Chicken Litter Soil Amendment Effects on Soilborne Microbes and *Meloidogyne incognita* on Cotton

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ABSTRACT

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The effects of chicken litter incorporated 28, 14, and 0 days before planting on *Meloidogyne incognita* in cotton and soil organisms were determined in the greenhouse. Treatments consisted of field soil amended with litter at rates of 0, 0.125, 0.25, 0.5, and 1% by weight. At 45 and 90 days after planting, numbers of *M. incognita* decreased as rates of litter increased. Microbivorous nematode densities increased as litter rates increased only in the first experiment. Plant growth increased as litter rates increased, regardless of when the litter was incorporated, or the presence or absence of *M. incognita*. Bacterial and fungal CFU fluctuated during both experiments, but generally had positive linear relationships with litter rate. Population densities of *M. incognita* decreased with increasing bacterial and fungal counts in amended soil. Bacterial genera identified from the litter-amended soil included *Arthrobacter*, *Bacillus*, *Cellulomonas*, *Micrococcus*, *Pseudomonas*, and *Rhodococcus*.

Additional keywords: biological control, manure, organic amendment, root-knot nematode

Root-knot nematodes are widely distributed in the southeastern United States and are a serious threat to vegetable and field crop production. *Meloidogyne incognita* (Kofoid & White) Chitwood race 3 is an important nematode parasite of cotton (*Gossypium hirsutum* L.) (19). This nematode also plays a primary role in disease complexes with soilborne fungi (26). Management of root-knot nematodes on cotton consists of chemical and cultural control, and plant resistance where available (19). Management practices for nematodes are changing due to increased costs and restrictions on nematicide use (25). Alternative management practices utilizing biological control and organic amendments are currently being investigated to replace or supplement currently available management practices (20–23).

Animal manures have been used since the beginning of agricultural food production to improve soil fertility, recycle nutrients, improve biological and physical properties of soil, and increase crop yield (22,24,27). Research with animal manures has shown that they have nematode-suppressive properties (11,17). The mode of action, however, has not yet fully been determined. The essential role played by the microbial community in nematode

control has been demonstrated (11,20). The application of manure enhances soil fertility, aids in controlling plant-parasitic nematodes, and provides a means for disposing of the manure. Poultry is an important segment of agricultural production in Georgia, and the poultry litter generated by this industry will require improved disposal methods as environmental regulations become more limiting. Chicken manure is potentially an environmental contaminant of water. Disposal by spreading over large land areas is a desirable option.

A common form of poultry manure is chicken litter, which consists of manure and pine shaving bedding. Chicken litter contains significant quantities of N, P, K, Ca, Mg, and micronutrients and can be used as a substitute for commercial fertilizers (16,24). Research with chicken litter as an organic amendment has shown that application to soil will lower population densities of plant-parasitic nematodes (9,20). Suppression of nematodes by chicken litter is probably a combination of enhanced microbial activity and constituent toxicity. The majority of the nitrogen in poultry manure is in the form of uric acid that can be rapidly converted to ammonium nitrogen if temperature, pH, and moisture are suitable for microbial activity (24). The ammonia produced has been shown to kill plant-parasitic nematodes (7). In addition, the transformation of organic nitrogen into inorganic nitrogen is essential in order to supply nitrogen that can be readily utilized by the plant (24). The presence of pine shavings in litter serves as a carbon source and reduces phytotoxicity caused by the

accumulation of ammonia and nitrates (10).

Organic and mineral constituents of litter stimulate the development of populations of microorganisms (15,20) which may include nematode antagonists or cause the proliferation of microbial species capable of producing substances toxic to nematodes (1). Incorporation of organic amendments into soils also has been shown to increase microbivorous nematode populations (15,17). Pine shavings also can stimulate the microbial community (20,21).

Timing of applications of litter may be important in the control of plant-parasitic nematodes, in nitrogen availability to the plant, and possible phytotoxic effects. An incubation period longer than 1 week was necessary between litter incorporation at a high rate and planting (20). However, at lower rates, an incubation period was not necessary. It is important to apply litter at rates in which nutrient uptake is maximized by the plant and nutrient leaching is minimized.

The objectives of this study were to investigate the effects of rate and time of application of chicken litter on population levels of *M. incognita* in cotton; to identify relationships among nematode, bacterial, and fungal, population densities in soil; and to identify bacteria from soil amended with chicken litter.

