Effect of compost addition and crop rotation point upon VAM fungi [1]

Abstract

Populations of vesicular–arbuscular mycorrhizal (VAM) fungi and mycorrhiza formation were examined in a field experiment studying the agricultural application of composted animal manures. The replicate experiment allowed each crop of a *Zea mays* > vegetable > small grain rotation to be sampled each of 3 years. Chicken litter/leaf compost and dairy cow manure/leaf compost enhanced spore populations of two VAM fungus species type groups (*Glomus etunicatum* type and the general *Glomus* spp. group, including *G. mosseae*) relative to those found in plots treated with raw dairy cow manure and conventional fertilizer. Populations of other groups were not affected by amendment, due likely to the large amount of P added in composts and manure relative to the conventional fertilizer applied. Crop rotation point had consistent, significant effects, with both lower populations of spores and less mycorrhizal infectivity of soil in plots after the vegetable crop (*Spinacea oleraceae* and/or *Capsicum annuum*) relative to maize and small grain (*Avena sativa* or *Triticum aestivum*). This was due to the non-mycorrhizal status and very low mycorrhization (approximately 1% root length colonized) of *S. oleraceae* and *C. annuum*, respectively. Future agricultural applications of compost and manure to provide N for crops need to consider the effects upon VAM fungi of other nutrients in these amendments. © 1997 Elsevier Science B.V.

Keywords: Vesicular–arbuscular mycorrhizal fungi; Sustainable agriculture; Compost; Manure; Preceding crop

1. Introduction

Increasing pressure to slow the flow of solid waste to landfills has caused municipalities to limit the disposal of yard wastes. Composting facilities exist in many cities as an alternative to the disposal of leaves and lawn clippings in landfills. Pollution of groundwater due to improper disposal of farmyard manure also has received regulatory attention as urban centers expand closer to areas of intensive agriculture. The percentage of wells in four south-eastern Pennsylvania, USA counties with water exceeding the 10 ppm NO_3–N standard set by the US Environmental Protection Agency ranged from 9 to 42% in a study conducted 10 years ago (Makuch, 1986).

Composting has been defined as "a biological process in which biological wastes are stabilized and converted into a product to be used as a soil condi-

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[1] Mention of a brand or firm name does not constitute an endorsement by the US Department of Agriculture over others not mentioned.

tioner and organic fertilizer (Willson, 1989). Co-composting of yard wastes and farmyard manures offers a solution to both problems outlined above (Reider et al., 1991). Municipalities would slow the waste stream to landfills and farmers would have a stabilized, organic source of nutrients for field application. In addition, an on-farm composting process was described recently which would allow for the utilization of waste straw (Churchill et al., 1996).

Vesicular–arbuscular mycorrhizal (VAM) fungi are obligate symbionts which colonize the roots of the majority of crop plants. Mycorrhizae are typically mutualistic symbioses, with the fungus receiving fixed carbon from the host and the host receiving a variety of benefits, most notably enhanced uptake of immobile nutrients from the soil (Harley, 1989). The current economic and environmental climate is causing agronomists to consider a shift from conventional, chemical-based agriculture to low-input, sustainable agriculture. Given the beneficial effects of mycorrhizae, the utilization of VAM fungi in sustainable agriculture should be essential. A first step in utilizing the indigenous community of VAM fungi of a site is to more thoroughly understand the effects of agronomic practices upon those fungi.

A long-term experiment was conducted to study crop response to and feasibility of the agricultural application of co-composted leaves and animal manures as the sole addition of nutrients to soil for plant growth. The study described here examined one aspect of this larger scale experiment: the effects of these applications upon populations of VAM fungi in the soil over the 3-year crop rotation compared to those found in soil treated with chemical fertilizer.

