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Responses of mycorrhizae and shoot phosphorus of maize to the frequency and timing of soil disturbance

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Abstract. In several growth chamber studies, both P absorption and mycorrhizal colonization of plants grown in soil left undisturbed after removal of the shoots of the previous crop were higher relative to plants in disturbed soil. However, in one of these studies the soil was disturbed only once instead of after each of three growth cycles, and this resulted in identical colonization in the undisturbed and disturbed treatments. The present study was conducted to systematically investigate the effect of varying the frequency and timing of soil disturbance on mycorrhizal colonization. Maize (*Zea mays* L.) was grown for four 3-week cycles in pots which initially contained disturbed soil. Five soil disturbance treatments were used to assess the impact of the frequency with which soil is disturbed and the impact of the timing of the disturbance. The frequency of soil disturbance had major effects on mycorrhizal colonization, while the timing of soil disturbance was more related to the reduction in shoot P absorption resulting from disturbance. These results suggest that the extraradical mycelium plays a key role in the mechanism by which soil disturbance reduces shoot P absorption.

Key words: Arbuscular mycorrhizae – Vesicular-arbuscular mycorrhizae (VAM) – Maize – Phosphorus – Soil disturbance – Hyphal length

Introduction

Several growth chamber studies conducted at the University of Guelph examined the effect of soil disturbance on P absorption by plants subsequently grown in that soil. The disturbance of the soil was by hand and by passage through a 5-mm sieve.

Two types of growth chamber studies have been reported. First, there have been studies which used undisturbed cores of soil collected from field plots. Plants were grown for 21 days in such undisturbed cores and also disturbed adjacent cores. The shoot P absorption and mycorrhizal colonization of plants grown in the undisturbed soil were higher when compared to plants in disturbed soil (O'Halloran et al. 1986; Evans and Miller 1988). It has been argued that the higher P absorption by plants in undisturbed soil is caused by improved mycorrhizal symbiosis in those plants compared with plants in the disturbed soil (Evans and Miller 1988).

Second, there have been studies which began with disturbed soil in pots in which maize was shown repeatedly to create a sequence of four growth cycles, each of 21 days. Shoots were clipped off at the soil surface at the end of each growth cycle, and the soil in the pots was either disturbed or not. Plants grown in soil left undisturbed after each growth cycle had a higher shoot P absorption and more extensive mycorrhizal development than plants grown in disturbed soil (Fairchild and Miller 1988, 1990).

McGonigle et al. (1990a) modified the second type of experimental system by disturbing the soil only once, immediately before the start of the fourth and final growth cycle. Again, plants in the undisturbed soil had a higher shoot P concentration than those in the disturbed soil. However, although mycorrhizal colonization of roots was extensive, it did not differ between the two disturbance treatments. This was surprising given that such a difference had always been found in previous work using both the first (O'Halloran et al.1986; Evans and Miller 1988) or the second (Fairchild and Miller 1988, 1990) type of experimental system described.

The study by McGonigle et al. (1990a) suggested that the frequency of soil disturbance is important in determining the differences in mycorrhizal colonization. It is important to note that the effect of soil disturbance on shoot P absorption is quite invariable: plants in less disturbed soil have always been found to have higher shoot P levels, irrespective of whether or not mycorrhizal colonization is changed by distur-

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bance. The experiment described here was designed to systematically investigate the effects of frequency and timing of soil disturbance on mycorrhizal colonization and P absorption of maize. We hoped that contrasting plants with similar levels of colonization but different disturbance histories would also throw light on the mechanism by which soil disturbance reduced shoot P absorption. Starting with disturbed soil in all pots and going through four cycles of growth, we compared five soil disturbance treatments at each of two P fertility levels.

Materials and methods

Maize (var. Pioneer 3949) was cultivated in soil in pots for four 3-week cycles of growth. The growth chamber was set to a 16-h day with 75 W m^{-2} and 21° C and an 8-h night at 13° C. There was a 7-day interval between clipping off the shoots at the end of one cycle and seeding the next. The soil was a silt loam with 3 ppm $NaHCO_3$-extractable P taken from the Elora Research Station, Ontario, Canada. Soil was broken up mechanically so that it passed a 5-mm sieve and was packed into pots of 20-cm diameter to a depth of 14 cm and at a bulk density of 1.1 g dry soil equivalent cm^{-3}. For each growth cycle, six seeds in the range 0.23–0.28 g per seed were planted in each pot and thinned to three seedlings per pot at 7 days. Pots were maintained gravimetrically at 25 g H_2O 100 g^{-1} dry soil.