MATERIALS AND METHODS

Chicken litter (chicken excrement and pine shaving bedding) was collected from a commercial broiler house in Oconee County, GA. Litter was collected from pens which were cleaned approximately once a month. Nitrogen analysis of the litter was performed by the Georgia Cooperative Extension Service Soil Testing Laboratory. Total N was extracted by persulfate digestion (18). Sandy-loam field soil (72% sand, 16% clay, 12% silt) was collected from the Plant Sciences Farm in Oconee County, GA. The soil was assayed to confirm the absence of root-knot nematodes and screened through a 2-cm sieve to remove rocks and debris. Soil and litter amendments at rates of 0, 0.125, 0.25, 0.5, and 1% litter by weight of soil (wt/wt) were mixed thoroughly for 15 min in a cement mixer and placed in 15-cm-diameter plastic pots 28, 14, and 0 days before planting. Pots were arranged on greenhouse benches in a randomized complete block design with four replications. Pots

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were inoculated by pipetting 12 ml of a suspension containing 5,000 eggs of *M. incognita* race 3 in the first trial and 40,000 eggs in the second trial. The eggs were pipetted into three trenches in the amended soil 28, 14, and 0 days before planting with cotton cv. DPL50. Nematode controls consisting of the same litter rates without *M. incognita* were set up on the same dates for assessment of plant growth effects. Pots that did not receive nematode inoculation were not used in the analyses of nematode-soil microbe interactions. At 45 and 90 days after planting, nematodes were extracted from 100-cm³ soil samples by elutriation and sucrose centrifugation. (2,3). Roots washed from soil samples were placed on funnels in an intermittent-mist chamber to collect nematodes that emerged from the roots (2). Ninety days after planting, cotton stems were cut at the soil line and the roots were removed from

the pots and washed free of soil. Fresh shoot, dry shoot (72 h at 60°C), and fresh root weights were recorded. Nematode eggs were collected from each root system with a 1% sodium hypochlorite (NaOCl) solution and counted (4). *M. incognita* second-stage juveniles (J2) and microbivorous nematodes were extracted from soil with elutriation and centrifugation (2,3) and counted.

Bacterial and fungal population densities were assayed 0, 45, and 90 days after planting. Counts of bacteria and fungi were determined by serially plating the soil-litter suspensions and controls onto selective media in 9-cm-diameter petri dishes with a Spiral Plater (Spiral Systems, Bethesda, MD). Soil (2 g) was weighed and placed into a whirlpac blender bag with 18 ml of deionized sterile water for the first dilution. Each sample then was placed in a Stomacher blender (Seward Medical, Lon-

don) and agitated for 30 s. Further dilutions were made in a laminar flow hood and the suspensions were plated onto selective media for microbial enumeration.

For fungal isolation, potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) amended with streptomycin (0.1 g/liter) and tetracycline (0.01 g/liter), and PDA amended with streptomycin (0.1 g/liter) and chloramphenicol (0.01 g/liter), were used. Nutrient agar with nystatin (0.05 g/liter) was used for isolating bacteria. Serial dilutions were made to 10⁻³ for fungi and 10⁻⁵ for bacteria. Bacterial CFU were counted after 3 days and fungal CFU were counted 4 to 5 days after incubation at 25°C in the dark.

After isolation, bacteria were stored at -80°C. Before storing, the bacteria were placed on a shaker at room temperature in 2-ml cryovials with 0.75 ml of trypticase soy broth (TSB) for 24 to 48 h. Each vial

Fig. 1. Response of *Meloidogyne incognita* counts 45 days after planting to increasing rates of litter. (A) In the first experiment, the response of $\log_e M. incognita$ (second-stage juveniles [J2] + 1) to increasing rates of litter was described by a linear model for litter incorporation 14 days before planting, $Y = 3.6 - 3.0X$, $R^2 = 0.41$, $P = 0.01$, and litter incorporated at planting; $Y = 5.3 - 2.5X$, $R^2 = 0.29$, $P = 0.05$. (B) In the second experiment, the response of $\log_e M. incognita$ (J2 + 1) decreased with increasing rates of litter incorporated 14 days before planting, $Y = 5.2 - 3.4X$, $R^2 = 0.40$, $P = 0.05$. For litter incorporation at planting, $\log_e M. incognita$ (J2 + 1) was best described by a quadratic model, $Y = 6.2 + 6.7X - 18.5X^2$, $R^2 = 0.55$, $P = 0.01$.

