

available at www.sciencedirect.comjournal homepage: www.elsevier.com/locate/apsoil

Fungal population levels in soils of commercial swine waste disposal sites and relationships to soil nutrient concentrations

R.G. Pratt*

U.S. Department of Agriculture, Agricultural Research Service, Waste Management and Forage Research Unit, P.O. Box 5367, Mississippi State, MS 39762, USA

ARTICLE INFO

Article history:

Received 18 January 2007

Received in revised form

5 October 2007

Accepted 18 October 2007

Keywords:

Soil microorganisms

Fungal populations

Animal waste

Swine waste

Soil nutrients

ABSTRACT

Commercial disposal of animal wastes by application to agricultural soils is well-known to increase soil nutrient concentrations and the potential for water pollution. Less is known of whether or how commercial animal waste disposal affects microbial populations in soil. This study was undertaken to determine whether commercial applications of liquid swine waste to pasture soils in Mississippi, USA, influence fungal population levels, and whether these are related to major differences in soil nutrient concentrations induced by commercial waste disposal. Colonies of culturable fungi were assayed by dilution plating from samples of soil, with and without applied swine waste, collected from pastures on three commercial swine farms early and late in the summer growing season in each of three years. Fungal population levels observed among 180 samples were $2.80\text{--}43.27 \times 10^4$ colony-forming units per gram of soil. Population levels of total fungi, or groups and species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Trichoderma*, did not differ significantly between waste treatments in more than 2 of 18 sampling events. Concentrations of P, K, and Na were always significantly greater, often by 5–10-fold, in waste-treated than in untreated soils; levels of Mg, Cu, and Zn were frequently greater; and levels of N, Ca, Fe, and Mn were seldom or never greater. However, fungal population levels in soil were not correlated with concentrations of any of these nutrients in more than 2 of 18 sampling events. Results indicate that culturable fungi represent a stable component of the soil microflora for which population levels seldom change in response to commercial swine waste disposal or the major differences in soil nutrient concentrations that result from it.

Published by Elsevier B.V.

1. Introduction

Concentrated animal production, in which large numbers of cattle, swine, and poultry are raised in year-around indoor confinement, has become commonplace in American agriculture since the middle of the 20th century. In the southeastern USA, frequently >250,000 chickens or 20,000 hogs are raised

indoors at single locations. The storage and disposal of large quantities of wastes generated from these animals daily, without causing environmental damage, is a major liability of production. Transportation of wastes to distant locations for use or disposal is uneconomical; therefore, most animal wastes are disposed by repeated applications to pastures at close proximity to production sites (Evers, 1996; Poore and Green, 1996).

* Tel.: +1 662 320 7424; fax: +1 662 320 7544.

E-mail address: rpratt@ars.usda.gov.

0929-1393/\$ – see front matter. Published by Elsevier B.V.

doi:10.1016/j.apsoil.2007.10.013

Application of animal wastes to the same soils for many years may profoundly affect soil chemistry. Although most animal manures are suitable for use as fertilizers, their nutrient contents do not correspond well to plant growth needs on account of high levels of P, K, and some micronutrients relative to N (Evers, 1996; Pierzynski et al., 2000). Consequently, as documented in numerous studies, applications of animal waste may increase P, K, and certain micronutrient levels in soils despite repeated harvests of hay intended to remove them (King et al., 1990; Sharpley et al., 1999). Movement of P from overloaded soils into surface waters then may lead to eutrophic pollution and loss of environmental quality (Evers, 1996; Pierzynski et al., 2000; Sharpley et al., 1999).

Less is known of whether or how commercial animal waste applications affect populations of microorganisms in soil. Although numerous studies have evaluated effects of animal wastes on soil microorganisms, in most of these, wastes were applied as individual treatments, usually along with other wastes, byproducts, organic materials, and fertilizers, in controlled experiments in the field (Acosta-Martinez and Harmel, 2006; Schnürer et al., 1985; Bulluck and Ristaino, 2002; Pérez-de-Mora et al., 2006; Ros et al., 2003; He et al., 1997) and laboratory (Lovell and Jarvis, 1996; Larkin et al., 2006; Entry et al., 1997) and not studied on actual commercial application sites. Effects of animal wastes on microbial populations and activities in controlled experiments have been evaluated by soil dilution plating (Bulluck and Ristaino, 2002); direct microscopic observation (Schnürer et al., 1985); quantification of organic C, N, and P (Acosta-Martinez and Harmel, 2006; Pérez-de-Mora et al., 2006; Ros et al., 2003; Schnürer et al., 1985; He et al., 1997; Lovell and Jarvis, 1996; Entry et al., 1997); respiration (Lovell and Jarvis, 1996; Ros et al., 2003; Schnürer et al., 1985); microbial enzyme activities (Pérez-de-Mora et al., 2006; Acosta-Martinez and Harmel, 2006; Schnürer et al., 1985); levels of fatty acid markers (Larkin et al., 2006; Acosta-Martinez and Harmel, 2006), and substrate utilization (Larkin et al., 2006; Ros et al., 2003).

