DIVISION S-3—SOIL MICROBIOLOGY & BIOCHEMISTRY

Growth of Vesicular-Arbuscular Mycorrhizal Mycelium through Bulk Soil

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ABSTRACT

Soil mycelia of vesicular-arbuscular mycorrhizal (VAM) fungi not only extend the range of plant roots for nutrient uptake but also may connect roots, allowing the transfer of small amounts of nutrients between plants. A greenhouse experiment was conducted to determine the range and the rate of advance of VAM hyphae in root-free bulk soil. Plants were grown in three-part containers with a VAM donor plant (soybean, *Glycine max* [L.] Merr.) and an initially non-VAM receiver plant (corn, *Zea mays* L.), separated by a soil bridge delimited on both sides by screens (44-μm openings). The screens permitted passage of the hyphae of the VAM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe between the donor and the receiver plants, but retained the roots. The length of the soil bridge (3, 6, or 9 cm) and the texture of the soil (1:2, 1:1, or 2:1 soil/sand) were varied. The advance of the hyphal front was estimated by timing colonization in the receiver plants. The rate of advance in the soil–sand mixes was 2.3 cm/wk with soil penetration of at least 90 mm. Spore production was inhibited in the heavier soils, and decreased in the soil of receiver plants with increasing distance between donor and receiver plant. Growth of the receiver plants was increased by early VAM development. Rates of hyphal growth may influence competitive relationships in plant groupings where mycorrhizal inoculum is sparse.

THE INTEGRITY of the soil mycelium of VAM fungi is of consequence in the ecology of plants (Newman, 1988). Exchange of nutrients between plants by means of viable (Sylvia, 1988) VAM hyphae may enhance seedling establishment (Read and Birch, 1988) and improve the nutritional status of adjacent plants (Van Kessel et al., 1985), although the extent of such exchanges has not been established unequivocally (Newman, 1988). On the other hand, soil disturbance (Evans and Miller, 1988) or fallowing (Thompson, 1987), which directly or indirectly destroy the continuity of the hyphae, may be detrimental to plants. The VAM soil mycelium, by extending beyond the rhizosphere into the bulk soil (Ames et al., 1983), is able to perform the dual function of enhancing plant nutrition and of promoting soil stability (Thomas et al., 1986) by serving as a source of C for soil organisms (Bethlenfalvay and Newton, 1990; Linderman, 1988).

There have been attempts to quantify the rate of advance of the fungal hyphae in the presence (Schelteme et al., 1985) or absence (Schüepp et al., 1987; Miller et al., 1989) of roots. The progress and range of VAM hyphae in soils of different resistance (texture or compaction) may be determined by inserting screened, root-free soil bridges (Warner and Mosse, 1983) of variable length between VAM plants and initially non-VAM receiver plants. The purpose of this experiment was to obtain an estimate of the rate of mycelial growth through soils of different densities and to relate growth and development of the fungus with the development of receiver plants as a function of the extent of separation (length of bridge) between plants.

MATERIALS AND METHODS

Donor and receiver plants were grown in identical compartments made of 6-mm-thick black acrylic sheet (15 by 15 by 20 cm, width by length by height) separated by a 15 by 20 cm bridge (same material) of 3-, 6-, or 9-cm length (Fig. 1). Compartment walls between the plant and bridge soils had 12 by 17-cm openings, covered with a fine-mesh (44-μm) polyester screen (TETKO Inc., Lancaster, NY) that was clamped between compartments. The screen was permeable to VAM hyphae, but not to roots. Shade barriers (15 cm high) limited aboveground interactions between plants.

The experiment was arranged in a randomized-block, 3 by 3 factorial design, with distance and soil texture as main effects. Distance (3, 6, or 9 cm) was varied by inserting bridge compartments of different lengths between donor (VAM) and receiver (non-VAM) plants. The soils of light (L), medium (M), and heavy (H) texture consisted of 1:2, 1:1, or