Fig. 2. Response of $\log_e M. incognita$ (second-stage juveniles [J2] + 1) population density to increasing rates of litter. (A) For litter incorporation 14 days before planting in the first experiment, $\log_e M. incognita$ (J2 + 1) showed a negative linear relationship to increasing litter rate, $Y = 4.6 - 3.2X$, $R^2 = 0.38$, $P = 0.01$. (B) In the second experiment, 90 days after planting, the relationship between $\log_e M. incognita$ (J2 + 1) and increasing litter rates incorporated 28 and 14 days prior to planting was described by quadratic models, $Y = 4.9 - 2.1X - 1.5X^2$, $R^2 = 0.52$, $P = 0.01$; and $Y = 5.4 - 6.0X - 13.2X^2$, $R^2 = 0.53$, $P = 0.01$, respectively.

received 0.25 ml of sterilized glycerol and then was placed at -80°C . Bacteria selected for identification based on colony morphological differences were transferred to a 5% trypticase soy agar (TSA; Difco Laboratories) for purification and fatty acid methyl-esters identification (FAME; 5,8,12).

The experiment was performed twice. Except where otherwise noted, all differences reported in the results were significant at the $P \leq 0.05$ level. Regression analysis was used to determine nematode responses to litter application, microbial population dynamics during the study, and plant growth response to the nematode and to litter amendments. Data also were analyzed by analysis of variance (ANOVA) and means were separated by Duncan's multiple-range test (SAS/STAT User's Guide, SAS Institute, Inc., Cary, NC). Nematode counts were transformed with $\log_e (X + 1)$ before analysis.

RESULTS

Significant interactions were observed between main effects and experimental runs for nematode population data; therefore, data were not combined across experiments for analysis. Interactions were observed in the significance of nematode responses to soil microbial counts from different litter application dates and nematode assay dates. Litter samples contained 3.17% total N in the first experiment and 5.18% total N in the second experiment.

In the first experiment, at 45 days after planting, the population density of *M. incognita* decreased in response to increasing rates of litter incorporated 2 weeks before planting and at planting (Fig. 1A). The mean nematode number decreased from 30 J2 per 100 cm^3 of soil to 1 J2 per 100 cm^3 of soil in the 1% rate where litter was applied 2 weeks before planting. Nematode population densities decreased from 310 J2 per 100 cm^3 of soil in the control to 1 J2 per 100 cm^3 of soil in the 1% rate where litter was applied at planting. Nematode population densities tended to be higher in the 0.125% litter rates in both experiments. No significant relationships between *M. incognita* density and litter rate were observed in the first experimental run when litter was incorporated 28 days before planting.

In the second experimental run, nematode counts at 45 days after planting decreased linearly with litter rate where litter was incorporated 14 days before planting (Fig. 1B). Decreased plant growth, compared with nonamended controls, was observed at the 1% rate of litter when incorporated at planting. Numbers of *M. incognita* decreased only at the 0.5% litter rate, whereas nematode population densities increased to levels above the control in the 0.125 and 0.25% litter treatments (Fig. 1B).

At 90 days after planting, in the first experimental run, a quadratic relationship was observed between numbers of *M. incognita* and litter rate incorporated 14 days before planting (Fig. 2A). No other significant relationships between litter rate and nematode number were observed at the other incorporation dates in the first experimental run. In the second experimental run, quadratic models best described the relationship between nematode counts and increasing litter rates at 14 and 28 days before planting (Fig. 2B), where a negative response to litter was observed for nematode counts in all rates except 0.125%.

At 90 days after planting, a quadratic relationship was observed between numbers of *M. incognita* eggs and increasing litter rate for incorporation 14 days before planting (Fig. 3). In the second experimental run, a quadratic model also best described the relationship between egg density and litter rate for litter incorporation 14 and 28 days before planting (Fig. 3). In both experiments, the 0.125% litter rate had higher numbers of nematodes than the nonamended control.