2. Materials and methods

Mycorrhizal fungi were examined in a field experiment initiated in 1993 to investigate the use of finished composts as substitutes for chemical fertilizer in agricultural crop production. The soil at the experimental site is predominately a Berks shaley loam (Typic Dystrochept). Prior to 1993, the field was cropped with a *Zea mays/Glycine max* rotation for 2 years, followed by 1 year in which the entire field was planted to sorghum–sudangrass (*Sorghum bicolor* (L.) Moench. × *S. sudanese* (Piper) Stapf). Soil chemical analyses were conducted in 1992, prior to amendment addition, and in 1995 at the end of this phase of the experiment. Soil pH (water), available P (0.025 N HCl, 0.03 N NH_4F extractant), and exchangeable K (1.0 N NH_4OAc) were determined as outlined in Black et al. (1965).

The 2.0-ha site was divided into four blocks on the basis of growth of the sorghum–sudangrass crop, slope, and soil organic matter, moisture, and rock fragment content. For the purpose of this experiment, each block was divided into four plots onto which the four treatments (see below) were applied. Each plot was further divided into three 21.5 × 6 m plots, one for each point of the 3-year crop rotation (Fig. 1) so that each crop was grown each year for each treatment. The crop rotation consisted of maize (*Z. mays* L.) > spinach (*Spinacea oleraceae* L.) and/or bell pepper (*Capsicum annuum* L.) > small grain. Maize cultivars used during the 3 years of this experiment were: Pioneer 3394 in 1993 and 1994, and Pioneer 3241 in 1994. Bell pepper cv Bell Tower was planted each year. The small grain rota-

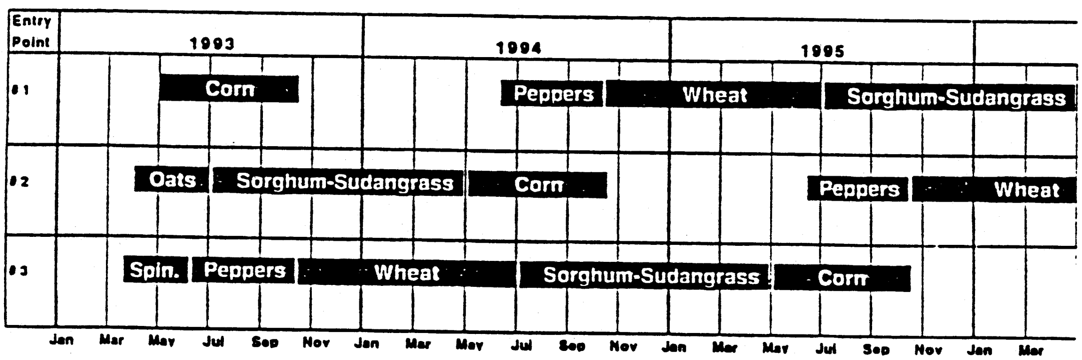

Fig. 1. Three-year crop rotation is utilized in this experiment. Entry points 1, 2 and 3 occurred each year for each treatment.

tion point was oats (*Avena sativa* L.) cv Ogle in 1993, and winter wheat (*Triticum aestivum* L.) cv Cardinal in 1994 and cv Madison in 1995.

Treatments examined included two composts, raw dairy cow manure, and conventional chemical fertilizer. Compost treatment one was a broiler chicken litter/leaf compost (1:3 [v/v]). The chicken litter consisted of chicken manure and coarse sawdust bedding. Compost treatment two was a dairy cow manure/leaf compost (1:4 [v/v]). The dairy cow manure included straw and newspaper bedding. The only variability from year to year in the production of composts was the number of turnings. Chemical analyses of the finished composts and manure at the time of application showed the chicken litter/leaf compost to be highest in P and the raw dairy cow manure to be highest in N, K, and C (Table 1). Chemical fertilizer was added to Pennsylvania State University recommended levels for the individual crops.