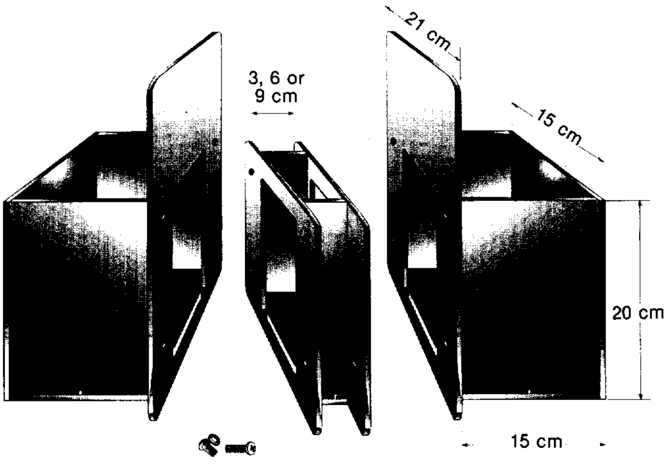

Fig. 1. Diagram of container for the study of vesicular-arbuscular mycorrhizal soil mycelium. Openings in the walls between compartments are covered by screens permeable to fungal hyphae but not to roots.

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2:1 mixtures (v/v) of soil/sand, respectively. Plant compartments contained 4.4 L, and bridge compartments 0.7, 1.5, or 2.4 L, of the growth media. There were three replicates of each distance/texture combination, for a total of 27 units. The results were evaluated by analysis of variance (ANOVA). Trends within significant ($P < 0.05$) main effects were evaluated by pooling soil or distance data and by testing for significance by polynomial contrasts (Chew, 1977).

A nonnodulating isoline (to avoid *Rhizorium* involvement) of soybean (cv. Clark) was used as the donor plant, and a dwarf variety of corn (cv. MM926) was the receiver plant. Seeds were germinated and seedlings were selected for uniformity prior to planting. Soils of the donor plants were inoculated with the VAM fungus *G. mosseae* WRRC Isolate no. 1 (Franson and Bethlenfalvay, 1989). The inoculum for each compartment, consisting of 50 mL of dry soil (stored for about 4 mo) containing approximately 700 sporocarps (1–5 spores) and 200 colonized root fragments (2–10 mm long), was mixed uniformly into the entire soil volume.