In *M. incognita*-infested soil, microbivorous nematode density 90 days after planting in the first experiment had a positive linear relationship with litter rate for litter incorporation 14 days before planting (Fig. 4A). Microbivorous nematode densities increased from 415 nematodes per 100 cm^3 of soil in the nonamended control to 1,291 nematodes per 100 cm^3 of soil in the 1% litter treatment. A quadratic model described the relationship between population

densities of microbivorous nematodes and litter rate when incorporated at planting (Fig. 4A), where the microbivorous nematode densities increased 311% from the nonamended control to the 1% litter treatment. Significant relationships between litter rate and microbivorous nematodes 90 days after planting were not observed for litter incorporation 28 days before planting in the first experiment. Ninety days after planting in the second experimental run, negative linear relationships were observed between microbivorous nematode counts and litter rate for litter incorporation 28 and 14 days before planting (Fig. 4B). Significant regressions for numbers of microbivorous nematodes with rates of litter incorporated at planting were not observed in the second experimental run.

Dry shoot weights in *M. incognita*-infested soil in the first experiment had a positive response to increasing litter rates (Fig. 5A). Shoot weights from the 1% litter treatments were 68, 63, and 80% greater than the nonamended controls for incorporation 28 and 14 days before planting and at plant, respectively. Dry shoot weights in the second experiment showed similar responses to increased litter rates (Fig. 5B).

Dry shoot weight also had a strong positive response to litter in soil without *M. incognita*. In the first experiment, a linear response for shoot weight to increasing litter rates was observed with regression models of $Y = 13.0 + 20.2X$, $R^2 = 0.80$, $P = 0.0001$; $Y = 13.2 + 21.7X$, $R^2 = 0.69$, $P = 0.0001$; and $Y = 14.8 + 15.6X$, $R^2 = 0.49$, P

Fig. 3. In the first experiment, 90 days after planting, the relationship between $\log_e$ *Meloidogyne incognita* (eggs + 1) and increasing litter rate in treatments with litter incorporated 14 days before planting, $Y = 10.2 - 7.3X + 3.6X^2$, $R^2 = 0.41$, $P = 0.05$; and, in treatments with litter incorporated 28 days before planting in the second experiment, $Y = 8.9 + 1.0X - 5.3X^2$, $R^2 = 0.58$, $P = 0.001$, were described by quadratic models.

= 0.001 at 28, 14, and 0 days before planting, respectively. A similar positive response for shoot weights to increasing litter rates in soil without *M. incognita* was observed in the second experiment (*data not shown*).

In the first experiment, fresh root weight had a positive relationship with litter rate when incorporated in the soil 28 and 0 days before planting in *M. incognita*-infested soil, with regression models of $Y = 8.32 + 41.13X - 29.11X^2$, $R^2 = 0.51$, $P = 0.01$; and $Y = 8.25 + 9.75X$, $R^2 = 0.37$, $P = 0.01$, respectively. Fresh root weight decreased slightly at the 1% litter rate, compared with the 0.5% rate, for incorporation 28 days before planting. In the second experiment, fresh root weight showed a positive linear relationship with litter rate only when the litter was incorporated at planting ($Y = 10.42 + 39.25X$, $R^2 = 0.37$, $P = 0.05$). No relationship was observed between root weights and litter rates in the absence of

M. incognita for incorporation at planting in the first experiment and at all three incorporation dates in the second experiment (*data not shown*).

Bacteria identified from the litter amended soil included *Arthrobacter aurescens*, *A. globiformis*, *A. ilicis*, *A. pascens*, *A. saperdae*, *Bacillus atrophaeus*, *B. subtilis*, *B. macerans*, *B. megaterium*, *B. pumilus*, *B. polymyxa*, *Cellulomonas fimi*, *Micrococcus kristinae*, *M. luteus*, *Pseudomonas chlororaphis*, *P. corrugata*, *P. putida*, *P. syringae*, *P. vesicularis*, and *Rhodococcus equi*. Species of *Arthrobacter*, *Bacillus*, and *Pseudomonas* were the most numerous species identified. Fungal and bacterial densities increased at 0, 45, and 90 days after planting as litter rate increased for all three dates of litter incorporation in the first experiment (Table 1). Relationships varied among day of litter incorporation and time of sampling. In the second experiment, similar trends were

observed as bacterial and fungal counts had positive relationships with litter rates throughout the length of the experiment, except that no significant relationships were observed for litter incorporation 28 days before planting (*data not shown*).