There were two soil P levels and five soil disturbance treatments arranged in a randomized complete block design with five replicates, giving 50 pots in all. The two P fertilizer rates were zero and 75 mg P kg^{-1} dry soil as monocalcium phosphate $Ca(H_2PO_4)_2$. H_2O powder, mixed thoroughly with the soil at the start of the study. Soil disturbance consisted of breaking up the soil by hand so that it could pass a 5-mm sieve. During soil disturbance, roots were cut into fragments about 2 cm long and thoroughly mixed with the soil. Each pot to be disturbed was handled in two 7-cm depth fractions that were kept separate and re-potted in order, the uppermost returning to the top.

There were five soil disturbance treatments: U (undisturbed) and D123, D12, D2, D3 where the numbers indicate the growth cycles followed by soil disturbance: D123 was disturbed after each of the first three cycles, and D12 after the first and second cycles; in D2 and in D3 the soil was disturbed only after the second or third cycle, respectively. Thus, the order D123, D12, D2, D3 and U represents a sequence of decreasing frequency of disturbance. On the basis of frequency, the D2 and D3 treatments could be placed in either order.

At the end of each cycle, shoots were dried for determination of mass and tissue concentrations of P and N (Thomas et al. 1967). A 33-mm diameter soil core extending downwards the full 14-cm depth was taken from each pot at the end of all four growth cycles. Cores were positioned mid-way between any two stem bases of adjacent plants. All cores were taken immediately following the clipping of shoots, and wooden dowels were used to plug the holes created by the core removal. When the soil in pots containing dowels was disturbed, the dowels were inserted upright into the pots before replacing the soil. Roots from each of the cores were washed free of soil against a 0.5-mm screen and stored in formyl acetic alcohol.

After rinsing, root samples were cut into pieces about 2 cm long. Where necessary, chopped root samples were subsampled by dispersal in water. Clearing and staining followed Brundrett et al. (1984), and mycorrhizal colonization was assessed by the procedure of McGonigle et al. (1990b) which gives values for arbuscular colonization (AC) and hyphal colonization (HC) as the proportions of root length colonized by arbuscules and hyphae, respectively. No attempt was made to distinguished between hyphae of mycorrhizal or other fungi.

A second set of 33-mm diameter soil cores, identical to those described above, was taken immediately after the final growth cycle and used to determine the length density of hyphae in soil. Soil samples were analyzed for hyphae using the following procedure, which incorporates some of the modifications of Miller and Jastrow (1992) to the protocol of Abbott et al. (1984). Samples for analysis of hyphae were homogenized by hand mixing after passing the soil through a 2-mm sieve. Roots were finely chopped using scissors and remixed with the sieved soil. A subsample of approximately 4 g dry mass equivalent of the mixture of homogenized soil and finely chopped roots was dispersed for 30 min in 280 ml 6.4 mM sodium hexametaphosphate $Na(PO_3)_6$. The dispersion was diluted by a factor of 50 in 6.4 mM $Na(PO_3)_6$ and 20 ml of the dilution was collected on a 0.020-mm nylon screen. Hyphae were stained for 30 min with 0.6% trypan blue in 1:2:2 lactic acid:glycerine:deionized water. After staining, hyphae were rinsed in deionized water, collected on a 0.012-mm cellulose nitrate membrane filter, air dried for 30 min and mounted in immersion oil. Estimation of hyphal length on the slide was as described by Abbott et al. (1984) at ×200 magnification. Whether the hyphae extracted from soil were mycorrhizal or not was unclear, and they were simply scored as brown pigmented or blue stained.

Means were compared using paired *t*-tests following analyses of variance. Separate analyses of variance were used at each harvest. Percentage AC and HC data were subjected to angular transformation prior to statistical analysis; back-transformed means of the transformed data are presented.

Results

P fertilization

From first to last, the four growth cycles are referred to as C1, C2, C3 and C4.