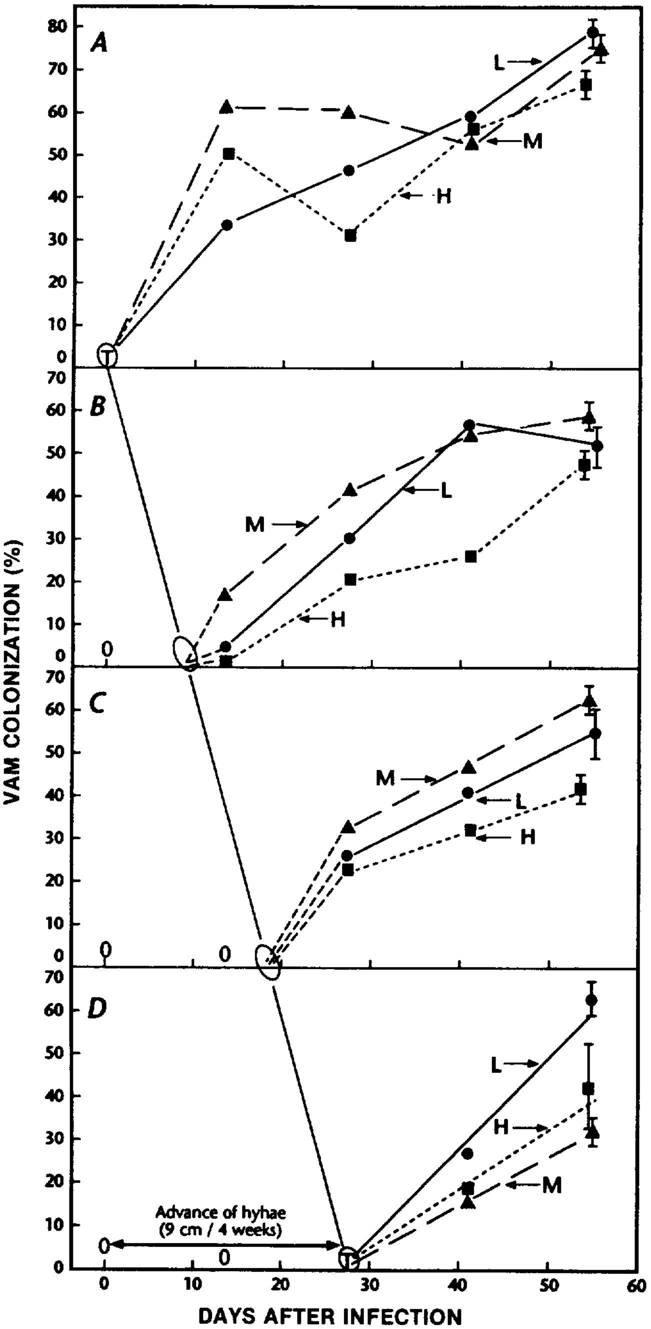

Fig. 2. Rate of advance of vesicular-arbuscular mycorrhizal (VAM) hyphae from donor (A) to receiver plants separated by root-free soil bridges 3, 6, and 9 cm long (B, C, and D, respectively). Samplings were made at 14-d intervals; trace (T) or no (0) infection is indicated along the x axis. Light, medium, and heavy soils (L, M, and H) were produced by mixing soil with sand (soil/sand 1:2, 1:1 and 2:1). Symbols represent the means of three replicates.

Donor plants were grown in a greenhouse in Albany, CA for 95 d (March–May). Midday light intensity (photosynthetic photon flux density, or PPFD) was 1500 μmol m^{-2} s^{-1}. Receiver plants were planted 14 d after donor plants and harvested with the donor plants. Temperatures and relative humidities varied between maxima and minima of 40 and 11 °C and 88 and 22%, respectively. The soil used was a calcareous silty-clay loam (Typic Xerorthent; 12% sand, 68% silt, 20% clay) of the Balcom series from Yolo County, CA; of pH 8, and of low N (0.6 g kg^{-1}), P ($NaHCO_3$–extractable, 3.3 mg kg^{-1}), and organic-matter (17 g kg^{-1}) content (Bethlenfalvay et al., 1985). It was mixed with fine sand, sterilized by autoclaving, and moistened 14 d prior to use. Plants were watered on alternate days, in a repeating sequence of one application of water followed by three of a nutrient solution containing 4.0 mM NH_4NO_3, 1.5 mM $CaCl_2$, 1.0 mM K_2SO_4, 0.2 mM KH_2PO_4, 0.25 mM $MgSO_4$, plus the following concentrations (μM) of micronutrients: B, 25; Co, 0.6; Cu, 0.5; Fe, 20; Zn, 2. The amount of liquid initially was 100 mL, and this was increased as the plants grew to keep soil water content from falling below 150 g kg^{-1}. Soil water content was determined by gypsum-block sensors (previously calibrated with a ceramic-plate water-potential apparatus) embedded in the soils.

Roots were sampled for VAM colonization at 14-d intervals starting with 28-d-old donor and 14-d-old receiver plants (two 30-mL soil cores per sampling). Root samples from replicate units were pooled and percent colonization of roots by the VAM fungus was determined by the grid-line-intersect method (Marsh, 1971) after staining with trypan blue (Koske and Gemma, 1989). Replicate determinations of root colonization were made only at the final harvest.

Soil in each of the bridge compartments was mixed thoroughly prior to sampling for hyphal and spore density. Hyphal density was determined according to Bethlenfalvay and Ames (1987): soil samples were suspended in water, disaggregated with sodium hexametaphosphate, and stained with trypan blue. A known volume of stained sample suspension (replicated four times) was placed in a petri plate for microscopic examination (160×). A camera-lucida attachment was used to superimpose an exact grid onto the field of view of the microscope. The density (m g^{-1}) of VAM hyphae (diameter >5 μm) was then estimated by the grid-line-intersect method of Marsh (1971) as modified by Tennant (1975). Spore density was determined by the wet-sieving and decanting technique (Daniels and Skipper, 1982). Water content of the bridge soil at sampling time was also determined so that hyphal and spore densities could be calculated on a dry-soil basis. Plant dry mass was determined after drying at 70 °C.

RESULTS

At first sampling, the 28-d-old donor plants (Fig. 2A) had traces (<1%) of VAM infection (initial entry of the fungus into the root), while the roots of 14-d-old receiver plants across the 3-cm bridge were not yet infected (Fig. 2B). At second sampling, 14 d later, there were low levels of colonization in these receiver-plant roots, while receiver plants 6 and 9 cm away were not infected (Fig. 2C and D). At third sampling, receiver-

