

Nutrient transfer between the root zones of soybean and maize plants connected by a common mycorrhizal mycelium

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The objective of the study was to determine whether nutrient fluxes mediated by hyphae of vesicular-arbuscular mycorrhizal (VAM) fungi between the root zones of grass and legume plants differ with the legume's mode of N nutrition. The plants, nodulating or nonnodulating isolines of soybean [*Glycine max* (L.) Merr.], were grown in association with a dwarf maize (*Zea mays* L.) cultivar in containers which interposed a 6-cm-wide root-free soil bridge between legume and grass container compartments. The bridge was delimited by screens (44 μ m) which permitted the passage of hyphae, but not of roots and minimized nonVAM interactions between the plants. All plants were colonized by the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe. The effects of N input to N-sufficient soybean plants through N₂-fixation or N-fertilization on associated maize-plant growth and nutrition were compared to those of an N-deficient (nonnodulating, unfertilized) soybean control. Maize, when associated with the N-fertilized soybean, increased 19% in biomass, 67% in N content and 77% in leaf N concentration relative to the maize plants of the N-deficient association. When maize was grown with nodulated soybean, maize N content increased by 22%, biomass did not change, but P content declined by 16%. Spore production by the VAM fungus was greatest in the soils of both plants of the N-fertilized treatment. The patterns of N and P distribution, as well as those of the other essential elements, indicated that association with the N-fertilized soybean plants was more advantageous to maize than was association with the N₂-fixing ones.

Key words – *Bradyrhizobium japonicum*, *Glomus mosseae*, *Glycine max*, nitrogen fixation, nutrient allocation, plant nutrition, vesicular-arbuscular mycorrhiza, *Zea mays*.

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Introduction

Colonization by vesicular-arbuscular mycorrhizal (VAM) fungi influences the transfer of nutrients between the root systems of associated plants (Chiariello et al. 1982, Francis et al. 1986, Heap and Newman 1980). The occurrence of VAM-mediated interplant transfer of C (Francis and Read 1984), N (van Kessel et al. 1985) and P (Ritz and Newman 1984) has been well-documented by the use of isotope-tracer methods. While nutrient fluxes of sufficient magnitude to cause quantitatively important changes in plant growth or nu-

trition have yet to be demonstrated (see Newman 1988), the potential implications of this phenomenon in understanding plant community structure have been recognized as profound (Fitter 1985) and suggest a concept of resource distribution in plant communities optimized by the movement of nutrients along concentration gradients between VAM donor and receiver plants (Read et al. 1985).

The input of N by legumes (Blevins et al. 1990) is known to be important in agro-ecosystem management (Gliessman 1990). In order for nonlegumes to benefit from this gain in N, mineralization of N-rich plant resid-

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ues was thought to be a pre-condition for the eventual utilization of biologically fixed N. This process involves only the 'soil pool pathway' (sensu Newman and Ritz 1986) of inter-plant nutrient transfer, but recent observations indicate that it is supplemented by a direct transfer pathway via VAM hyphae (for review, see Newman 1988). While the resource distribution concept of Read et al. (1985) describes a plausible mechanism for undisturbed ecosystems, where direct nutrient fluxes between plants associated through their symbiotic fungi of long-established compatibility may facilitate long-term equilibria, its validity is more problematic in agro-ecosystems. Here, soil disturbance can inhibit nutrient uptake by disrupting VAM soil hyphae (Evans and Miller 1990), while rotation of crop plants (However et al. 1987) may routinely hinder the establishment of compatible host-endophyte combinations. Intercrops, such as the model legume-grass system, may pose an additional problem by requiring the shared VAM mycelium to be compatible in terms of nutrient-transfer capability with different, arbitrarily-chosen host plants.

The purpose of our experiment was to determine whether VAM-mediated nutrient transfer occurs between the root zones of a legume-grass intercrop system, and whether this transfer is affected by the source of N input to the system under conditions where nutrient exchange by means other than the VAM hyphae is minimized.

Abbreviations – NOD, nodulated; VAM, vesicular-arbuscular mycorrhizal.

Materials and methods

The experimental unit, experimental design and statistics

Associations of soybean [*Glycine max* (L.) Merr. cv. Clark] and maize (*Zea mays* L. cv. MM296) were grown in 3-compartment containers (Fig. 1). The root compartments of the associated plants were separated by a soil bridge (6 cm wide) delimited by screens (44 μ m openings) on both sides of the bridge. The screens permitted the passage of VAM hyphae, but not of roots. The root-free zone was interposed between the associated plants to minimize nutrient transfer by exudation, diffusion and root anastomosis, processes which are unavoidable if roots are allowed to intermingle (Haystead et al. 1988, van Kessel et al. 1985).