Principal results from these and similar studies are that animal wastes, when applied to soil alone or with other wastes and amendments, usually increase the following parameters of microbial biomass and activity: (1) microbial biomass C, N, and/or P (Acosta-Martinez and Harmel, 2006; Pérez-de-Mora et al., 2006; Ros et al., 2003; Schnürer et al., 1985; He et al., 1997; Lovell and Jarvis, 1996); (2) population levels, observable structures, or fatty acid concentrations of microorganisms (Schnürer et al., 1985; Bulluck and Ristaino, 2002; Acosta-Martinez and Harmel, 2006; Larkin et al., 2006); (3) substrate utilization and respiration (Ros et al., 2003; Lovell and Jarvis, 1996; Larkin et al., 2006); and (4) microbial enzyme activity (Acosta-Martinez and Harmel, 2006; Pérez-de-Mora et al., 2006). Increases in microbial biomass or activity also were related to increased soil organic matter (Schnürer et al., 1985), organic C (Pérez-de-Mora et al., 2006; Ros et al., 2003), mineral N (Lovell and Jarvis, 1996; Entry et al., 1997), and cellulose degradation (Entry et al., 1997). However, some studies have reported different results for several of these parameters (Acosta-Martinez and Harmel, 2006; Entry et al., 1997), and in nearly all studies, significant differences between various waste treatments were observed.

Several studies have specifically evaluated effects of animal wastes on fungi in soil, and results were remarkably similar despite major differences in location, environment, animal wastes, and assay methods for fungal population levels or biomass (Bittman et al., 2005; Bulluck and Ristaino, 2002; De Vries et al., 2006; Entry et al., 1997; Marschner et al., 2003; Schnürer et al., 1985). In all instances, application of animal wastes either caused no increases in total measurable fungi, fungal structures, or indicator compounds, or else ratios of fungi to bacteria decreased as levels of organic or inorganic N increased. When bacteria were measured, in all instances their populations increased in response to animal wastes or N levels, whereas populations of total detectable fungi in soil did not. In one study, propagules of *Trichoderma* greatly increased in response to a swine manure amendment, but populations of *Fusarium* or total culturable fungi did not increase (Bulluck and Ristaino, 2002).

Although most field studies on effects of animal wastes on fungi in soil were conducted as controlled experiments with replicated plots, in two instances, evaluations were performed in situations that corresponded more closely to commercial conditions. Parham (2003) evaluated plots to which cattle manure had been applied for over a century; application of manure increased populations of bacteria but not fungi in soil relative to inorganic fertilizers. Acosta-Martinez and Harmel (2006) applied poultry litter to whole pastures and cultivated fields by conventional agricultural practices; bacteria increased at low rates of litter application, but fungi, as determined by fatty acid levels, increased only at the highest rates.

Although animal wastes usually are not known to strongly affect total fungal populations in soil, numerous reports have described the inhibition and even eradication of a wide range of individual, plant pathogenic fungi in soil by application of animal wastes and other organic amendments as biocontrol materials (e.g., Conn et al., 2005; Weller et al., 1988; Aryantha et al., 2000). Mechanisms of inhibition usually involve stimulation of antagonistic or mycoparasitic bacteria and fungi by the applied materials (Weller, 1988), but they also may involve direct release of toxic compounds such as ammonia and volatile fatty acids (Conn et al., 2005). Soils in which such suppression of pathogens or diseases occurs either naturally or after application of organic amendments are often referred to as “suppressive soils” (Hornby, 1983; Weller, 1988). Inhibition of fungal diseases in soil also may be caused by forms or levels of macro- and micronutrients (Engelhard, 1989), and such inhibition is sometimes considered to represent soil suppressiveness.