The dry mass, P concentration and P content of shoots were strongly increased by P fertilization at all harvests (Tables 1, 2, 3; Fig. 1a–c). In the absence of P fertilizer, the shoots were P deficient, with concentrations reaching only 1.34 g kg^{-1} at the end of the C4 cycle (Fig. 1b). Addition of 75 mg P kg^{-1} dry soil raised shoot P concentrations to 1.45–2.66 g kg^{-1} at the end of the experiment (Fig. 1b). In contrast, shoot N concentration was significantly reduced by P fertilization (Fig. 1d).

Roots in all treatments were mycorrhizal at all harvests. AC was unaffected by P fertilization at all times of sampling (Tables 1, 2, 3; Fig. 2a), whereas P fertilization reduced HC after the first cycle (Table 1) but not subsequently (Tables 2, 3; Fig. 2b).

Table 1. Shoot dry mass, shoot nutrient status and colonization of roots at the end of the C1 growth cycle. Means in any row followed by different letters are significantly different at the 5% level

	No P added	P fertilized
Number of pots	25	25
Dry mass (g $shoot^{-1}$)	0.32a	1.32b
Shoot [P] (g kg^{-1})	1.04a	1.63b
P content (mg $shoot^{-1}$)	0.36a	2.12b
Shoot [N] (g kg^{-1})	28.6a	29.1a
Arbuscular colonization (%)	26.4a	26.8a
Hyphal colonization (%)	74.2b	55.5a

Table 2. Shoot dry mass, shoot nutrient status and colonization of roots at the end of the C2 growth cycle. Means in any row followed by different letters are significantly different at the 5% level

	No P added		P fertilized	
	Disturbed after C1	Undisturbed after C1	Disturbed after C1	Undisturbed after C1
Number of pots	10	15	10	15
Dry mass (g shoot^{-1})	0.33a	0.36a	0.91b	0.88b
Shoot [P] (g kg^{-1})	1.09a	1.16a	1.58b	1.87c
P content (mg shoot^{-1})	0.36a	0.39a	1.38b	1.65b
Shoot [N] (g kg^{-1})	29.8a	29.6a	29.1a	30.1a
Arbuscular colonization (%)	17.2a	27.2b	21.0a	27.3b
Hyphal colonization (%)	55.2a	72.2b	46.8a	66.5b

Table 3. Shoot dry mass, shoot nutrient status and colonization of roots at the end of the C3 growth cycle. Means in any row followed by different letters are significantly different at the 5% level

	No P added			P fertilized		
	Disturbed after C1 and C2	Disturbed after C2 only	Undisturbed after C1 and C2	Disturbed after C1 and C2	Disturbed after C2 only	Undisturbed after C1 and C2
Number of pots	10	5	10	10	5	10
Dry mass (g shoot^{-1})	0.32a	0.32a	0.39a	0.68b	0.69b	1.18c
Shoot [P] (g kg^{-1})	1.07a	1.09a	1.16b	1.39c	1.41c	1.88d
P content (mg shoot^{-1})	0.34a	0.35a	0.46a	0.97b	0.97b	2.09c
Shoot [N] (g kg^{-1})	28.0c	27.9c	26.9bc	25.0ab	24.8ab	22.4a
Arbuscular colonization (%)	11.2a	15.6ab	22.9b	16.8ab	16.2ab	21.8b
Hyphal colonization (%)	39.8a	50.9b	70.2c	42.9ab	51.3b	64.7c

The hyphal length density (HLD) of brown-pigmented hyphae did not differ among treatments and had an overall mean ±sd of 6.1±3.1 m g^{-1} for $n=50$. The occurrence of hyphae stained blue was greater, ranging from 40.7 m g^{-1} to 69.3 m g^{-1} (Fig. 2c). The HLD of hyphae stained by trypan blue was significantly reduced by P fertilization in the U treatment, but not in other soil disturbance treatments (Fig. 2c).