Compost and manure application rates were adjusted, based upon chemical analyses of the final material (conducted by A&L Eastern Labs, Richmond, VA), to supply the same available N to the particular crop as the recommended level of chemical fertilizer. Fifty percent of the N in the raw dairy manure was considered to be available to plants based upon published values for manure incorporated into the soil on the day of application (Penn State Agronomy Guide 1991–1992, 1991). Forty percent of the N in the composts was considered available to plants. This was based upon a 2-year trial experiment comparing yields of maize receiving variable levels of chicken litter/leaf compost with those receiving fertilizer N (Reider et al., 1992). Therefore, the recommended application rate of 146 kg N ha^{-1} for maize yielded target application rates of 365 kg compost N and 292 kg manure N per ha^{-1}. Similarly, to achieve the recommended application rate of 112 kg N ha^{-1} for bell peppers, 281 kg of compost N and 225 kg of manure N were the target rates. Amendments were applied prior to planting oats in 1993, but not before winter wheat in 1994 and 1995. The recommended rate of 84 kg N ha^{-1} for oats yielded target application rates of 210 kg compost N and 168 kg manure N ha^{-1}. Mean yearly actual applications of N are shown in Table 2 and differed from these target rates due to problems in spreading the composts and manures uniformly.

Adjusting compost and manure application rates solely on the target available N application led to significant addition of other nutrients (Table 2). Several fold greater applications of P occurred with the organic amendments relative to the conventional fertilizer applied at recommended rates.

Table 2
Mean yearly application of N, P, and K (kg ha^{-1}) for each ammendment at each of the three rotation points

Treatment	Nutrient	Maize	Pepper	Oats
Broiler litter + leaf compost	N	356 ± 10	299 ± 57	298
	P	186 ± 9	158 ± 25	136
	K	224 ± 21	191 ± 37	170
Dairy manure + leaf compost	N	537 ± 167	423 ± 70	253
	P	176 ± 52	140 ± 21	86
	K	265 ± 84	224 ± 28	106
Raw dairy manure	N	391 ± 52	271 ± 87	239
	P	123 ± 21	69 ± 32	91
	K	257 ± 72	173 ± 74	214
Conventional fertilizer	N	146 ± 0	149 ± 37	84
	P	34 ± 0	0	0
	K	102 ± 47	93 ± 46	139

Means of 3 years ± SEM, except for oats which were only grown 1 year (no ammendments applied to winter wheat grown the other 2 years).

Table 1
Composition of manure and composts, 1993–1995

Amendment	% Dry Weight			
	N	P	K	C
Broiler litter + leaf compost	2.11 ± 0.35	1.09 ± 0.12	1.31 ± 0.16	27.6 ± 5.2
Dairy manure + leaf compost	1.59 ± 0.06	0.53 ± 0.01	0.79 ± 0.08	29.3 ± 3.4
Raw dairy manure	4.17 ± 0.39	0.75 ± 0.04	1.88 ± 0.05	50.6 ± 0.2

Mean analyses of finished composts or manure prior to spreading each of 3 years.

Four soil samples were collected from a depth of 16 cm from each block × treatment × rotation point combination subplot on April 27, 1993 prior to the application of amendments and commencement of the experimental rotation to establish baseline data for populations of VAM fungus spores. Samples then were collected in late autumn for 3 successive years: November 22, 1993; December 1, 1994; and December 5, 1995 to quantify treatment effects upon spore populations and inoculum levels. Spores were isolated from 50 cm^3 soil samples via wet sieving and centrifugation (Gerdemann and Nicolson, 1963; Jenkins, 1964), characterized and counted.

Mycorrhizal infectivity of the field soil was quantified in greenhouse/growth chamber bioassays. After completion of spore isolation and quantification, the four soil samples from each block × treatment × rotation point combination were pooled and mixed 1:1 [v/v] with vermiculite and potted in conical plastic pots (Super Cell C-10, Stuewe and Sons, Corvallis, OR, USA). One bahiagrass (*Paspalum notatum* Flugge) seedling (1993, 1994) or sorghum–sudangrass seedling (1995) was transplanted into each cone. Plants were grown for 3–6 weeks after which, roots were cleared and stained with trypan blue (Phillips and Hayman, 1970) and assayed for percentage root length colonized by VAM fungi using the gridline intersect method (Newman, 1966). Roots of crop plants were collected from the field at various times in the growing season and assayed for mycorrhizae as above.