Commercial applications of animal wastes to agricultural crops are performed in order to rapidly dispose, at minimal expense, large quantities of manure that are generated daily at production sites. This commercial waste disposal may create environmental conditions that are more unique, harsh, variable, and extreme than would occur in most experimental situations. For swine waste in particular, manure is disposed by daily washing from production houses into anaerobic holding lagoons, and the resulting liquid slurries are then sprayed repeatedly over the same pastures throughout the growing season for many years by center-pivot, overhead irrigation (King et al., 1990).

Features and consequences of commercial swine waste application that are likely to differ from experimental applications of solid animal wastes include the following: (1) most N in manure is lost as a result of volatilization of ammonia from the liquid suspension before and shortly after waste application (Al-Kaisi and Waskom, 2002); (2) nutrients from liquid waste applications may be imbibed immediately into dry soil, or washed and leached from saturated soil, without more long-term, slow release as occurs during decomposition of solid waste; (3) potential use of swine waste as a substrate for colonization and increase by soil fungi does not exist with liquid slurries; (4) repeated, long-term application of liquid swine waste to soils for many years results in abnormally high levels of P, K, and Mg, and imbalances in soil nutrient profiles (King et al., 1990; Pierzynski et al., 2000). Any of these conditions might affect population levels, activity, and survival of microorganisms differently in soils that receive commercial swine waste applications than in controlled experimental applications.

Soil fungi are involved in numerous activities related to nutrient cycling, plant growth, and plant health (Christensen, 1989; Pierzynski et al., 2000). These include decomposition of organic matter with release of soluble nutrients (Frey et al., 2003; Pierzynski et al., 2000), solubilization of nutrients from minerals (Asea et al., 1988), translocation of elements and nutrients within the soil profile and from soil to plants (Christensen 1989; Frey et al., 2003), uptake of nutrients and incorporation into the fungal biomass (Frey et al., 2003), creation of secondary soil structure (Frey et al., 2003; Pierzynski et al., 2000), and causation and suppression of disease in plants (Englehard 1989; Weller et al., 1988). Therefore, factors that affect populations and activities of fungi in soil may significantly affect soil productivity and environmental quality.

The extent to which the unique conditions that result from commercial swine waste disposal affect soil fungi and other microorganisms is largely unknown. Therefore, this study was undertaken to determine: (1) whether commercial swine waste applications negatively impact population levels of total culturable fungi or randomly selected groups in a manner comparable to their effects on soil nutrient composition; (2) whether population levels of soil fungi are related to abnormally high soil nutrient concentrations caused by commercial swine waste applications; and (3) whether discrepancies exist between results obtained from commercial swine waste disposal and animal waste applications performed under experimental controlled conditions.

2. Materials and methods

2.1. Swine waste application sites and collection of soil samples

Soil samples were collected from three commercial swine farms in northeast Mississippi (Pratt, 2006). Site 1 was located in Clay County, MS, on an Ora loam (fine-loamy, siliceous, semiactive, thermic Typic Fragiuudult); Site 2 was located in Lowndes County on a Brooksville silty clay (fine, smectitic, thermic Aquic Chromudert); Site 3 was located in Chickasaw County on an Atwood silt loam (fine-silty, mixed, thermic

Typic Paleudolf. At each site, swine waste was applied to mixed-grass pastures of bermudagrass (*Cynodon dactylon* [L.] Pers.), johnsongrass (*Sorghum halepense* [L.] Pers.), dallisgrass (*Paspalum dilatatum* Poir.), bahiagrass (*P. notatum* Flugge), broadleaf signalgrass (*Brachiaria platyphylla* [L.] Beauv.), yellow foxtail (*Setaria glauca* L.), and other minor grasses and broadleaf weeds. On all sites, waste was washed from swine production houses into holding lagoons, and liquid slurries from these were applied to pastures in fixed patterns by overhead, center-pivot irrigation 1–3 times weekly or more from May to October. All pastures also contained areas with the same soil types and vegetation that were located beyond spray patterns and remained free of applied swine waste. Areas of pastures with and without applied swine waste were cut for hay at the same times and otherwise received the same management practices.