In the first experimental run, *M. incognita* population densities had only negative relationships with fungal and bacterial counts for litter incorporated at planting (Table 2). Numbers of *M. incognita* assayed at 45 and 90 days after planting decreased as bacterial and fungal numbers enumerated at 45 days increased. In the second experiment, both positive and negative relationships were observed between *Meloidogyne incognita* population densities and bacterial and fungal counts (Table 2). Nematode numbers were positively related to microbial population densities for litter incorporation 28 days before planting but were negatively related for litter incorporation at 14 days before

Fig. 4. Response of microbivorous nematodes to increasing rate of litter. (A) In the first experiment, the number of microbivorous nematodes per 100 cm³ of soil increased with litter rate when litter was incorporated 14 days prior to planting, $Y = 612 + 703X$, $R^2 = 0.21$, $P = 0.05$. When litter was incorporated at planting, the relationship between log_e (microbivorous nematodes + 1)/100 cm³ and litter rate was described by a quadratic model, $Y = 5.6 + 2.9X - 2.1X^2$, $R^2 = 0.32$, $P = 0.05$. (B) In the second experiment, 90 days after planting, microbivorous nematodes per 100 cm³ showed a negative linear relationship with litter rate when incorporated 28 days before planting, $Y = 7.7 - 0.7X$, $R^2 = 0.23$, $P = 0.05$, and when incorporated 14 days before planting, $Y = 7.1 - 0.8X$, $R^2 = 0.20$, $P = 0.05$.

Fig. 5. (A) Dry shoot weight in the first experiment increased with increasing litter rate when litter was incorporated 28 days before planting, $Y = 12.3 + 22.0X$, $R^2 = 0.68$, $P = 0.0001$; litter incorporated 14 days before planting, $Y = 12.3 + 17.5X$, $R^2 = 0.46$, $P = 0.001$; and litter incorporated at plant, $Y = 7.2 + 19.6X$, $R^2 = 0.73$, $P = 0.0001$. (B) In the second experiment, dry shoot weight increased with increasing rates of litter when litter incorporation occurred 14 days before planting, $Y = 6.9 + 41.0X$, $R^2 = 0.73$, $P = 0.0001$; and litter incorporated at plant, $Y = 1.5 + 25.8X$, $R^2 = 0.78$, $P = 0.0001$.

planting. Microbivorous nematode population densities increased as bacterial and fungal numbers increased for both experimental runs (*data not shown*).

M. incognita population densities at 45 and 90 days after planting were affected by the rate of litter and the date at which litter was incorporated, but there was no interaction between these effects (Table 3). At 90 days after planting, *M. incognita* egg numbers were affected by the time of litter incorporation before planting but not by application rate. Bacterial population densities were influenced solely by the rate of litter incorporated into the soil (Table 4). Fungal counts, however, were affected by rate and time of litter application and by the date of assay. Further, a significant interaction was observed between litter rate and the date of assay for fungal counts, indicating that the rate response detected depended on the date of observation.

DISCUSSION

Regression models from this work showed that *M. incognita* population densities decreased in response to increasing rates of chicken litter soil amendment, and

that population counts of bacteria and fungi increased in response to increasing litter applications. As a result of these inverse relationships, *M. incognita* numbers generally decreased in response to increasing counts of bacteria and fungi. This response may have been partially due to covariation in the counts in response to increasing litter rates, but there also may have been direct antagonism to *M. incognita* among certain of the microbial species enumerated. The addition of organic amendments to the soil has previously been shown to increase microbial diversity (14,15). With this increase in microbial diversity and numbers, it becomes more likely that bacteria and fungi capable of parasitizing specific nematode life stages or producing substances toxic to nematodes may be involved in the resulting nematode control (1). Soil was artificially infested with nematode inoculum in this greenhouse-pot study, and it is likely that a different community of biota would be present in naturally infested field soil. Our study is directed toward an evaluation of the effects of chicken litter and microbes associated with the litter amendments on population

densities of *M. incognita*. The relationships among nematode population densities and numbers of microbes may be different in a natural soil environment.

Bacteria identified from the litter-amended plots were species that have commonly been found in soil. Bacteria are quite diverse in nutritional requirements, and several species identified in the genera *Arthrobacter*, *Bacillus*, *Cellulomonas*, and *Micrococcus* have the ability to use ammonium salts or nitrates as their sole source of nitrogen (13). Poultry litter has high levels of nitrogen in the organic and inorganic forms (6,24) that would be readily available to these organisms. Others bacteria, such as *B. macerans* and *B. polymyxa*, use ammonia to fix N₂ under anaerobic conditions (6). In addition, the genus *Cellulomonas* has been isolated from substrates high in cellulose and has the ability to reduce nitrate to nitrite (6). Chicken litter is composed of chicken excrement and pine shaving bedding, which would provide both cellulose and nitrogen to *Cellulomonas* spp.