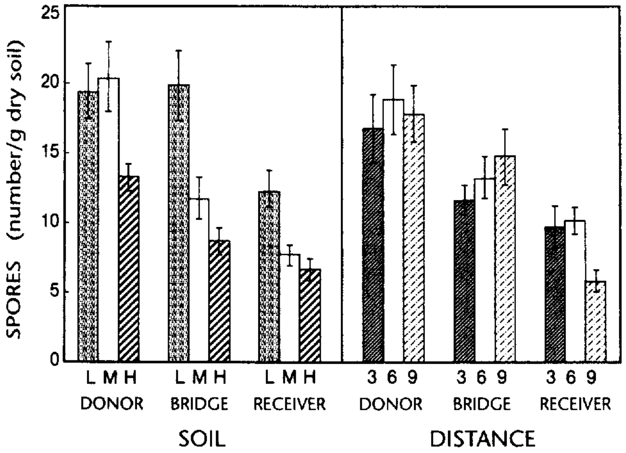

Fig. 3. Spore production by the vesicular-arbuscular mycorrhizal fungus in light (L), medium (M), and heavy (H) soils of the donor and receiver plant compartments and in 3-, 6-, and 9-cm root-free soil bridges. Bars represent the means of nine replicates.

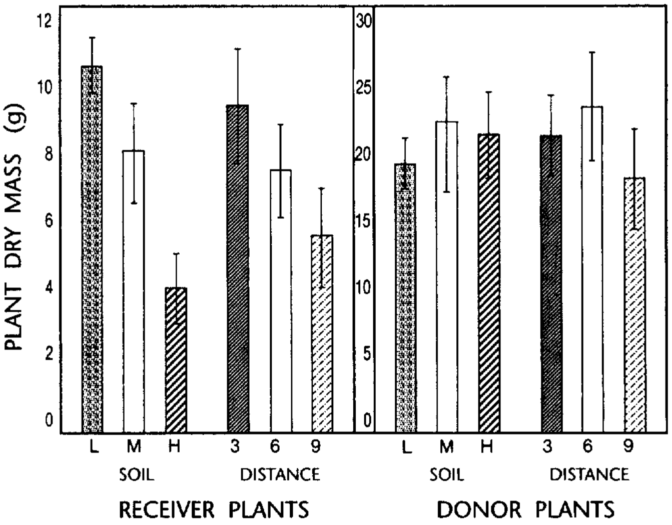

Fig. 4. Comparison of light (L), medium (M), and heavy (H) soil and 3-, 6-, and 9-cm bridge distance effects on receiver and donor plant dry mass. Bars represent the means of nine replicates.

plant roots at 6 cm were colonized (20–30%), while roots at 9 cm had only traces of infection. Connecting the points of trace colonization in donor plants and in the receiver plants 9 cm distant (Fig. 2) permitted extrapolation of the onset of colonization in the receiver plants at 3 and 6 cm. Assuming a constant growth rate and immediate infection of the roots contacted, the rate of advance of the hyphal front was 0.3 cm d^{-1}.

At harvest, the containers were disassembled, and the absence of root penetration through the screens was verified. Analysis by ANOVA revealed no interaction between the main effects (soil and distance) for any of the parameters measured. Root colonization of donor and receiver plants at harvest was analyzed by polynomial contrasts. The first-order contrasts were different among soils, with means as follows. Donor plants: L, 84; M, 70; H, 66; $P = 0.004$; and receiver plants: L, 57; M, 51; H, 46; $P = 0.046$. Root colonization was inhibited with increasing soil content of the sand–soil growth medium. Distance effects pooled over soil types were not significant by linear contrasts. Even the receiver plants at the greatest distance from the donor, which were colonized late and had the shortest time to form mycorrhizae (28 d), attained colonizations levels similar to those of plants at shorter distances, indicating rapid development of colonization once it was initiated (Fig. 2).

Linear contrasts revealed a soil effect on spore production in the donor ($P = 0.015$), bridge ($P = 0.0001$), and receiver ($P = 0.0006$) compartments, with the smallest spore density in the heaviest soil (Fig. 3). The distance effect on spore production was significant only for the receiver plants (donor, $P = 0.656$; bridge $P = 0.108$; receiver, $P = 0.007$).

Density of the VAM soil hyphae (diameters >5 μm, Ames et al., 1983; Fitter, 1985) in the bridge compartments did not have a significant trend with either factor by linear contrasts. Averaged over distance, soil effects on VAM hyphal densities (m g^{-1} were significant (L, 584; M, 907; H, 757, means; $P = 0.021$). Averaged over soil types, distance effects on VAM hyphal densities were not significant.