Soil disturbance treatments

The higher shoot P concentration of plants in the undisturbed soil first appeared after the C2 cycle for P-fertilized pots (Table 2), but not before the third harvest under low-P conditions (Table 3). A greater shoot dry mass for plants in undisturbed soil was not seen until after the C3 cycle for those pots given P fertilizer (Table 3) and not before the final harvest for the low-P soil (Fig. 1a). Disturbance of soil after both the C1 and C2 cycles affected shoot growth and P absorption at the third harvest to the same extent as disturbance imposed only after the C2 cycle (Table 3). At both levels of soil fertility, there were larger differences in shoot P content between the plants in the U and the D123 treatments at the end of the C4 cycle than at the earlier harvests (Table 4).

At the final harvest, the higher shoot dry mass and P concentration associated with the U treatment was more pronounced at the higher soil P level (Fig. 1a, b), with a factor of two difference in shoot P content between D123 and U under low soil P, but a fourfold difference between the corresponding treatments in P-fertilized soil (Fig. 1c).

In the low-P soil, the growth and P absorption of plants in the D12, D2, D3 and D123 treatments were indistinguishable (Fig. 1a, b, c). At the higher soil P, level, the D12, D2 and D3 treatments produced shoot dry masses intermediate between the D123 and U treatments (Fig. 1a). In the P-fertilized soil, the D12 and D2 treatments produced shoot P concentrations intermediate between the D123 and U treatments, but the D3 treatment produced a lower shoot P concentration more similar to the D123 treatment (Fig. 1b).

Shoot N concentrations for plants in the low-P soil were unaffected by disturbance (Tables 2, 3; Fig. 1d). In the P-fertilized soil, shoot N concentration at the final harvest was lower in the U and D3 treatments than in the D12 and D123 treatments (Fig. 1d).

At both soil P levels, AC and HC at the C2 harvest were reduced by soil disturbance (Table 2). In the unfertilized system, AC and HC at the C3 harvest were lower in soil which had been disturbed after the C1 and C2 cycles than in undisturbed soil (Table 3). At the final harvest, there was a gradual increase in both AC and HC with decreasing frequency of soil disturbance (Fig. 2a, b).

In the low-P soil, the HLD of blue-stained hyphae was significantly higher in the U treatment compared to the D123 treatment (Fig. 2c). There was also a positive relationship between the HLD of blue-staining hyphae and the HC of the roots, but again only in the low-P soil (Fig. 3).

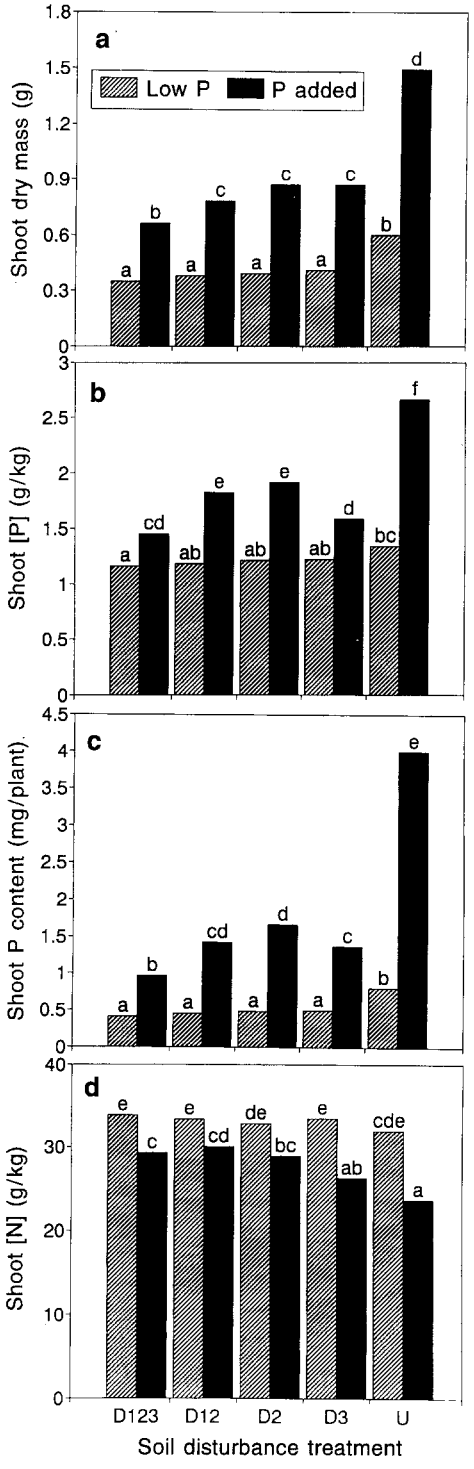

Fig. 1. **a** Shoot dry mass, **b** shoot P concentration, **c** shoot P content and **d** shoot N concentration, at the end of the final (C4) growth cycle. Disturbance treatments (D123, D12, D2, D3 and U) are explained in the text. Means surmounted by different letters are significantly different at the 5% level ($n=5$)