Nematode inoculum was increased eightfold in the second experiment because *Meloidogyne incognita* population densities were low in the first experiment. However, this higher inoculum rate did not result in higher nematode numbers at 90 days after planting when litter was incorporated 28 or 14 days before planting. *M. incognita* is an obligate parasite, but J2 can survive in a quiescent state for a period limited by the extent of their food reserves. Thus, even with the high inoculum level in the second experiment, a large proportion of the nematodes in treatments with litter incorporated 28 and 14 days before planting died before a susceptible host was available. This may be representative of early-season field applications of litter, where the treatment was applied before planting a susceptible host for *M. incognita*.

Table 1. Regression models of the linear relationship between bacterial and fungal CFU per gram of soil and increasing litter rate in soil amended with litter and infested with *Meloidogyne incognita* in the first experiment

DAP ^a	Date ^b	Independent variable ^c	Intercept	Slope	R ²	P
0	28	Bacteria	15.90	2.89	0.86	0.0001
	14	Fungi	11.73	1.03	0.29	0.05
	0	Bacteria	16.29	1.79	0.81	0.0001
45	28	Bacteria	16.57	0.63	0.22	0.05
	28	Fungi	11.85	0.98	0.50	0.001
	14	Fungi	11.73	1.03	0.29	0.05
	0	Bacteria	16.28	0.98	0.63	0.001
	0	Fungi	11.79	0.84	0.28	0.05
90	28	Fungi	11.85	0.98	0.50	0.001
	14	Fungi	11.83	1.02	0.44	0.01

^a DAP = days after planting.

^b Date of litter incorporation before planting.

^c Bacterial counts log × 10⁷ CFU per gram of soil and fungal counts log × 10⁵ CFU per gram of soil.

Table 2. Regression models describing the relationship between *Meloidogyne incognita* counts and bacterial and fungal CFU per gram of soil in the first and second experiments

Experiment, date ^a	Days after planting		Microbe ^b	Intercept	Slope	<i>R</i> ²	<i>P</i>
	Microbial enumeration	<i>M. incognita</i> counts					
First experiment, J2 ^c							
0	45	45	Fungi	18.05	−1.30	0.32	0.05
0	45	90	Bacteria	38.38	−2.07	0.29	0.05
Second experiment, J2							
28	45	45	Bacteria	−4,245	264.4	0.26	0.05
28	90	45	Bacteria	−5,820	355.5	0.31	0.05
28	90	45	Fungi	−11,551	963.1	0.27	0.05
14	90	45	Fungi	3,832	−291.8	0.30	0.05
14	45	45 ^c	Bacteria	27.14	−1.34	0.36	0.05
14	90	45 ^c	Bacteria	35.3	−1.81	0.44	0.01
Eggs ^d							
14	45	90	Bacteria	16.7	−0.90	0.53	0.01
14	45	90	Fungi	15.14	−1.11	0.33	0.05
14	90	90	Bacteria	18.2	−0.98	0.43	0.01
14	90	90	Fungi	1.8	−0.13	0.34	0.05

^a Date of litter incorporation before planting; J2 = second-stage juveniles. The 1% litter rate was removed from analyses due to phytotoxicity.

^b Bacterial counts log × 10⁷ CFU per gram of soil and fungal counts log × 10⁵ CFU per gram of soil.

^c J2 counts were log transformed for analysis.

^d Eggs counts × 10⁴.

A statistical interaction was observed among main effects and experimental run. A part of this interaction resulted from models that were significant for specific litter application and observation dates in one run but not in the other experimental run. Chicken litter varies in the stage of decomposition and source composition. This variability will be a limiting factor in the calculation of optimal application rates for growers. Time-related effects of chicken litter amendment varied from one run to the next in this study. This dynamic characteristic of the media was part of the research design, where litter was incorporated at different times before planting and assays were conducted at several intervals. Although specific time-related responses differed, there was a general trend toward lower population densities of *M. incognita* at higher rates of chicken litter, and with higher microbial population densities. Also, data presented for each experimental run showed that application of chicken litter 14 days before planting was optimal for effects on nematode population densities, compared with application 28 days before planting, and for limiting the degree

of phytotoxicity, compared with application at planting.