Distance effects on the growth of the receiver plants were significant: shoot ($P = 0.005$) and root ($P = 0.012$) mass and shoot height ($P = 0.042$) declined with increasing distance, reflecting delayed colonization. Means at 3, 6, and 9 cm, respectively, were as follows: shoot mass: 8.7, 6.8, and 3.6 g; root mass: 1.6, 1.1, and 0.5 g; and shoot height, 45.1, 42.3, and 30.8 cm.

A comparison of soil and distance effects in receiver and donor plants showed that receiver plant growth was inhibited both by heavier soils and by distance-related, delayed VAM colonization (Fig. 4). Linear contrasts in receiver plants showed significant soil effects pooled over distance, and distance effects pooled over soil types. Analysis of variance did not show any significant interaction between distance and soil texture in receiver plants, indicating that the soil response of receiver plants was independent of distance. The distance response was found only in the receiver plants and was apparently due to the delay in VAM colonization. Donor plants did not respond significantly ($P > 0.05$) to either factor. This lack of response to the soil factor by the donors indicates that dilution of nutrients by the addition of sand was not important and was masked by the provision of nutrients in solution.

DISCUSSION

Few data are available on the distances VAM mycelia can grow in soils (Schüepp et al., 1987) or on their rates of advance. The maximum distance clearly defined by double-screened, root-free zones in soil was 55 mm (Ames et al., 1983). Fungi grown by Warner and Mosse (1983) did not cross a zone wider than 20 mm, and these workers concluded that VAM fungi can infect only over relatively small interroot distances. Our results extend previous estimates of the range of VAM mycelia in bulk soil.

We suggest that the distance VAM fungi can cross in soil depends, to some extent, on the capacity of the host plant to supply the ectophytic portion of its endophyte (i.e., the soil mycelium) with reduced C and the ability of the hyphae to transport C compounds toward the bulk soil. The integrity of the soil mycelium is also affected by physical disturbance (Fairchild and Miller, 1990), by the abiotic component of the soil (Day et al., 1987) and, perhaps most importantly, by the activities of the soil biota (Ames et al., 1989; Linderman, 1988). The rate of hyphal advance from the VAM roots toward the roots of other plants or toward zones of favorable moisture or nutrient concentrations in the soil are likely to depend on some of the above factors, and are probably modified by specific plant–fungus compatibilities (Gianinazzi-Pearson et al., 1988), and by differences in the uptake capabilities of different fungal species or edaphotypes for individual nutrients (Bethlenfalvay et al., 1989).

The importance of considering the characteristics of VAM fungi, including the development of their soil mycelia, before introducing them into an agroecological system to resolve problem situations has been emphasized (Abbott and Robson, 1990; Jeffries, 1987). In systems where the VAM fungi are reduced in density or composition, such as by fire (Gibson and Daniels Hetrick, 1988), fallowing (Thompson, 1987) or fertilization (Thomson et al., 1986), the effects of newly introduced fungal isolates will depend on the effectiveness of the soil mycelium to colonize its new habitat (Abbott et al., 1983).

As an indication of the potential of VAM fungi to affect bulk soil, our data show the capability of the VAM soil mycelium to traverse a zone of root-free soil at least 90 mm wide. This is about 50 times the width of the rhizosphere (Curl and Truelove, 1986). Our findings also provide an estimate of the overall rate of hyphal advance through soil, although our method did not permit measurement of the constancy of hyphal growth rates. Presumably, fungal growth will decrease in proportion to the distance from the host root, and will be affected importantly in nonsterile soils by the mycoparasitic soil biota.

The large number of spores in the root-free bridge compartment was an indicator of the extent of soil colonization, and serves as a measure of the potential of the fungi to influence soil nutrition and aggregation (Bethlenfalvay and Franson, 1990). Delayed growth in non-VAM receiver plants as a function of distance from a VAM donor plant is reminiscent of the soil disturbance effects documented by Fairchild and Miller (1988), in that both are related to the absence of an effective soil mycelium. Since the rapidity of colonization may confer a competitive advantage of a fungal species due to its prior occupancy of roots (Abbott and Robson, 1984), the rate of advance of the soil mycelium is expected to be a factor in determining the effectiveness of VAM fungi.