Three treatments, each with 6 replications, were arranged in a completely random design. The soybean plants of the soybean-maize experimental units were either nodulated (NOD treatment), not nodulated but N-fertilized (+N treatment) or not nodulated and not fertilized (-N treatment). Both the soybean and maize plants of the -N treatments were used as a reference for comparing the effects of N input by N_2 fixation or fertilization on VAM-fungus-mediated N transfer between soybean and maize compartments. Because of

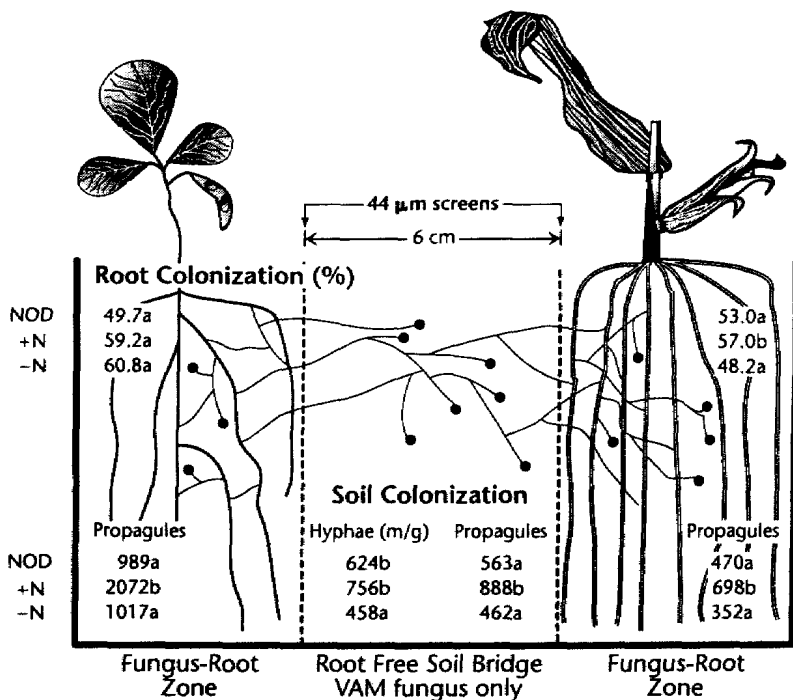


Fig. 1. Root and soil colonization of soybean and maize plants associated by shared VAM-fungal mycelium. Plants were grown in a three-part container. Screens (44 μ m), permeable to hyphae but not to roots, separated the root zones of a 6 cm root-free soil bridge. Soybean plants of the soybean-maize associations were nodulated (NOD), fertilized with NH_4NO_3 (+N) or kept N-deficient (-N). Propagules represent spores and sporocarps per 100 g of soil. Numbers are the means of 6 replications and are significantly ($P < 0.05$) different (linear contrasts) when followed by different numbers.

differences in the morphology and physiology of non-VAM and VAM plants (see Bethlenfalvai and Newton 1991), and because available soil volume (as affected by impermeable barriers to separate associated plants) is an important modifier of growth response (Stevenson 1967), use of a mycorrhizal -N treatment as the reference for evaluation was thought to provide more valid comparisons than the use of nonVAM plants or plants separated by solid barriers as controls.

The differences between the donor and receiver plants of the 3 treatments were evaluated by analysis of variance and linear contrasts. Actual significance values are presented instead of the arbitrary 5% level in order "to give a more precise reading of the probability and to permit the reader to attach his or her own perception of the weight of the evidence to the results" (Nelson 1989). Probability values up to $P=0.10$ were interpreted as being within the range of statistical significance.

Biological materials

A dwarf cultivar (MM296) of maize was used in all treatments. A nodulating isolate of Clark soybean was used for the NOD treatment and a nonnodulating one for the +N and -N treatments. Seeds were germinated for 3 days and selected for uniformity. The nodulating soybean seedlings were inoculated with 15 ml of a suspension (10^9 cells ml^{-1}) of *Bradyrhizobium japonicum* (strain USDA 110) in yeast-mannitol broth. All plants were inoculated with the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd and Trappe, WRRC Isolate No 1, collected from a site without a history of soybean or maize cultivation (Bethlenfalvai et al. 1984). The inoculum (50 g of dry soil stored for 6 months and containing approximately 700 sporocarps and 200 colonized root fragments) was mixed uniformly into the soils of each plant compartment.

Growth conditions

Plants were grown for 65 days in a greenhouse in Albany, CA, from late September to early December, 1989. Automatic control systems were operating at temperatures above 25°C and below 18°C, minimizing day-to-day variations. Supplementary lights (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, General Electric 1000 W metal halide lamps) extended the daylight to 14 h. The soil utilized was a loamy sand of pH 7.7, bulk density of 1.5 g cm^{-3} , organic matter content of 0.2%, and available (NH_4HCO_3 -DTPA extract) nutrient concentrations (mg kg^{-1}) of: N, 4.8; P, 5.7; K, 51; Cu, 0.7; Fe, 13.5; Mn, 8.7; and Zn, 13.4. The watering schedule was the same for all 3 compartments of the soil containers and solution amount was proportional to the volumes of the compartments (plant compartments, 4 l; soil bridge, 1.5 l). Soil solution fluxes were monitored with soil moisture sensors embedded in both the soybean and maize root compartments of the growth containers. Movement of

N between compartments was determined by applying fertilizer N (99.99% $^{14}\text{N-NH}_4\text{NO}_3$) to the soybean compartment of the +N treatment and testing for its appearance in the bridge and maize compartments.