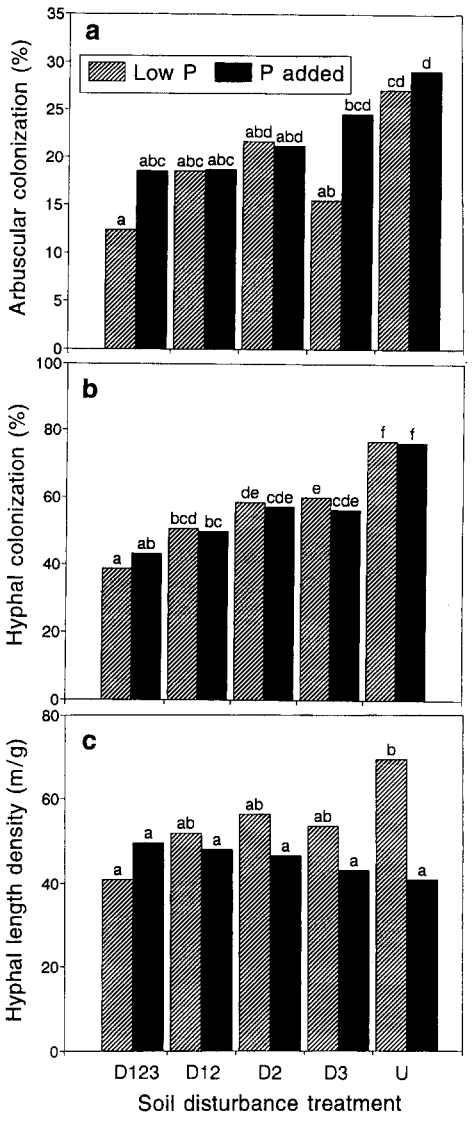

Fig. 2. **a** Arbuscular colonization, **b** hyphal colonization and **c** length density in the bulk soil of hyphae which stain with trypan blue, at the end of the final (C4) growth cycle. Treatments and statistical comparisons as in Fig. 1

Discussion

In references to previous studies, we will use the notation for soil disturbance treatments outlined in the Materials and methods section above rather than used in the original papers.

In the growth chamber study of McGonigle et al. (1990a), AC was higher than that found in the present study, although roots were extensively colonized with hyphae in both cases. There was no change from one harvest to the next in the extent of colonization by arbuscules or hyphae. This consistency contrasts with the data of Fairchild and Miller (1988), who found colonization increased from the earlier to the later growth cycles. This may be explained by the colonization intensity rating used by Fairchild and Miller (1988), if there is little change in the proportion of root length colonized from cycle to cycle, but instead an increase in the density of fungal proliferation within colonized regions.

There are two interpretations of the weak correspondence between HC and HLD. First, the fungi in the soil outside the roots are only partly represented by those inside the roots. Second, if the fungi outside

Table 4. Absolute and percentage differences in mean shoot P content between the U and the D123 treatments at the harvests at the end of the C4, C3 and C2 growth cycles

Harvest	No P added		P fertilized	
	U-D123 (mg shoot^{-1})	100 × (U-D123)/U (%)	U-D123 (mg shoot^{-1})	100 × (U-D123)/U (%)
End of C4	0.39	48.8	3.03	75.9
End of C3	0.12	26.1	1.12	53.6
End of C2	0.03	7.7	0.27	16.4