Soil samples were collected from the three sites both early (June) and late (September–October) in the summer growing season in 2003, 2004, and 2005. Swine waste was applied intermittently at all sites during these times. In each sampling event, samples of topsoil approximately 1.5–2 l in volume were taken from the upper 15 cm of soil within 0.5 m² areas at 5 randomly selected points beyond the zone of waste application and at 5 points within it. All sampling points were 15–30 m or more apart. Locations of sampling points were not precisely defined but were described generally according to landmarks in and around pastures; consequently, all comparable samples are believed to have originated at points within the same areas of approximately 25–50 m² at all six sampling times during the study. Samples were spread on a greenhouse bench, air-dried to 10–13% moisture content, and stored in sealed plastic bags at room temperature for up to 2 weeks prior to assay of fungal population levels.

2.2. Determination of fungal population levels and nutrient concentrations in soil

Population levels of total culturable fungi in each soil sample were estimated by dilution plating on agar. Portions of soil equivalent to 10 g oven-dry weight were mixed in 90 ml sterile distilled water and diluted serially at 1/10 dilutions with sterile distilled water, after 20 min stirring at each dilution, to a concentration of 10⁻³ g soil/ml suspension. Aliquots of 0.1 ml were then streaked evenly over surfaces of three agar plates, and plates were inverted on a laboratory bench at 23–25 °C.

Soil dilution plating was performed with unamended potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) in 2003 and on Sabouraud's agar + chloramphenicol (SAC) (Remel, Inc., Lexia, KS) in 2005. In 2004, plating was performed on both PDA and SCA. When soil suspensions were plated on PDA, surfaces were washed free of bacteria after 3 days by gently rubbing under running tapwater. Fungal colonies growing in agar then were observed under a dissecting microscope at 10–30×. Colonies were marked at 3, 4, and 5 days, and total colonies were recorded at 5 days. Fungal colonies on SAC were marked and recorded in a similar manner without washing the surface of agar.

Population levels of 6 individual fungal species or groups that formed distinctive and consistently recognizable colonies, on the basis of coloration, morphology, and sporulation,

on SAC (*Aspergillus* spp. [*A. niger* group], *Penicillium purpurogenum* Stoll, *Fusarium oxysporum* Schlecht. Emend. Sny. & Hans., *F. semitectum* Berk. & Rav., *Fusarium* sp., *Trichoderma* spp.) also were determined by soil dilution plating. Equal quantities of the 5 soil samples of each waste treatment, collected at each sampling event, were mixed to produce a composite soil sample. Three experiments to assay population levels then were performed with each of the 36 composited samples (18 sampling events \times 2 waste treatments). Population levels of each fungal taxon or group in each sample were assayed on 3 plates of SAC in each of the 3 experiments.

Total concentrations of 10 macro- and micronutrients and elements were determined in each of 180 soil samples. Concentrations of total N were determined by Dr. T.E. Fairbrother and M. Hardy with a Thermoquest C/N analyzer (C.E. Elantec, Lakewood, NJ). Total concentrations of all other elements were determined by Dr. Fairbrother with an inductively coupled dual axial argon plasma spectrophotometer (ICP) (Therma-Jarrell-Ash model 1000, Franklin, MA).

2.3. Statistical methods

Mean population levels of total fungi in 5 samples of soil with and 5 without applied swine waste from each of 18 sampling events (3 years \times 2 seasons \times 3 locations) were evaluated for significant differences at $P = 0.05$ by t-tests with the Microsoft Excel program. Significance of main effects and interactions of year, time, and waste treatment were determined by factorial analysis of variance according to procedures described by Steele and Torrie (1960). For 6 individual fungal taxa, mean population levels observed in 3 individual experiments with composited samples of waste-treated and untreated soils from each sampling event were used as replicates in t-tests to evaluate significance of differences between the waste treatments.

Mean concentrations of 10 nutrients and elements, observed in 5 individual samples of waste-treated and untreated soil from each sampling event, were compared by t-tests at $P = 0.05$. Linear correlations between total fungal population levels and concentrations of the nutrients and elements in 10 soil samples across waste treatments at each sampling event were evaluated

at $P = 0.05$. Consistency of results obtained for fungal population levels on the two different agar media in 2004 also was evaluated by linear correlation.

3. Results

3.1. Population levels of total culturable filamentous fungi and seven taxa in soils

Colonies of most fungi on SAC were well-defined, dense, with a wide range of colors and hues, and often with frequent to profuse sporulation. Most commonly 10–30 colonies were recorded on each plate. Colonies of bacteria and actinomycetes were absent or few and small on SAC. On PDA, colonies of bacteria were larger and more numerous; these sometimes limited development of surface mycelium, but fungal growth within the agar could be observed after bacteria were removed by washing.