Nutrient regimes were based on calculations from seed, soil and (projected) plant N content at harvest. These indicated that our NOD or +N soybean plants, scheduled to receive the majority of their N input from N_2 fixation at natural ^{15}N abundance (0.3663% ^{15}N) or ^{15}N -depleted fertilizer (0.01% ^{15}N), would have significantly different ^{15}N contents after 6 weeks of growth under their respective N regimes. We assumed that this difference would only be detectable in associated maize plants if nutrient transfer by VAM hyphae connecting both plant compartments were appreciable. We further reasoned that N fluxes from soybean to maize would be enhanced through maximizing source strength in soybean by N-deficiency, using N-deficient (low source strength) soybean as a basis for comparison.

All soybean plants received a nutrient solution 1 mM in NH_4NO_3 until root nodules (of the NOD plants) became functional (3 weeks). Thereafter, NOD and -N soybeans did not receive fertilizer N. The +N soybeans continued to receive N with concentrations adjusted (see below) to keep the +N and NOD plants comparable in size. Maize plants of the 3 treatments were grown under identical conditions, except for their association through VAM hyphae with soybean plants of differing N status. Maize received 2 mM NH_4NO_3 for the first 4 weeks only to relieve initial N deficiency until N transfer from the donor plants might start. The estimate for this time span was based on an approximate rate of advance of a VAM hyphal front through soil of 2 cm week^{-1} , observed previously under similar experimental conditions (Camel et al. 1991). All N fertilizer used was depleted in ^{15}N (99.99% ^{14}N). Other N inputs (soil, seed, air) were at natural abundance (Bremer and van Kessel 1990).

The nutrient solution for all plants initially contained KH_2PO_4 (0.2 mM, first 3 weeks; 0.1 mM, fourth week) until VAM colonization became established. Thereafter, P was not supplied. The solution used for the entire growth period contained other elements at the following concentrations (mM): CaCl_2 , 1.5; K_2SO_4 , 1.0; MgSO_4 , 0.25; and micronutrients (μM): B, 25.0; Co, 0.6; Cu, 0.5; Fe, 20.0; Mn, 2.0; Mo, 0.5; and Zn, 2.0.

The input of N derived from the solution was calculated from the concentrations and the amounts of solution applied. The amount of N derived from N_2 fixation was calculated from the final N content of the nodulated plants. Total N input (mg) available to the plants was: NOD soybean: seed 10.0, soil (available N) 11.0, solution 59.4, air 338.2; -N soybean: seed 7.4, soil 11.0, solution 59.4; +N soybean, seed 7.4, soil 11.0, solution 1120; maize: seed 3.1, soil 11.0, solution 156.4.

Assays

Analyses of soil and plant-tissue ^{15}N concentrations were performed by Isotope Services, Inc. (Los Alamos, NM). Our use of ^{15}N -depleted fertilizer resulted in a deficit in ^{15}N abundance, instead of the atom % excess ^{15}N more frequently used in experimentation (see Hauck 1982). It was calculated as:

atom % deficit $^{15}\text{N} = 1 - (\text{atom \% actual } ^{15}\text{N}/\text{atom \% natural } ^{15}\text{N})$, in analogy with atom % excess values.

Plant tissues were harvested separately, dried at 70°C for 1 day and weighed. Nutrient analyses were performed by the Research Extension Analytical Laboratory, Ohio State University, Wooster, OH. Root length was determined from subsample and total root mass by the grid-line intersect method (Marsh 1971), and percent VAM colonization according to Koske and Gemma (1989). Spore densities in the plant-compartment soils and spore and hyphal densities in the bridge compartments were determined and referenced as described in detail elsewhere (Camel et al. 1991).

Results

Nitrogen isotope studies

The 3 treatments were designed to have different ^{15}N abundances: while all soybean plants received some ^{15}N -depleted fertilizer during their first 3 weeks of growth, the main input of N was as follows: to NOD from the air, to $-N$ from soil and seed and to $+N$ from the ^{15}N -depleted solution. Soybean leaf ^{15}N concentration reflected the expected distribution: it was 13 times higher in NOD than in $+N$ leaves, with $-N$ leaves intermediate (Tab. 1). The small amount of ^{15}N -depleted fertilizer given during the first 3 weeks was not detectable at harvest in NOD soybean, but decreased ^{15}N concentration in $-N$ soybean by 37%. The use of large amounts of ^{14}N in $+N$ soybean resulted in a 92% dilution of ^{15}N (Tab. 1). Maize plants followed the pattern shown by soybean. Before the establishment of VAM hyphal connections, ^{15}N concentrations in maize plants of all treatments were determined by N input from seed and soil (0.3663% ^{15}N) and from 4 weeks of fertilizer application (0.01% ^{15}N). Following the period of VAM-mediated association with its soybean partner, NOD maize showed an increase (15%) and $+N$ maize a decrease (18%) in ^{15}N abundance compared to the $-N$ controls (Tab. 1). As a result of extreme N deficiency of the $-N$ soybean leaves (6.9 mg g^{-1} actual vs 48.0 mg g^{-1} reported for field-grown plants by Hallmark et al. 1987), $-N$ soybean was unlikely to have functioned as an N donor. Atom % deficits ^{15}N of soybean and maize plants (Fig. 2) correlated significantly ($r = 0.9937$, $P = 0.071$), indicating the effects of VAM-mediated N exchange between the root zones of associated plants.