Nitrogen and P concentrations of the shoots of plants were measured in the 1995 growing season. Shoots of winter wheat were sampled on May 23, maize on June 14, and bell pepper on July 11. Four samples for each crop per block × treatment combination were oven-dried (80°C), ground to pass a 20 mesh sieve, and digested with H_2SO_4–H_2O_2 at 225°C. Total N was determined colorimetrically via the method of Wall and Gehrke (1975) and P according to Murphy and Riley (1962).

Data were analyzed using analysis of variance after arcsin (percentage root length colonized) or square root of $x + 1$ (spore count) transformations. Measures for which significant treatment effects were found were characterized further using Tukey's method of multiple comparisons.

3. Results

3.1. Soil and plant analysis

Compost and manure addition to supply N for crop growth increased available P and K in the soil over the course of the experiment (Table 3). Levels of these nutrients in soils treated with chemical fertilizer tended to remain constant relative to those at the start of the experiment. This is not surprising given the wealth of research upon which fertilizer recommendations are based and that the chemical composition can be readily adjusted to suit the needs of a crop or deficiencies of a soil.

Shoot N and P concentrations of maize and wheat were not significantly different among treatments when sampled early in the 1995 growing season. Nitrogen concentrations ranged from 2.3 to 2.7% dry weight in maize and 1.1 to 1.4% in wheat. Phosphorus concentrations ranged from 0.407 to 0.437% dry weight in maize and 0.258 to 0.283% in wheat.

Table 3
Results of soil analyses conducted prior to amendment addition (1992) and after 3 years of additions (1995)

Sample	pH	Available P (kgP_2O_5 ha^{-1})	Exchangeable K (kg K_2O ha^{-1})
1992	6.5 ± 0.1	709 ± 32	191 ± 12
Broiler litter + leaf compost	6.9 ± 0.1	787 ± 29	239 ± 37
Dairy manure + leaf compost	6.7 ± 0.1	854 ± 85	277 ± 39
Raw dairy manure	6.7 ± 0.1	833 ± 20	248 ± 28
Fertilizer	6.4 ± 0.1	713 ± 12	219 ± 15

Means of pooled samples collected from a depth of 20 cm ± SEM; pH measured in water. P extracted with 0.025 N HCl and 0.03 N NH_4F. K extracted with 1.0 N NH_4OAc.

Treatment	%P	%N
Broiler litter + leaf compost	0.427[a]	3.660[a]
Dairy manure + leaf compost	0.463[a]	3.801[a]
Raw dairy manure	0.389[b]	3.366[a]
Fertilizer	0.383[b]	3.393[a]

Means of 12, numbers in the same columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's method of mutiple comparisons).

Shoot phosphorus levels were significantly higher in pepper plants grown in the composts compared to raw dairy cow manure or conventional fertilizer (Table 4). Nitrogen concentrations were higher in pepper than maize or wheat, but not significantly different among treatments.

No spores which could be readily identified as *Entrophospora* or *Acaulospora* were found. Members of the Gigasporineae which were seen included *Gigaspora gigantea* (Nicol. and Gerd.) Gerdemann and Trappe and, rarely, *Scutellospora pellucida* (Nicol. and Schenck) Walker and Sanders. The Glomineae spores which were found were divided into five type groups: *Glomus geosporum* (Nicol. and Gerd.) Walker (brown spores, 150 μm diameter); *G. occultum* Walker and *G. occultum*-like spores (hyaline, < 100 μm diameter), *G. etunicatum* Becker and Gerdemann and *G. etunicatum*-like spores (small yellow, approximateký 100 μm diameter, including *G. intraradices* Schenck and Smith and *G. fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske; a large hyaline *Glomus* sp. (150–200