Mean population levels of total culturable fungi observed in soil samples with and without applied swine waste in the 18 sampling events ranged from $2.80\text{--}42.27 \times 10^4$ colony-forming units (cfu)/g of soil when assayed on the SAC medium. These means differed significantly between soils of the two waste treatments in only 2 of the 18 sampling events from early 2004 (data not presented). When mean fungal population levels in soils with and without swine waste at each site were used as replicates in factorial analysis of data, significant ($P = 0.05$) variation was attributed to years but not to sampling times, waste treatments, or interactions. In 2004, when assays were performed on both SAC and PDA, mean population levels observed across sites were consistently 8–20% higher on PDA than on SAC. Results for 30 individual soil samples assayed with these two media were highly significantly correlated ($P < 0.01$) across sites and waste treatments both early and late in the year ($r = 0.71$ and 0.72 , 28 d.f., respectively).

Mean population levels of the six fungal groups and taxa observed in composited soil samples, with and without applied swine waste, over 18 sampling events and three dilution series, were $0\text{--}5.67 \times 10^4$ cfu/g (Table 1). For each

Table 1 – Population levels of individual groups, genera, or species of fungi in soils with and without applied swine waste collected from commercial cotton farms during eighteen sampling events^a

Fungal group, genus, or species ^b	Range of mean population levels ^c (cfu/g soil $\times 10^{-4}$)	Number of sampling events with significant ($P = 0.05$) differences between treatments ^d	
		(+)-waste soil > (-)-waste soil	(-)-waste soil > (+)-waste soil
<i>Fusarium oxysporum</i>	0.00–5.67	1	1
<i>F. semitectum</i>	0.11–3.89	0	1
<i>F. sp.</i>	0.00–1.22	1	1
<i>Penicillium purpurogenum</i>	0.00–2.67	1	1
<i>Trichoderma</i> spp.	0.00–1.33	0	1
<i>Aspergillus</i> spp. (black-spored) ^e	0.00–0.78	0	1

^a 18 sampling events = 3 farms \times 3 years \times 2 seasons.

^b Fungi selected randomly based on formation of distinctive colonies that were consistently recognizable on soil dilution plates.

^c cfu = colony-forming units. Each value is the mean number of colonies observed on 3 replicate plates in each of 3 dilution series with soil from a single sampling event.

^d Significance based on t-tests of population levels in 3 dilution series from (+)- and (-)-waste soils at each sampling event.

^e Black-spored species *in toto*.

Table 2 – Concentrations of nutrients and elements in soils with and without applied swine waste collected from commercial swine farms during eighteen sampling events^a

Element	Unit	Range of mean concentrations ^b	Number of sampling events with significant (P = 0.05) differences between treatments ^c	
			(+)-waste soil > (-)-waste soil	(-)-waste soil > (+)-waste soil
N	g/kg (0.1)	0.72–3.41	1	0
P	g/kg (10)	0.01–0.27	18	0
K	g/kg (10)	0.05–2.10	18	0
Na	g/kg (10)	0.02–0.58	18	0
Mg	g/kg (10)	0.01–0.24	14	0
Cu	mg/kg (10)	0.13–5.29	6	2
Zn	mg/kg (10)	2.16–9.82	6	1
Mn	mg/kg	3.28–20.42	0	4
Ca	g/kg (10)	0.70–5.56	2	0
Fe	mg/kg (0.1)	1.38–3.59	0	0

^a 18 sampling events = 3 farms × 3 years × 2 seasons.

^b Values are mean concentrations of nutrients and elements determined in 5 replicate soil samples. N concentrations were determined with a ThermoQuest C/N analyzer (CE Elantec, Inc., Lakewood, NJ); all other elements were determined with an ICP spectrophotometer (Thermo Jarrell-Ash Model 1000, Franklin, MA).

^c Significance based on t-tests of differences between concentrations in 5 replicate samples from (+)- and (-)-waste soils at each sampling event.

group of fungi, population levels differed significantly between soils of the two waste treatments in no more than one or two of the 18 sampling events. For all six fungal groups, significant differences in population levels between the waste treatments were observed in only 9 of 108 possible instances (18 sampling events × 6 fungal groups). In 2 of these instances, population levels were higher in soils with applied waste, and in 6 they were higher in soils without it (Table 1).