To estimate the occurrence of N diffusion, the concentrations of ^{15}N in the soils of the $+N$ soybean, bridge

Tab. 1. Abundance of ^{15}N in soybean and maize plants connected only by VAM soil hyphae. Soybean plants of the soybean-maize association received N through N_2 fixation (NOD), from N fertilizer ($+N$), or were N-deficient ($-N$). Numbers are means and \pm SE of 6 replications.

Treatment	Atom % ^{15}N		
	Soybean		Maize
	Leaf	Nodule	Leaf
NOD	0.369 ± 0.001	0.365 ± 0.001	0.166 ± 0.014
$+N$	0.028 ± 0.002		0.116 ± 0.001
$-N$	0.232 ± 0.016		0.141 ± 0.025

and maize compartments were determined at harvest. The ^{15}N content of soil in the $+N$ soybean compartment, which had received 1120 mg of ^{15}N -depleted fertilizer N, was diluted significantly from $0.3736 (\pm 0.0054 \text{ SE})$ to 0.3304 atom % $^{15}\text{N} (\pm 0.0051)$, while ^{15}N abundance in the soils of the bridge and maize compartments were 0.3726 ± 0.0045 and 0.3727 ± 0.0034 at harvest, respectively. Thus, movement of the N fertilizer applied to the $+N$ donor plants was not detectable in the bridge and maize compartments. Soil moisture sensors embedded in the soybean and maize root compartments consistently registered lower soil water potentials in the donor compartment. This was due to more rapid soil water depletion by the larger donor plants (Tab. 2). Thus, water fluxes, if any, were from maize to soybean and counterfluxes of N from soybean to maize were unlikely.

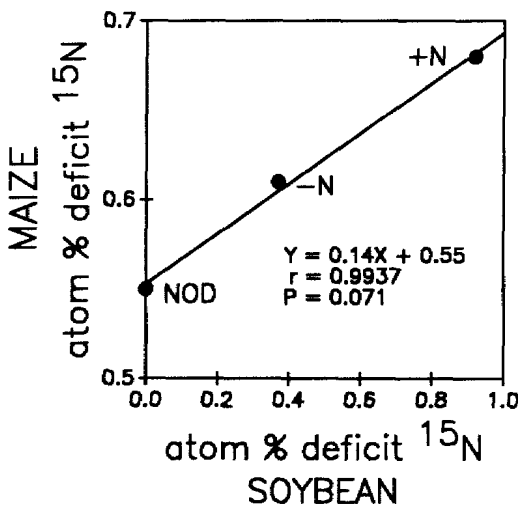


Fig. 2. Correlation of atom % deficits ^{15}N of associated soybean and maize plants of the NOD, $+N$ and $-N$ treatments. Deficits are derived as: atom % deficit $^{15}\text{N} = 1 - (\text{atom \% actual } ^{15}\text{N}/\text{atom \% natural } ^{15}\text{N})$. Treatment codes are as in Tab. 1.

Tab. 2. Development of soybean and maize plants connected only by VAM hyphae. For treatment code see Tab. 1. Probabilities show the differences between treatments by linear contrasts.

Parameter	Treatment			Probability		
	NOD	+N	-N	NOD vs +N	NOD vs -N	+N vs -N
<i>Soybean</i>						
Plant dry mass (g)	25.1	21.8	3.9	0.03	<0.01	<0.01
Root length (m)	737	441	145	0.01	<0.01	<0.01
Root colonization (%)	49.7	59.2	60.8	0.22	0.15	0.82
Nodule dry mass (g)	0.8					
<i>Maize</i>						
Plant dry mass (g)	6.9	8.6	7.2	0.06	0.79	0.09
Root length (m)	190	228	164	0.18	0.22	0.02
Root colonization (%)	53.0	57.0	48.2	0.60	0.52	0.25
Ear dry mass (g)	0.11	0.53	0.27	<0.01	<0.01	<0.01