Table 5
Effect of compost or fertilizer treatment upon AM fungus spore populations at the Rodale Research Center. Soil was collected on November 22, 1993; December 1, 1994; and December 4, 1995

Treatment	Spore type group					
	GGGT	LOCT	*Glomus*	LETC	LHG	LGEO
Year 1						
Broiler litter + leaf compost	1.1[a]	31.5[a]	17.5[a]	11.4[a]	1.2[a]	0.3[a,b]
Dairy manure + leaf compost	8.1[a]	27.4[a]	22.3[a]	12.2[a]	0.7[a]	0.1[b]
Raw dairy manure	0.3[a]	26.3[a]	24.9[a]	13.8[a]	0.4[a]	0.6[a]
Fertilizer	1.8[a]	28.9[a]	11.0[a]	7.7[a]	3.7[a]	0.6[a]
Year 2						
Broiler litter + leaf compost	0.4[a]	38.7[a]	34.6[a]	17.9[a]	5.1[a]	0.4[a]
Dairy manure + leaf compost	2.4[a]	35.4[a,b]	31.8[a]	17.4[a]	0.9[a]	0.2[a]
Raw dairy manure	0.2[a]	26.9[a,b]	21.4[b]	7.4[b]	3.1[a]	0.3[a]
Fertilizer	1.3[a]	19.9[b]	14.8[c]	7.1[b]	8.2[a]	0.5[a]
Year 3						
Broiler litter + leaf compost	0.2[a]	25.5[a]	19.4[b]	10.7[a,b]	11.3[a]	0.6[a]
Dairy manure + leaf compost	2.0[a]	31.9[a]	26.2[a]	13.8[a]	2.5[a]	0.2[a]
Raw dairy manure	0.1[a]	23.2[a]	9.8[c]	8.3[b]	2.6[a]	0.2[a]
Fertilizer	0.3[a]	30.1[a]	10.9[c]	7.6[b]	13.7[a]	0.6[a]

Spores 50 cm^{-3}, means of 36 observations. Numbers in the same column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's method of multiple comparisons). GGGT = *Glomus gigantea*, large, yellow 400 μm diameter; LOCT = *Glomus occultum* group, small hyaline < 100 μm diameter; LETC = *Glomus etunicatum* group, small yellow 100 μm diameter; LGEO = *Glomus geosporum* group, brown, 150 μm diameter; LHG = large hyaline *Glomus* 150–200 μm diameter; *Glomus* = other *Glomus* spp.

Treatment	%P	%N
Broiler litter + leaf compost	0.427[a]	3.660[a]
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Dairy manure + leaf compost	8.1[a]	27.4[a]	22.3[a]	12.2[a]	0.7[a]	0.1[b]
Raw dairy manure	0.3[a]	26.3[a]	24.9[a]	13.8[a]	0.4[a]	0.6[a]
Fertilizer	1.8[a]	28.9[a]	11.0[a]	7.7[a]	3.7[a]	0.6[a]
Year 2						
Broiler litter + leaf compost	0.4[a]	38.7[a]	34.6[a]	17.9[a]	5.1[a]	0.4[a]
Dairy manure + leaf compost	2.4[a]	35.4[a,b]	31.8[a]	17.4[a]	0.9[a]	0.2[a]
Raw dairy manure	0.2[a]	26.9[a,b]	21.4[b]	7.4[b]	3.1[a]	0.3[a]
Fertilizer	1.3[a]	19.9[b]	14.8[c]	7.1[b]	8.2[a]	0.5[a]
Year 3						
Broiler litter + leaf compost	0.2[a]	25.5[a]	19.4[b]	10.7[a,b]	11.3[a]	0.6[a]
Dairy manure + leaf compost	2.0[a]	31.9[a]	26.2[a]	13.8[a]	2.5[a]	0.2[a]
Raw dairy manure	0.1[a]	23.2[a]	9.8[c]	8.3[b]	2.6[a]	0.2[a]
Fertilizer	0.3[a]	30.1[a]	10.9[c]	7.6[b]	13.7[a]	0.6[a]