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Element Uptake by Mycorrhizal Soybean from Sewage-Sludge-Treated Soil

D. H. Lambert* and T. C. Weidensaul

ABSTRACT

Components of sewage sludge that reduce the availability of P or are toxic to mycorrhizal fungi will alter the uptake of P, Cu, and Zn by plants. A greenhouse study was conducted to (i) determine effects of sewage sludge on P availability, (ii) determine the effects of sludge on mycorrhizal (MR) uptake of P, Cu, and Zn, and (iii) confirm MR supression of Cu and Zn uptake by P. 'Beeson' soybean (*Glycine max* [L.] Merr.) was grown for 7 wk in soil (Ravenna, a fine-loamy, mixed, mesic Aeric Fragiudalf) treated factorially with (i) 0, 60, 150, or 270 mg/kg P as monocalcium phosphate; (ii) four sources of sewage sludge providing 100 mg/kg P or a nontreated control; and (iii) *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske as MR inoculum or no inoculum. Sludge reduced P uptake at 150 mg/kg P or higher in nonmycorrhizal (NMR) plants with little difference in plant growth among sludges. In MR treatments, growth and P-uptake responses to sludge ranged from very beneficial with two sludges to a complete inhibition of the MR response with another sludge. This inhibition was persistant and apparently due to suppression of MR fungi by toxic levels of NH_4^+. Mycorrhizae substantially increased shoot Cu and Zn uptake only at low soil-P levels. Foliar Mn concentrations increased with P in NMR plants, but not in MR treatments. Our conclusions are that (i) sludge may reduce the availability of fertilizer P, (ii) sludge may exert differential effects on MR uptake of minerals, and (iii) inhibition of MR activity by P is the major reason for P-induced decreases in Cu and Zn for soybean.

A PRIMARY OBJECTIVE of sewage treatment is the removal of P from wastewater. This is accomplished by precipitation with excess Fe, Al, Ca, or polymers and by microbial uptake. Phosphorus in these forms is usually less available than standard fertilizer phosphates (Pastene and Corey, 1980). Uptake of native or fertilizer P from soil treated with sewage sludge might likewise be decreased by these precipitation agents.

Components of sludge that affect the activity of mycorrhizal fungi will also alter uptake of slowly diffusing elements. Mycorrhizae have increased P, Cu, and Zn uptake in most plants studied (Tinker and Gildon, 1984). Mycorrhizal activity is generally suppressed as plant P increases (Thompson et al., 1986), decreasing uptake of P, Cu, and Zn (Gildon and Tinker, 1983; Rhodes et al., 1978; Ross, 1971; Timmer and Leyden, 1980). Elements often abundant in sludge, such as N and heavy metals, can also reduce infection and sporulation of mycorrhizal fungi (Angle and Heckman, 1986; Chambers et al., 1980; Gildon and Tinker, 1983; Hepper and Smith, 1976; Lambert et al., 1985; McIlveen and Cole, 1979).

The objectives of this study were to: (i) determine if sludge source affects nutrient uptake via a vesicular-arbuscular mycorrhizal (VAM) fungus, (ii) determine if sludge source affects the availability of fertilizer phosphate, and (iii) confirm P suppression of VAM activity as a major mechanism of P-induced suppression of Cu and Zn uptake.

MATERIALS AND METHODS

The design of the primary experiment was a completely randomized factorial with 10 replicates of three factors. The factors were: mycorrhizal inoculation (added or not added); monocalcium phosphate (MCP) at 0, 60, 150, or 270 mg P/kg; and five sludge treatments (four sludges and a control without sludge). Ravenna fine loam, with an initial pH of 5.2 (1:1 water), 6 mg/kg Bray no. 1 P, was pasteurized with aerated steam at 60 °C for 2 h and treated with K_2SO_4 and hydrated lime to achieve amounts of exchangeable Ca, Mg, and K equivalent to 75, 20, and 5% of the initial cation-exchange capacity (CEC) of 13 meq/100 g. *Glomus fasciculatum*, isolated from *Trifolium pratense* L. by L. Rhodes and confirmed by J. Gerdemann, was used to produce mycorrhizal inoculum. Spores and infected wheat (*Triticum aestivum* L.) roots, sieved from 20 g of soil, were mixed with each kilogram of soil for inoculated treatments. The silt and clay fraction of the same inoculum was passed through a brass sieve with 45-μm openings to remove roots and the large *G. fasciculatum* spores. An equivalent amount of this fraction was added to nonmycorrhizal treatments to make all treatments as similar as possible with respect to content of bacteria and smaller-spored, nonmycorrhizal fungi.

Zimpro process (heat-sterilized) sludges were obtained from Canton and N. Olmsted, OH. A lime-ferric, anaerobically digested sludge was obtained from Lakewood, OH, and a heat-dried activated sludge (Milorganite) was obtained

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