Plant and symbiont development

Plant dry masses and root lengths of NOD and +N soybean were several times greater than those of -N soybean (Tab. 2). Root colonization was statistically the same in the soybean plants of all treatments (Tab. 2),

but the number of VAM-fungal propagules (spores and sporocarps) was twice as high in +N soybean soil than in the soils of the other 2 treatments (Fig. 1). In maize, the difference between the dry masses and root lengths of the NOD and -N treatments were not significant (Tab. 2). The number of propagules was significantly

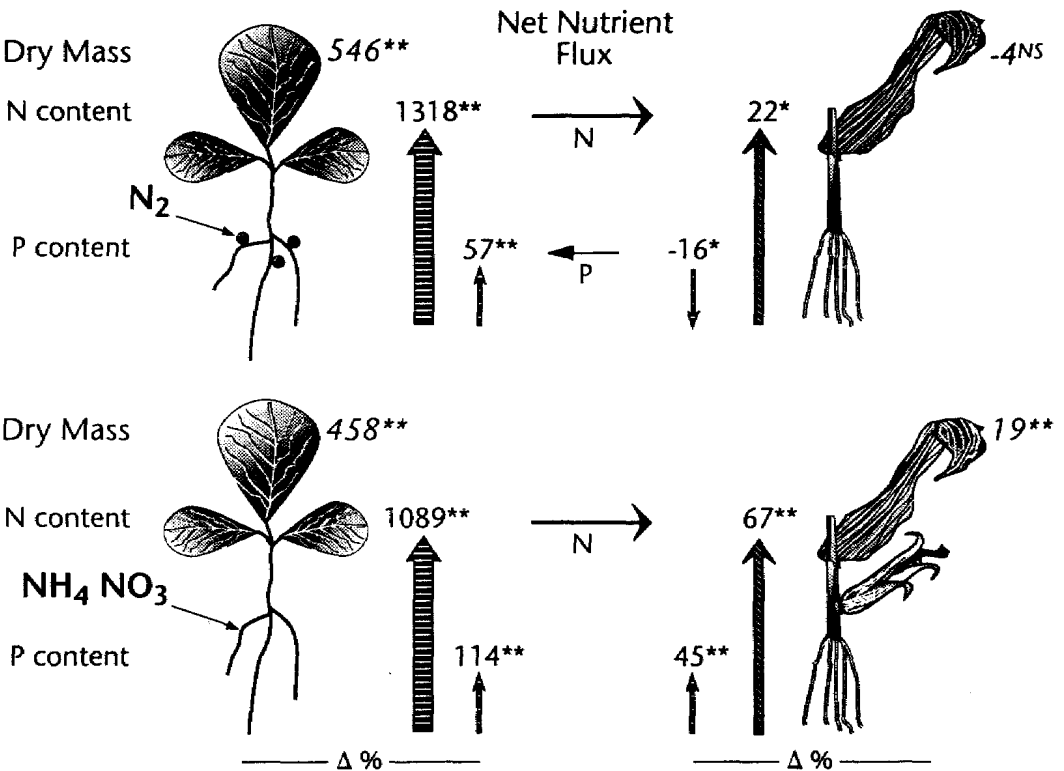


Fig. 3. Biomass and N and P content of N-sufficient soybean (NOD and +N) and associated maize plants relative to N-deficient associations (-N). Numbers represent percent difference ($\Delta\%$) between N-sufficient (NOD and +N) and N-deficient (-N) treatments based on the means of 6 replications. Notation (**, $P < 0.01$; *, $0.10 > P > 0.01$; NS, $P > 0.10$) indicates the significance of differences between the parameter means of N-sufficient and N-deficient treatments.

Tab. 3. Changes (%) in plant and soil parameters of the N-sufficient (NOD and +N) treatments vs the N-deficient (-N) control. Differences between the means are shown as: $P > 0.10$, NS; $0.10 > P > 0.05$, *; $0.05 > P > 0.01$, **; $P < 0.01$, ***.

Parameter	Percent change			
	NOD vs -N		+N vs -N	
	Soybean	Maize	Soybean	Maize
Root colonization	-18 NS	10 NS	-3 NS	18 NS
Soil VAM propagules	-2 NS	34 *	104 ***	69 ***
Leaf dry weight	288 ***	-2 NS	199 ***	4 NS
Leaf concentration				
N	308 ***	10 NS	347 ***	77 ***
P	-57 ***	-12 NS	-9 NS	33 ***
K	-20 ***	-11 **	0 NS	-15 ***
Ca	-33 ***	2 NS	-30 ***	38 ***
Mg	-27 ***	9 NS	-15 **	44 ***
B	-22 ***	2 NS	-5 NS	34 ***
Cu	-53 ***	-4 NS	-43 ***	-7 NS
Fe	0 NS	-7 NS	-30 **	9 NS
Mn	12 NS	-15 *	-4 NS	31 ***
Zn	-71 ***	17 NS	-55 ***	69 **

higher (Fig. 1) and ear development was both earlier and greater in the +N maize plants than in those of the other treatments (Tab. 2).