3.2. Concentrations of 10 nutrients and elements in soil and relationships to fungal population levels

Major and significant differences in concentration between waste-treated and untreated soils were observed for some of the 10 elements assayed, while few or no differences were observed for others. The greatest and most consistent differences occurred with P, K, and Na. Concentrations of each of these elements were significantly greater in waste-treated than in untreated soils in all 18 sampling events (Table 2), and magnitudes of increases ranged up to 8-, 15-, and 4-fold for P, K, and Na, respectively. Concentrations of Mg were significantly greater in waste-treated soils in 14 sampling events with up to a 22-fold increase over concentrations in untreated soils. Concentrations of Zn and Cu were commonly but not consistently greater in waste-treated soils (seven and six sampling events, respectively); concentrations of C, Ca, and N were occasionally greater (two, two, and one sampling events, respectively), and concentrations of Fe and Mn were never greater. In 2, 4, and 1 of 18 sampling events with Cu, Mn, and Zn, respectively, concentrations in untreated soils were significantly greater than in waste-treated soils (Table 2).

Among the 18 sets of 10 soil samples, that included both waste-treated and untreated soils collected at each sampling event, total fungal population levels were significantly (P = 0.05) correlated with soil nutrient levels in only 13 of 180 possible instances (18 sampling events × 10 nutrients). Twelve of these significant correlations were positive and one was negative. Fungal population levels were positively

correlated with concentrations of N, K, and Fe in two sampling events, and with concentrations of C, Mg, Na, P, Mn, and Zn in one sampling event each. Also in one sampling event, fungal population levels were negatively correlated with concentrations of Zn.

4. Discussion

Results of this study demonstrate that repeated commercial applications of liquid swine waste to pasture soils on three farms in Mississippi for 8 or more years prior to assay did not negatively impact population levels of total fungi or six randomly selected groups and species. This conclusion is based upon the fact that few significant differences in population levels of either total culturable fungi, or the individual groups and species, were ever observed between soils that received frequent overhead applications of liquid swine waste throughout each summer growing season, and adjacent soils that received no waste applications. When significant differences were observed in some sampling events, higher levels were found in both the waste-treated and untreated soils (Table 1). Therefore, these results indicate that the well-known, strong impacts of commercial swine waste disposal on soil nutrient contents and water quality (King et al., 1990; Sharpley et al., 1999) are not paralleled by comparable effects on soil fungal populations. Results further indicate that negative effects of swine waste applications on survival of specific plant pathogenic fungi in soil, as observed previously (Conn et al., 2005; Pratt, 2006), do not indicate any broader inhibitory effects on soil fungi in general. Results of this study are unique in that they were obtained under variable and extreme conditions of commercial swine waste disposal rather than from more uniform and moderate conditions of most controlled experiments.

Although results of this study indicate few significant effects of commercial swine waste disposal on fungal population levels in soil, these results may not be entirely

conclusive because many of the fungi assayed were relatively fast-growing, heavily sporulating organisms that commonly colonize organic matter in soil. Several individually assayed fungi (e.g., *Aspergillus* spp., *Trichoderma* spp.) may fall in this category. If such fungi do predominate on isolation plates, then more rare, slow-growing, and weakly sporulating organisms may be under-represented in colony counts. Such fungi may have contributed to higher colony counts observed on PDA than on SAC in 2004 as a result of suppression of the fast-growing species by bacterial antibiosis, which in turn reduced their tendency to overgrow and obscure other, more slow-growing and infrequent fungi.

Most previous studies of fungal populations, biomass, or activity in animal waste-amended soils have not evaluated concentrations of the full array of macro- and micronutrients present in soil and their relationships to parameters of fungal populations. In this study, where total concentrations were evaluated, the well-known effects of long-term commercial swine waste applications on soil nutrient composition (King et al., 1990; Pierzynski et al., 2000) were again clearly illustrated. In all 18 sampling events, levels of P, K, and Na were significantly greater in waste-treated than in untreated soils, and magnitudes of increases ranged up to 4–15-fold. Levels of Mg were similarly greater in 14 sampling events with up to a 22-fold increase (Table 2). However, these major differences in concentrations of nutrients in soil did not affect fungal population levels as indicated by few significant differences between (+)- and (–)-waste treatments, and by few significant correlations of fungal population levels with concentrations of any individual nutrients.