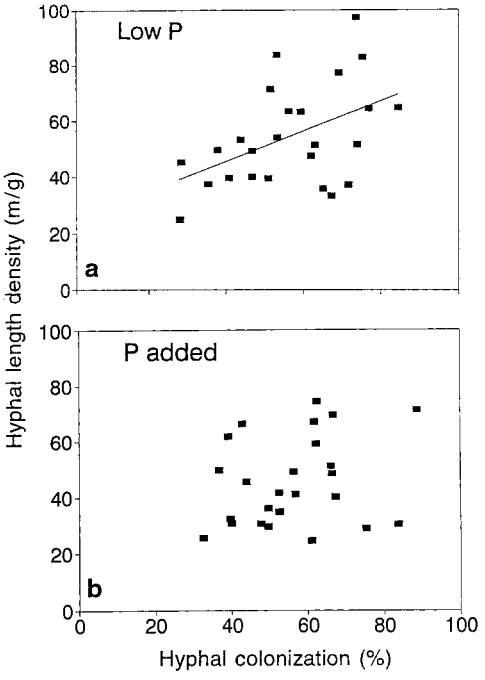

Fig. 3. Plots of length density in the bulk soil of trypan blue-staining hyphae against hyphal colonization of the roots at the end of the final (C4) growth cycle, **a** with no P fertilizer added and **b** for pots with 75 mg P added kg^{-1} dry soil. For the low P soil there is a significant relationship ($F_{1,23}=6.67$; $P=0.0166$; $r^2=0.22$) for which the regression line ($y=23.4+0.547\ x$) has been drawn in. There is no significant relationship for the P-fertilized soil ($F_{1,23}=1.38$; $P=0.25$; $r^2=0.06$)

and inside the roots are mostly the same, then the external and internal phases of those fungi responded differently to the soil disturbances treatments which produced the range of colonization.

As found previously (Fairchild and Miller 1990; McGonigle et al. 1990a), the soil at the Elora field site responded little to P fertilization in terms of reduced mycorrhizal colonization.

The higher shoot P concentration and mycorrhizal colonization in the U treatment compared to the D123 treatment were expected from earlier work (Fairchild and Miller 1988, 1990). The larger difference in shoot P content between the U and D123 treatments at the end of the C4 cycle, compared to those between the corresponding pots at the earlier harvests, was also reported by Fairchild and Miller (1988).

The data here confirms the results of McGonigle et al. (1990a), who found that D3 plants had a lower shoot P concentration than U plants, but that both treatments had similar AC values. For plants grown under glasshouse conditions during the summer, Miller and McGonigle (1992) also found AC to be the same for the U and D3 treatments but lower in the D123 treatment, while shoot P concentration was highest in the U treatment.

Considering plants in the P-fertilized soil, at the final harvest the D123 and D3 treatments had lower shoot P concentrations than the D12 and D2 treatments (Fig. 2). Both the D123 and D3 treatments employed disturbance of soil immediately prior to the start of the final growth cycle; thus the timing of soil disturbance may be important in determining shoot P concentration. In contrast, the frequency of soil disturbance had a greater effect on the colonization of the roots. The gradual increase in AC and HC from the D123 to the U treatments (Fig. 2a, b) shows that the number of times the soil is disturbed is important in determining the extent of root colonization. A similar effect of the frequency of soil disturbance on colonization can be seen at the harvest at the end of the C3 cycle (Table 3).

The mechanism by which disturbance of soil affects colonization is unclear. Plants subjected to a higher frequency of disturbance had less colonization and it would at first seem plausible to attribute this to a greater accumulation of inoculum in pots disturbed less frequently. However, this supposed role of inoculum accumulation contradicts the finding that colonization did not increase from the first to the final cycle of growth. The extraradical phase of the mycorrhizal fungi is certainly involved in the mechanism by which disturbance of soil reduces colonization (Jasper et al. 1989a, b; Evans and Miller 1990). Evans and Miller (1990) disturbed a soil volume from which roots but not extraradical hyphae were excluded. Disturbance of this soil volume led to lower colonization of plants compared to plants grown in similar but undisturbed root-free compartments.

In the D12 and D2 treatments, the rooting volume may be considered to have had intact extraradical mycelium which was carried over from the C3 to the C4 cycle, whereas in the D123 and D3 treatments this extraradical mycelium would have been broken up prior to the start of the final growth cycle. The outcome of the experiment presented here is, therefore, consistent with the interpretation that P absorption is lower in disturbed soil because of the breaking up of the extraradical mycelium of the mycorrhizal fungi.

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