The large increase in dry mass of NOD vs -N soybean plants (546%) was not shown by the NOD maize plants which were statistically the same as the controls (-4%, $P = 0.75$; Fig. 3). There was a lack of response in VAM propagule formation to N input from N_2 fixation in NOD soybean relative to the -N plants (Tab. 3, Fig. 1). The increase in the N content of NOD vs -N

soybean plants (1318%, Fig. 3) and of leaf N concentration (308%, Tab. 3) was large. Yet this N did not enhance propagule formation by the fungus. Although the maize plants of the NOD treatment did not show a significant increase over the -N plants in leaf N concentration (10%, $P = 0.49$, Tab. 3) and only a marginally significant increase in plant N content (22%, $P = 0.10$; Fig. 3), this increase in N concentration was related to an increase in propagule production (34%, $P = 0.09$; Fig. 1, Tab. 4). The N contents of maize plants and associated propagule densities were significantly correlated ($r = 0.9993$, $P = 0.02$) over the 3 treatments.

The +N vs -N comparisons were quite different from the NOD vs -N comparisons. Large and highly significant changes in dry mass and N content (458% and 1089%, respectively; Fig. 3) and leaf N concentration (347%, Tab. 3) in +N soybean were reflected in a significant increase in maize dry mass (19%, $P = 0.09$; Fig. 3) and highly significant increases in plant N content (67%, $P < 0.01$; Fig. 3) and leaf N concentration (77%, $P < 0.01$; Tab. 3). Increases in +N vs -N VAM propagule production to accompany these increases in plant N status were two-fold in both the soybean and maize soils (Fig. 1). The N made available to the +N system from NH_4NO_3 application apparently became readily available to the VAM fungus, resulting in increased sporulation.

Plant nutrition

All nutrient contents of the NOD and +N soybean plants were significantly greater than those of the con-

Tab. 4. Nutrient contents of soybean and maize plants connected by VAM hyphae. For treatment code see Tab. 1. Probabilities are as in Tab. 1.

Nutrient (mg)	Treatment			Probability		
	NOD	+N	-N	NOD vs +N	NOD vs -N	+N vs -N
Soybean						
N	418.6	351.0	29.5	0.16	<0.01	<0.01
P	24.9	33.8	15.8	<0.01	<0.01	<0.01
K	240.1	227.2	72.0	0.07	<0.01	<0.01
Ca	239.4	212.4	48.9	0.02	<0.01	<0.01
Mg	174.4	120.9	31.1	<0.01	<0.01	<0.01
B	0.82	0.67	0.14	<0.01	<0.01	<0.01
Cu	1.06	1.28	0.30	<0.01	<0.01	<0.01
Mn	2.44	2.02	0.59	0.11	<0.01	<0.01
Zn	0.67	0.73	0.22	0.03	<0.01	<0.01
Maize						
N	39.5	54.8	32.6	0.02	0.10	<0.01
P	9.0	16.4	10.7	<0.01	0.10	<0.01
K	118.7	129.1	124.9	0.15	0.38	0.54
Ca	22.0	28.9	20.0	0.06	0.38	<0.01
Mg	28.5	37.3	26.0	0.03	0.49	<0.01
B	0.10	0.13	0.10	<0.01	0.94	<0.01
Cu	0.29	0.37	0.25	<0.01	0.02	<0.01
Mn	0.51	0.76	0.38	<0.01	0.03	<0.01
Zn	0.14	0.22	0.12	<0.01	0.62	<0.01

Tab. 5. Leaf nutrient concentrations of plants connected by VAM hyphae. For treatment code see Tab. 1. Probabilities as in Tab. 2.

Nutrient	Treatment			Probability		
	NOD	+N	-N	NOD vs +N	NOD vs -N	+N vs -N
Soybean						
N (mg g ⁻¹)	28.4	30.8	6.9	0.20	<0.01	<0.01
P	1.2	2.4	2.7	<0.01	<0.01	0.30
K	12.5	15.7	15.8	<0.01	<0.01	0.93
Ca	14.4	15.1	21.6	0.33	<0.01	<0.01
Mg	4.8	5.6	6.6	0.05	<0.01	0.02
B (µg g ⁻¹)	53.0	64.2	67.7	<0.01	<0.01	0.37
Cu	9.7	11.7	20.7	<0.01	<0.01	<0.01
Fe	114.5	80.0	115.0	<0.01	0.96	<0.01
Mn	109.7	94.2	97.7	0.26	0.38	0.79
Zn	30.0	47.8	106.7	<0.01	<0.01	<0.01
Maize						
N (mg g ⁻¹)	5.5	8.8	5.0	<0.01	0.49	<0.01
P	1.9	2.9	2.2	<0.01	0.24	<0.01
K	20.2	19.2	22.7	0.35	0.02	<0.01
Ca	5.6	7.5	5.5	<0.01	0.76	<0.01
Mg	4.6	6.0	4.2	<0.01	0.33	<0.01
B (µg g ⁻¹)	39.5	53.8	40.2	<0.01	0.86	<0.01
Cu	9.6	9.3	10.0	0.92	0.82	0.80
Fe	47.3	55.2	50.7	0.42	0.72	0.64
Mn	137.8	210.8	161.3	<0.01	0.10	<0.01
Zn	17.3	24.8	14.7	0.09	0.53	0.03

