Although traces of radioactivity were also detected in non-mycorrhizal receiver

shoots, these were much lower and were negligible compared to the levels measured in mycorrhizal receiver shoots.

Phosphorus transfer rates were calculated according to the intensity of ³²P radioactivity, and the results are shown in Table 8. Transfer rates in non-mycorrhizal treatments were much lower than those in mycorrhizal treatments. In mycorrhizal treatments, transfer rates at the low P addition level were slightly higher than those at 100 mg P kg⁻¹. In addition, transfer rates from clover plants to ryegrass plants were significantly higher than those in the opposite direction, although rates in both directions were low.

4. Discussion

Colonisation of plant roots by AM fungi varies greatly [16], especially when comparison is made between dicots and monocots [17]. In general, colonisation rates of mono-

Table 5
Phosphorus uptake by donor and receiver plant shoots in the presence and absence of hyphal links

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass (mg pot ⁻¹)		Ryegrass to clover (mg pot ⁻¹)	
		Donor	Receiver	Donor	Receiver
50	Non-mycorrhizal	0.86	5.01	5.06	0.83
	Mycorrhizal	1.93	5.87	5.98	1.40
100	Non-mycorrhizal	0.96	6.58	4.57	1.14
	Mycorrhizal	2.23	6.97	6.61	2.43
Standard error				0.205	
Significance ^a due to:					
Mycorrhizal colonization				***	
Direction				**	
P addition level to receiver soil				***	
Plant species				***	
Mycorrhiza × plant species				NS	
Mycorrhiza × P level				NS	
P level × plant species				NS	
Direction × mycorrhiza				NS	
Direction × P level				NS	
Direction × plant species				*	

NS, not significant.

^a By analysis of variance.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 7

Total radioactivity of ^{32}P in donor and receiver plant shoots in the presence and absence of AM hyphal links

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass ($\times 10^4$ cpm pot ⁻¹)		Ryegrass to clover ($\times 10^4$ cpm pot ⁻¹)	
		Donor	Receiver	Donor	Receiver
50	Non-mycorrhizal	12.15	1.22	90.93	0.10
	Mycorrhizal	13.20	0.47	68.65	0.37
100	Non-mycorrhizal	40.45	0.10	68.05	0.10
	Mycorrhizal	12.82	0.25	81.57	0.30
Standard error				3.710	

Significance^a due to:

Mycorrhizal colonization

*

Direction

P addition level to receiver soil

NS

Plant species

Mycorrhiza $\times$ plant species

NS

Mycorrhiza $\times$ P level

NS

P level $\times$ plant species

*

Direction $\times$ mycorrhiza

NS

Direction $\times$ P level

*

Direction $\times$ plant species

NS, not significant.

^a By analysis of variance.* $P < 0.05$.*** $P < 0.001$.

cots tend to be lower than those of dicots. In our experiments, colonisation rates of ryegrass plants were about 10% lower than clover plants as donors (Table 2). A greater ability of ryegrass roots to absorb P, as indicated by their higher shoot P concentrations (Table 4), may have contributed to this effect. Plant species may also differ in root length and it is possible that a greater length of mycorrhizal roots in ryegrass than clover was also a contributory factor. At the same added P level of 50 mg kg⁻¹, both plant species showed higher colonisation rates as donors than as receivers, and this may have been due, at least in part, to the placement of fungal inoculum in the donor compartments and not to the receivers.

Nutrient transfer via hyphal links between plants of the same species or of different species has been confirmed in many experiments. In the present experiment, ^{32}P was applied to detect directly the transfer of P. In the non-mycorrhizal treatments, traces of ^{32}P were detected in receiver plants, suggesting that the 3-cm buffer zone used was not wide enough to prevent completely the transfer of ^{32}P labelled root exudates. Despite this, much higher levels of ^{32}P radioactivity were detected in receiver plants in the mycorrhizal treatments (Table 7). This indicates that ^{32}P was transferred from donor to receiver plants via AM hyphal links. In general, the amount of P transferred was very low,

Table 8

Phosphorus transfer rate from donor to receiver plants, calculated as the ^{32}P activity of receiver shoots as a percentage of that of donor shoots

Receiver P level (mg kg ⁻¹)	Mycorrhizal status	Clover to ryegrass (%)	Ryegrass to clover (%)
50	Non-mycorrhizal	1.03	0.11
	Mycorrhizal	3.49	0.56
100	Non-mycorrhizal	0.33	0.15
	Mycorrhizal	2.08	0.38
Standard error		0.354	0.052

Significance^a due to:

Mycorrhizal colonization

P addition level to receiver soil

*

NS

Mycorrhiza $\times$ P level

NS

NS

NS, not significant.

^a By analysis of variance.* $P < 0.05$.*** $P < 0.001$.

varying from 0.3×10^4 to 0.5×10^4 cpm pot⁻¹ (Table 7), and this may be due to the relatively low colonisation rates and comparable P concentrations in donor and receiver plants. The study would have been more complete with inclusion of root DM yields and root P concentrations and uptake values. Unfortunately, however, roots are notoriously difficult to recover quantitatively from experiments in which soil is used as the growth substrate, and root P analysis is also unreliable because it is difficult to remove soil contamination of the roots by washing techniques.

Earlier studies have shown that numerous factors can affect the amount of nutrient transferred between plants via hyphal links, including the status of hyphal development between plants [2] and differences in nutrient concentrations between plants [18]. In the present experiments, the P transfer rate from red clover to ryegrass was higher than that from ryegrass to clover. Both the intensity of ³²P radioactivity in receiver shoots and the P transfer rate were higher when 50 mg P kg⁻¹ were applied to the donor compartment compared with 100 mg kg⁻¹ added P (Tables 7 and 8). Table 4 shows that P concentrations in non-mycorrhizal ryegrass shoots were much higher than in non-mycorrhizal clover shoots, irrespective of P addition level or donor or receiver status. This indicates that the internal P requirement of ryegrass was higher than that of clover, leading to a lower apparent P transfer rate from ryegrass to clover than from clover to ryegrass. Francis et al. [18] also suggested that the effectiveness of nutrient transfer between connected roots would clearly vary from species to species according to their nutrient demands. However, our data indicate that hyphae are an important link between the plants but under these experimental conditions no appreciable net transfer of P occurred. Thus the amount of P that a receiver plant would obtain for growth would be similar irrespective of whether the donor was ryegrass or clover.

The available P level in the soil would also have contributed to the P transfer rate affecting the P concentration in the plant. A higher P application to the receiver compartment increased the P concentration in the receiver plants, resulting in lower P transfer rates via hyphal links. Although the colonisation rates of ryegrass and clover plants were investigated in the present study, the status of hyphal development (especially the total hyphal length in the donor compartment and the status of the hyphal links) was not examined. Further studies are required on the development of hyphal links to further elucidate the mechanisms of P transfer from donor to receiver plants.

5. Conclusions

The results indicate that ³²P transfer via AM hyphal links took place both from red clover to perennial ryegrass and in the opposite direction. However, the apparent rate of transfer was higher from clover to ryegrass than from ryegrass to clover. Furthermore, the ³²P transfer rate was influenced by the soil P supply to the receiver plants, with a tendency for

higher soil available P status to decrease the transfer rate. Traces of ³²P were detected in non-mycorrhizal receiver plants, indicating that some P transfer from donors to receivers had occurred by mechanisms other than through hyphal links, for example, mass flow and diffusion. The data indicate that the mycorrhizal hyphae were an important link between the plant species but no net transfer of P occurred between the plants under these experimental conditions and may not be likely to occur in the field.

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