Fresh and dry shoot weight and root weight all were stimulated by the addition of litter, with or without nematode infestation. Agronomic studies on the plant growth-enhancing effects of poultry manures have shown benefits from manure applications in the absence of plant-parasitic nematodes (24,27). The rate at which dry shoot weight increased in response to increasing rates of litter in *M. incognita*-infested and noninfested soil were nearly identical. Chicken litter is a nutrient rich medium, containing significant quantities of N, P, K, and micronutrients (6,24). The litter rates used in this study, of 0.125, 0.25, 0.5, and 1% wt/wt, were equivalent to 5, 10, 21, and 42 metric tons/ha, respectively, based on dry weight of soil to a depth of 20 cm. High nitrogen contents in the forms of ammonia, nitrate, and nitrite can lead to a reduction in seed germination or a decrease in emergence (24). Decreased plant growth was observed in the 1% litter rate treatments incorporated at planting, compared with nonamended pots, possibly because of the high nitrogen content of the

litter. Incorporation before planting was generally found to be beneficial, with 2 weeks before planting apparently an optimum time of litter incorporation. Application of litter at a rate and time which maximizes nematode control, increases soil fertility, and minimizes phytotoxicity or environmental hazards, will be an important consideration in management.

These results demonstrated that chicken litter soil amendments decreased population densities of *M. incognita* and partially elucidated the effects of these amendments on other microbial populations. Regression relationships further indicated that increases in soilborne microbial populations may have an essential role in nematode-suppressive mechanisms. More detailed studies of microbial population dynamics in litter-amended soils are needed to identify the specific roles that fungi and bacteria may have in nematode control. The use of poultry litter as a nematode management tool would be most efficient when used in combination with other management practices that are currently available. Litter, if used appropriately, can be substituted for mineral fertilizers. Use of such organic amendments would not only enhance soil fertility, but also would increase microbial diversity, decrease population densities of root-knot nematodes, and ultimately increase crop yields.

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Table 3. Effects of chicken litter application rates and time of application on *Meloidogyne incognita* population densities

Source ^a	df	SS ^b	MS	F	Pr > F
J2 45					
Rate	3	28.5	9.5	3.01	0.0348
Day	2	132.1	66.1	20.94	0.0001
Rate × day	6	6.42	1.07	0.34	0.9141
J2 90					
Rate	4	51.5	12.9	4.60	0.0021
Day	2	58.9	29.4	10.53	0.0001
Rate × day	8	12.1	1.5	0.54	0.8240
Eggs ^c					
Rate	4	15.3	3.2	0.69	0.5983
Day	2	110.4	55.2	10.04	0.0001
Rate × day	8	30.8	3.8	0.70	0.6909

^a J2 45 and J2 90 = *M. incognita* second-stage juveniles 45 and 90 days after planting, respectively; log-transformed *M. incognita* J2 counts per 100 cm³ of soil. Day = date of litter incorporation days before planting. Litter was incorporated at rates of 0, 0.125, 0.25, 0.5, and 1% by weight of soil at 28 and 14 days before planting, and at planting.

^b SS = sum of squares; MS = mean square; Pr = probability of a greater F value.

^c Log transformed *M. incognita* eggs per pot 90 days after planting.

Table 4. Effects of chicken litter amendment rates, time of application, and date of assay on bacterial and fungal population densities

Source ^a	df	SS ^b	MS	F	Pr > F
Bacterial counts					
Litter rate	3	37.48	12.48	28.03	0.0001
Day ^c	2	0.58	0.29	0.65	0.5207
Sampling date ^d	2	1.52	0.76	1.71	0.1826
Litter rate × sampling date	6	3.95	0.66	1.48	0.1864
Fungal counts					
Litter rate	3	14.2	4.74	6.07	0.0005
Day	2	12.9	6.44	8.26	0.0003
Sampling date	2	15.6	7.78	9.97	0.0001
Litter rate × sampling date	6	19.9	3.30	4.24	0.0004

^a Bacterial counts log × 10⁷ CFU per grams of soil and Fungal counts log × 10⁵ CFU per grams of soil.

^b SS = sum of squares; MS = mean square; Pr = probability of a greater F value.

^c Sampling date for microbes.

^d Date of litter incorporation before planting. Litter was incorporated at rates of 0, 0.125, 0.25, 0.5, and 1% by weight of soil at 28 and 14 days before planting and at planting.

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