controls (Tab. 4). Soybean plants of the NOD treatment had higher nutrient concentrations than the +N plants except for P and Zn, which were lower, and N, which was statistically the same (Tab. 4). In maize, the trend was the opposite: all nutrient contents in the NOD plants were lower than those of the +N plants, except for K, which was the same. The NOD vs -N and the +N vs -N comparisons were also different. In NOD maize plants, N, Cu and Mn contents were significantly higher, and P content significantly lower than in the controls, while in the +N plants all nutrient contents were significantly higher, except for K, which was the same. The decrease in NOD vs -N maize P content (Tab. 4) cannot be explained by dilution (Jarrell and Beverly 1981), since the dry masses of the 2 plants were statistically the same ($P=0.79$; Tab. 2).

The datum on lowered P content in NOD maize was related to the significantly lower ($P<0.01$) P concentration in NOD vs +N soybean leaves (Tab. 5). These data together indicate that P-deficient NOD soybean was a stronger sink for P than +N soybean, and that the maize plants associated with NOD soybean served as sources for that P. As to the other nutrient concentrations (Tab. 5), only N was higher in NOD and +N soybean leaves than in the controls. The NOD soybean leaves were significantly lower in all other nutrient concentrations, except for Fe and Mn, which were the same. In the +N soybean leaves also, all nutrient concentrations (other than N) were the same or significantly lower than in the controls. This phenomenon (except for the P data) can be explained by dilution of nutrients (Jarrell

and Beverly 1981) in the larger NOD and +N plants which were not subject to N limitation, or by nutrient concentration in the N-limited and smaller -N plants.

The leaves of NOD maize had significantly lower N, P, Ca, mg, B, Mn and Zn concentrations than those of the +N maize plants (Tab. 5). Compared to -N maize leaves, NOD leaves had the same concentrations in all nutrients, except for K and Mn, which were lower. On the other hand, all nutrient concentrations of +N maize leaves were significantly higher than those of the -N leaves, except for Cu and Fe, which were the same. Clearly, association with the N-fertilized soybean plants was more advantageous for maize plants than association with those fixing N₂.

Discussion

Direct VAM nutrient transfer vs the soil pool pathway

In the older literature (see Haynes 1980), N transport from legume to grass is reported to occur only via the 'soil pool pathway', where the mineralization of N-rich legume-plant residues eventually permits the uptake of N by the associated grass (Heichel and Barnes 1984). During the past decade, however, increasing evidence has pointed towards the existence of a direct pathway, where nutrient transfer is mediated by VAM hyphae that connect the plants (see Newman 1988). However, unequivocal proof that transfer can take place solely through VAM hyphae is elusive. Most experiments have made use of intermingled root systems, where positive

VAM effects (Francis et al. 1986, Haystead et al. 1988, van Kessel et al. 1985) may have been due to changes in root exudation and the movement of exudates and other nutrients between plants through the soil. Where the experimental design precluded the intermingling of roots (Schüppel et al. 1987, Warner and Mosse 1983), VAM effects on nutrient transfer were not reported.

The occurrence of soil-mediated nutrient transfer can be minimized by interposing wide, screened, root-free soil bridges between the plants connected by VAM hyphae across the bridges. Since the extent of separation of associated roots and the continuity of the hyphal connections are inversely related, a balance must be found between the requirements of minimum soil transfer and maximum hyphal transfer by varying the width of the soil bridge (Camel et al. 1991). In our present experiment, a bridge width in excess of any reported previously, and watering regimes which favored fluxes of soil solution, if any, from the N sink (maize) to N source (soybean) minimized N transfer from soybean to maize through soil diffusion. Since such N movement could not be detected by ^{15}N measurements, we concluded that it was in fact minimized. However, this design did not provide safeguards against a flow of other ions from maize to soybean.

Nodulating vs nonnodulating legumes in N transfer

The weak performance of nodulating soybean as an N source (Fig. 3) was reminiscent of the report by Hamel et al. (1990), who did not find N movement from their nodulated soybean plants to the VAM mycelium. Their conclusion that N nutrition of the fungus is independent of the host plant (and that it consequently derives its N from the host soil) was supported by our data on propagule proliferation in the N-fertilized (soybean) soil (Fig. 1). It was not supported, however, by similar hyphal densities (NOD vs +N) in the bridge soils. The significant ($P = 0.02$) correlation between maize-plant N content and soil propagule density over all treatments suggested a causal relationship between the 2 parameters, which may be interpreted as N exchange between this host (maize) and its symbiont. The lack of such a relationship in soybean, where the NOD and -N treatments were associated with a VAM soil mycelium of equal spore density (Fig. 1), indicated that it is nodulated soybean and not plants in general that has impaired N-exchange capacity with its fungal endophyte. Such antagonistic interactions between nodulation and VAM colonization in soybean have been described earlier (Bethlenfalvay et al. 1985).