Spores 50 cm^{-3}, means of 36 observations. Numbers in the same column followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's method of multiple comparisons). GGGT = *Glomus gigantea*, large, yellow 400 μm diameter; LOCT = *Glomus occultum* group, small hyaline < 100 μm diameter; LETC = *Glomus etunicatum* group, small yellow 100 μm diameter; LGEO = *Glomus geosporum* group, brown, 150 μm diameter; LHG = large hyaline *Glomus* 150–200 μm diameter; *Glomus* = other *Glomus* spp.

No spores which could be readily identified as *Entrophospora* or *Acaulospora* were found. Members of the Gigasporineae which were seen included *Gigaspora gigantea* (Nicol. and Gerd.) Gerdemann and Trappe and, rarely, *Scutellospora pellucida* (Nicol. and Schenck) Walker and Sanders. The Glomineae spores which were found were divided into five type groups: *Glomus geosporum* (Nicol. and Gerd.) Walker (brown spores, 150 μm diameter); *G. occultum* Walker and *G. occultum*-like spores (hyaline, < 100 μm diameter), *G. etunicatum* Becker and Gerdemann and *G. etunicatum*-like spores (small yellow, approximateky 100 μm diameter, including *G. intraradices* Schenck and Smith and *G. fasciculatum* (Thaxter) Gerdemann and Trappe emend. Walker and Koske; a large hyaline *Glomus* sp. (150–200

Vesicular–arbuscular mycorrhizal fungus colonization of oats, maize, and pepper plants during the first year of ammendment addition

Treatment	Oats	Maize	Pepper
Broiler + Leaf Compost	14.4[a]	49.7[a]	0.9[a]
Raw Dairy	16.5[a]	41.5[b]	0.9[a]
Fertilizer	16.3[a]	36.1[b]	1.6[a]

Means of 16 samples collected on July 20, 1993. Numbers in the same column followed by the same letter are not significantly different ($\alpha = 0.10$).

to the effects of the previous crop of maize on those plots in 1994 (Fig. 1). Infectivity of soils from plots on which a crop of pepper was grown was significantly lower than other plots after the 1995 growing season (Table 8). This reflects the effect of the low VAM fungus colonization of peppers upon soil borne inoculum of the mycorrhizal fungi.

4. Discussion

Compost addition in the experiment reported here increased the numbers of spores of the *Glomus* spp. and *G. etunicatum* type groups only (Table 5). Larger and more consistent increases in populations of VAM fungus spores typically are seen with sustainable agricultural practices (Limonard and Ruissen, 1989; Sattelmacher et al., 1991; Douds et al., 1993, 1995;