Possibly one significant relationship between soil nutrients and fungal population levels in this study might have involved N. In contrast to P and K, levels of N differed significantly between (+)- and (–)-waste soils in only 1 of 18 sampling events (Table 2). This near absence of differences in N contents of soils might possibly be related to the near absence of differences in fungal population levels. The absence of increased N in waste-treated soils likely resulted from its loss to ammonia volatilization prior to, during, and after application of liquid swine waste to soil (Al-Kaisi and Waskom, 2002; Whitehead and Cotta, 2004). Results of several other studies indicate or suggest that parameters of fungal populations in animal waste-amended soil may be positively related to N contents (Larkin et al., 2006; Schnürer et al., 1985), but others did not indicate such relationships (Acosta-Martinez and Harmel, 2006; De Vries et al., 2006; Entry et al., 1997; Marschner et al., 2003). If the occurrence of few differences in both fungal population levels and certain nutrient concentrations may be related, then Ca and Fe would also have to be considered, along with N, for such potential relationships with soil fungal populations (Table 2).

One purpose of this study was to determine whether discrepancies exist between results on fungal population levels in soils where swine wastes were applied commercially, and soils to which animal wastes were applied in controlled experiments. Some discrepancies are apparent between results from the commercial and experimental situations, but those are not consistent because results and conclusions from the different controlled experiments with animal wastes themselves were inconsistent. Applications of liquid swine

and dairy manure to soils in the laboratory increased fungal population levels (Larkin et al., 2006), and applications of poultry litter and farmyard manure to field plots increased fungal parameters over controls (Acosta-Martinez and Harmel, 2006; Schnürer et al., 1985). However, other studies in the laboratory and field indicated no responses of fungal parameters to applied animal wastes (Bulluck and Ristaino, 2002; Entry et al., 1997) or even negative responses (Bittman et al., 2005; De Vries et al., 2006). In two studies that involved long-term prior applications of animal wastes to soil, no positive responses of fungal parameters were observed (Marschner et al., 2003; Parham, 2003). In one specific comparison, Bulluck and Ristaino (2002) observed no significant increases in total fungal population levels after solid swine waste was applied to field plots while populations of *Trichoderma* spp. did increase. In this study, no significant increases in *Trichoderma* populations were observed with the commercial swine waste applications. In general, results of experimental studies in which fungal parameters were evaluated only after repeated, long-term applications of animal wastes to soil appear to correspond most closely to results of this study in which the same kind of situation was represented.

Bacteria were not evaluated in this study, so their possible responses to commercial swine waste applications remain undetermined. However, bacteria in holding lagoons and soil may have exerted a major influence on results because these are known to convert nitrogenous compounds in animal wastes into ammonia which is lost to volatilization (Whitehead and Cotta, 2004). Therefore, it appears likely that the absence of differences in N contents of soils observed in this study, and any possible relationships of those to fungal population levels, may have resulted from enzymatic action of bacteria that removed N from liquid swine waste before, during, and after its application to soil.

Overall results of this study indicate that culturable fungi comprise a highly stable component of the soil microflora because their population levels are not positively or negatively impacted by commercial swine waste disposal or the major differences in soil nutrient concentrations that result from it.

Acknowledgements

The author is grateful to T.E. Fairbrother and M.E. Hardy for performing analyses of soil nutrient concentrations; to J. Perry, K. Kennedy, J. Fitzgerald, and E. López-Gourley for assisting with assays of fungal population levels; and to E. López-Gourley for assisting in data analysis.

REFERENCES

- Acosta-Martinez, V., Harmel, R.D., 2006. Soil microbial communities and enzyme activities under various poultry litter application rates. *J. Environ. Qual.* 35, 1309–1318.
- Al-Kaisi, M.M., Waskom, R.M., 2002. Estimating ammonia loss from sprinkler-applied swine effluent. *Agron. J.* 94, 1156–1162.
- Aryantha, I.P., Cross, R., Guest, D.I., 2000. Suppression of *Phytophthora cinnamomi* in potting mixes amended with