The striking response of maize to association with soybean of the +N treatment (Fig. 3) may be interpreted as plant-to-plant nutrient fluxes or as nutrient uptake from the N-fertilized soil of the soybean compartment by the VAM mycelium common to both plants. Uptake of N by VAM fungi has been shown to occur in sporadic reports in the VAM literature, pre-

senting variable results (Ames et al. 1983, Barea et al. 1989, Karunaratne et al. 1987, Smith et al. 1985). We are hesitant to attribute the increases in growth and nutrition of our +N maize plants entirely to VAM-mediated soil-N uptake, since the +N vs NOD comparisons (Tab. 5) showed markedly higher leaf concentrations in the +N (vs NOD) maize plants in almost all other nutrients, which, unlike N, were not preferentially enriched in the +N treatment.

More work is needed to clarify whether plant growth responses to VAM colonization result from VAM-mediated nutrient transfer from the roots themselves or from the root zone of an associated plant. In fact, we feel that this distinction may be artificial. Since both the root and its surrounding soil volume are permeated by the same VAM mycelium, it may be more realistic to think of them as of a unit. From the point of view of the associated receiver plant, the donor's root, rhizosphere and bulk soil together represent a potential source of nutrients: the mycorrhizal nutrisphere.

If plant-to-plant N flux were an important mode of transfer in legume associations, the different initial N-assimilation products of N_2 -fixing vs N-fertilized soybean would suggest a mechanism for the apparent difference in N-donation capacity of these plants reported here. The N-assimilation products of soybean nodules are ureides (Harper 1987), for which the exchange of N between host and fungus, as was postulated for amino acids by Smith and Smith (1990), may not be operative. It remains to be seen if amino acids produced directly by nodules of other legumes are more readily available to the fungus for export from the plant than are amino acids eventually recirculated to the roots in soybean following ureide catabolism and the satisfaction of other sink requirements for N in the shoot.

A direct effect between plants, which could not be attributed to uptake by the continuous VAM mycelium between the two plant compartments was the highly significant ($P < 0.01$) difference in the P contents and concentrations of the NOD and +N maize plants (Tabs 4 and 5). The significant ($P = 0.10$) decline in NOD maize-plant P content (vs -N) did not occur in +N maize. We may interpret it therefore as a function of sink demand by the NOD soybeans. Due to their high P demand (Israel 1987), root nodules may function as such a sink: nodule P concentration was markedly higher than that of other soybean tissues (leaf, 1.2; root, 0.9; nodule, 3.3 mg g^{-1}).

In contrast to NOD maize, the P content of +N maize increased by 59% relative to the controls probably as a result of relief from N limitation through N import from the soybean side of the container. The general pattern of no significant changes or declines in NOD vs -N maize plants in all leaf nutrient concentrations (Tab. 3) supports our hypothesis of P limitation (export of P) and no relief of N limitation as a factor in NOD-maize response. On the other hand, significant increases or no change in the leaf nutrient concentrations of +N maize

vs the control indicate relief from N limitation and lack of P limitation as influencing the response of +N maize plants to VAM nutrient transport.

Impact on intercrop and crop rotation systems

The effects of soybean-maize crop rotation are generally favorable for maize yields (see Johnson 1987), although the fertilizer-N equivalent derived from soybean is considerably lower than that provided by some other legumes (see Heichel and Barnes 1984). In simultaneous plantings the results reported are mixed, and range from improved yields (Crookston and Hill 1979) to decreases in the grasses associated with legumes (Hall 1978, Wahua and Miller 1978).

The effectiveness of N input into the intercrop system by the legume component has been associated with the availability of P (Voss and Schrader 1984), reminiscent of the P effect observed in our experiment. Hall (1978) ascribed poor response to VAM by ryegrass (*Lolium perenne* L.) associated with clover (*Trifolium repens* L.) to intra-specific competition for soil P. Significantly, clover benefited greatly from the presence of ryegrass (at the expense of the latter) in that study. Under different conditions, however, it was the legume (N₂ fixation by *Medicago sativa* L.) whose function was impaired, apparently as a result of competition with ryegrass (Barea et al. 1989). Such results can be explained by VAM-mediated nutrient fluxes, which may contribute to inter-plant competitive relationships. As to the direction of these fluxes, recent findings in our laboratory (R. L. Franson and C. Hamel, unpublished data) suggest that the stage of development (sink size and activity) of associated plants is a factor of influence.

Conclusions

Our findings indicate that: (1) VAM-mediated N transfer from the root zone of soybean to maize varies with the mode of N input, (2) transfer of nutrients other than N is variable and can be significant and bi-directional and (3) the direction of flow is related to source-sink relationships. The results also suggest the availability of P and high root-nodule demand for P as factors influencing plant responses between associated soybean and maize plants.

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