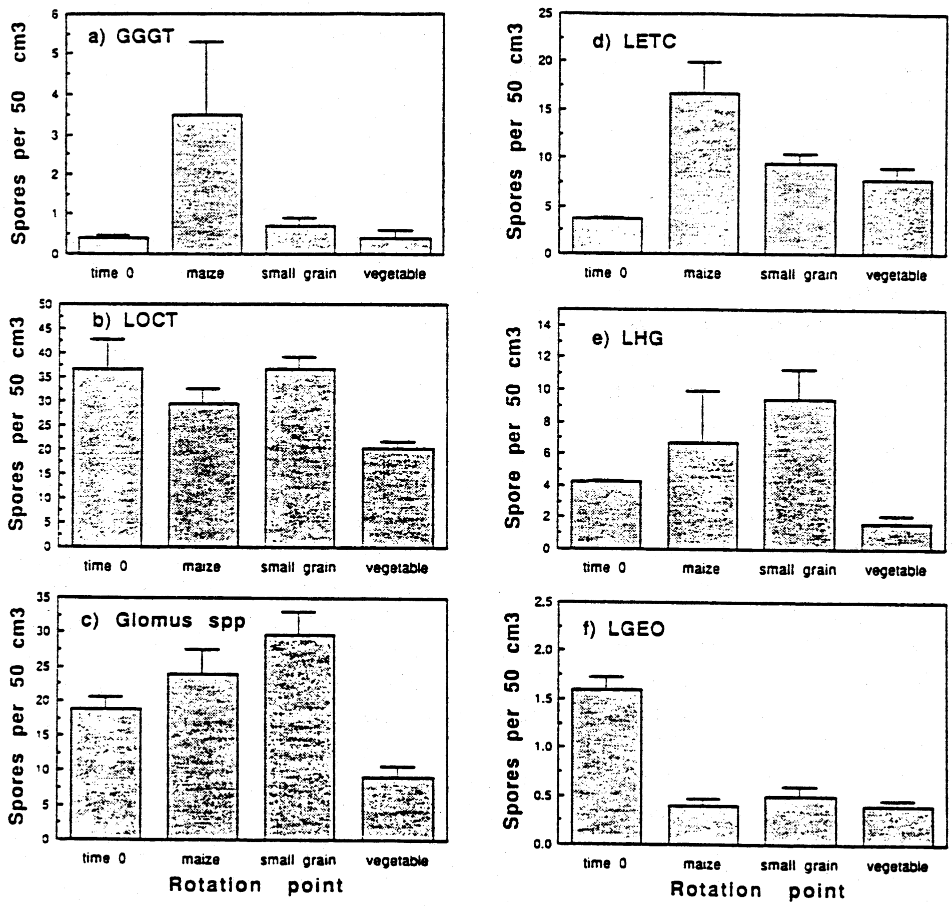

Fig. 2. Initial VAM fungus spore populations and those found following each point of the 3-year crop rotation. Means ± SEM. (a) *G. gigantea*, (b) *G. occultum* type group, (c) *Glomus* spp., (d) *G. etunicatum* type group, (e) large, hyaline *Glomus*, and (f) *G. geosporum*. See Table 5 for descriptions of spore groups.

colonization needed for sporulation by some VAM fungi at this site. Colonization of pepper in a variety of field and greenhouse experiments has ranged from 1–4% (Davies and Linderman, 1991) to 70–80% root length colonized (Hirrel and Gerdemann, 1980).

Co-composted farmyard manure and municipal yard wastes have utility for decreasing both the waste stream to landfills and the potential for pollution of groundwater by livestock operations (Reider et al., 1991). However, application rates of composts to agricultural fields calculated to be the sole source of N for optimal crop yield may be excessive in light of carryover of other nutrients and may not be good sustainable agriculture practice. Further, practitioners of sustainable agriculture should be wary of including non-mycorhizal and sparsely mycorrhizal plants (under their field conditions) in rotations because of their effects upon levels of inoculum of VAM fungi.

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colonization needed for sporulation by some VAM fungi at this site. Colonization of pepper in a variety of field and greenhouse experiments has ranged from 1–4% (Davies and Linderman, 1991) to 70–80% root length colonized (Hirrel and Gerdemann, 1980).

Co-composted farmyard manure and municipal yard wastes have utility for decreasing both the waste stream to landfills and the potential for pollution of groundwater by livestock operations (Reider et al., 1991). However, application rates of composts to agricultural fields calculated to be the sole source of N for optimal crop yield may be excessive in light of carryover of other nutrients and may not be good sustainable agriculture practice. Further, practitioners of sustainable agriculture should be wary of including non-mycorhizal and sparsely mycorrhizal plants (under their field conditions) in rotations because of their effects upon levels of inoculum of VAM fungi.

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