- uncomposted and composted animal manures. *Phytopathology* 90 (7), 775–782.
- Asea, P.E.A., Kucey, R.M.N., Stewart, J.W.B., 1988. Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. *Soil Biol. Biochem.* 20 (4), 459–464.
- Bittman, S., Forge, T.A., Kowalenko, C.G., 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. *Soil Biol. Biochem.* 37, 613–623.
- Bulluck, L.R., Ristaino III, J.B., 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. *Phytopathology* 92 (2), 181–189.
- Christensen, M., 1989. A view of fungal ecology. *Mycologia* 81 (1), 1–19.
- Conn, K.L., Tenuta, M., Lazarovits, G., 2005. Liquid swine manure can kill *Verticillium dahliae* microsclerotia in soil by volatile fatty acid, nitrous acid, and ammonia toxicity. *Phytopathology* 95 (1), 28–32.
- De Vries, F.T., Hoffland, E., van Eekeren, N., Brussaard, L., Bloem, J., 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. *Soil Biol. Biochem.* 38, 2092–2103.
- Engelhard, A.W. (Ed.), 1989. *Soilborne Plant Pathogens: Management of Diseases with Macro- and Microelements*. Am. Phytopathol. Soc., St. Paul, Minnesota, p. 217.
- Entry, J.A., Wood, B.H., Edwards, J.H., Wood, C.W., 1997. Influence of organic by-products and nitrogen source on chemical and microbiological of an agricultural soil. *Biol. Fertil. Soils* 24, 196–204.
- Evers, G.W., 1996. Overview of recycling nutrients from animal waste through forages. In: *Proc-South-Pasture-Forage-Crop-Improv-Conf.* New Orleans: ARS (Southern Region), vol. 52, USDA, 1974, pp. 59–64.
- Frey, S.D., Six, J., Elliot, E.T., 2003. Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil-litter interface. *Soil Biol. Biochem.* 35, 100–1004.
- He, Z.L., Wu, J., O'Donnell, A.G., Syers, J.K., 1997. Seasonal responses in microbial biomass carbon, phosphorus, and sulphur in soils under pasture. *Biol. Fertil. Soils* 24, 421–428.
- Hornby, D., 1983. Suppressive soils. *Annu. Rev. Phytopathol.* 21, 65–85.
- King, L.D., Burns, J.C., Westerman, P.W., 1990. Long-term swine lagoon effluent applications on 'Coastal' bermudagrass: II. Effect on nutrient accumulation in soil. *J. Environ. Qual.* 19, 756–760.
- Larkin, R., Honeycutt, C., Griffin, T., 2006. Effect of swine and dairy manure amendments on microbial communities in three soils as influenced by environmental conditions. *Biol. Fertil. Soils* 43 (1), 51–61.
- Lovell, R.D., Jarvis, S.C., 1996. Effect of cattle dung on soil microbial biomass C and N in a permanent pasture soil. *Soil Biol. Biochem.* 28 (3), 291–299.
- Marschner, P., Kandeler, E., Marschner, B., 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biol. Biochem.* 35, 453–461.
- Parham, J.A., 2003. Long-term cattle manure application in soil II. Effect on soil microbial populations and community structures. *Biol. Fertil. Soils* 38, 209–215.
- Pérez-de-Mora, A., Burgos, P., Madejón, E., Cabrera, F., Jaekel, P., Schloter, M., 2006. Microbial community structure and function in a soil contaminated by heavy metals: effect of plants growth and different amendments. *Soil Biol. Biochem.* 38 (2), 327–341.
- Pierzynski, G.M., Thomas Sims, J., Vance, G.F., 2000. *Soils and Environmental Quality*. CRC Press, New York, 459 pp.
- Poore, M., Green, J., 1996. Forage systems utilizing swine effluent swine effluent: dream...or nightmare? In: *Proc. Southern Pasture and Forage Crop Imp. Conf.* 52. pp. 109–116.
- Pratt, R.G., 2006. Comparative survival of conidia of eight species of *Bipolaris*, *Curvularia*, and *Exserohilum* in soil and influences of swine waste amendments on survival. *Appl. Soil Ecol.* 31, 159–168.
- Ros, M., Hernandez, M.T., Garcia, C., 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biol. Biochem.* 35 (3), 463–469.
- Schnürer, J., Clarholm, M., Rosswall, T., 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biol. Biochem.* 17 (5), 611–618.
- Sharpley, A.N., Daniel, T., Sims, T., Lemunyon, J., Stevens, R., Parry, R., 1999. Agricultural phosphorus and eutrophication. U.S. Dept. Agric., Agr. Res. Service AR-149, 37.
- Steele, R.G.D., Torrie, J.H., 1960. *Principles and Procedures of Statistics*. McGraw-Hill, New York, 481 pp.
- Weller, D.M., 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* 26, 379–407.
- Whitehead, T.R., Cotta, M.A., 2004. Isolation and identification of hyper-ammonia producing bacteria from swine manure storage pits. *Curr. Microbiol.* 48, 20–26.