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Soil Biology & Biochemistry 39 (2007) 2302–2316

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Long-term effects of organic and synthetic soil fertility amendments on soil microbial communities and the development of southern blight

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Received 2 July 2006; received in revised form 16 March 2007; accepted 12 April 2007
Available online 7 May 2007

Abstract

The effects of tillage and soil fertility amendments on the relationship between the suppressiveness of soils to southern blight and soil physical, chemical and biological factors were examined in experimental station plots in North Carolina. Main plots were either tilled frequently or surface-mulched after one initial tillage. Organic soil amendments including composted cotton gin trash, composted poultry manure, an incorporated rye–vetch green manure, or synthetic fertilizer were applied to subplots in a split-plot design experiment. Incidence of southern blight was lower in surfaced-mulched than tilled soils. Incidence of southern blight was also lower in soils amended with cotton gin trash than those amended with poultry manure, rye–vetch green manure or synthetic fertilizer. Soil water content was negatively correlated with the incidence of disease in both years. Disease incidence was negatively correlated with the level of potassium, calcium, cation exchange capacity (CEC), base saturation (BS) and humic matter in 2002, and net mineralizable nitrogen in 2001. Although, populations of thermophilic organisms were significantly higher in soils amended with cotton gin trash than the other three fertility amendments in each year, there was no significant correlation between the populations of thermophiles and incidence of the disease. Bacterial community diversity indices based on community-level physiological profiling (CLPP) and denaturing gradient gel electrophoresis (DGGE) were significantly higher in soils amended with cotton gin trash than those amended with poultry manure, green manure or synthetic fertilizer. There was a significant negative correlation between the incidence of southern blight, and CLPP and DGGE diversity indices. Greater differences in the richness of bacterial functional groups than genotypes were observed. These results demonstrate that organic soil fertility amendments and cotton gin trash in particular, reduced the development of the disease and affected soil physical, chemical and biological parameters.
Published by Elsevier Ltd.

Keywords: *Sclerotium rolfsii*; Soil physical, chemical and biological parameters; Organic and synthetic fertility amendments; Disease; Microbial communities; BIOLOG; DGGE

1. Introduction

Sclerotium rolfsii Sacc. is a soil-borne plant pathogen responsible for significant economic losses on a wide range of agronomic host plants that include 500 plant species in over 100 plant families (Punja, 1985). This pathogen can cause severe disease on tomato (*Lycopersicon esculentum* Mill.), leading to major economic losses. The pathogen infects all aboveground portions of the plant in contact with the soil and sclerotia can remain viable for many years and provide the primary inoculum for epidemics (Gurkin and Jenkins, 1985; Punja, 1985). Control of *Sclerotium* diseases is difficult and depends on a combination of chemical, cultural and biological approaches (Munnecke et al., 1982; Punja, 1985, Ristaino et al., 1991).

Various chemicals, including fumigants such as methyl bromide, chloropicrin, or metam sodium, have been used to control southern blight. However, these chemicals are restricted-use products, and their toxicity and cost limit the usefulness in many situations. In addition, application is not practical in many field situations because of the large quantities of chemicals required and effectiveness is not always consistent from year to year (Gorodicki and Hadar,

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0038-0717/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.soilbio.2007.04.001

1990; Munnecke et al., 1982). Farmers are under increasing pressure to reduce pesticide use and implement sustainable crop protection practices. Integrated pest and disease management strategies that employ manipulation of cultural practices in combination with reduced quantities of pesticides are commonly used to help achieve optimal crop yield and quality (Hadar and Gorodecki, 1991). Organic soil amendments not only influence soil physical and chemical properties but also impact soil biological communities (Canullo et al., 1992; Knudsen et al., 1999; Kundu and Nandi, 1985; Trankner, 1992; van Elsas et al., 2002).

Organic soil amendments, including composted or uncomposted plant residues, animal manures and green manure, have widely different effects on the balance of soil microflora and plant diseases depending on the nature of the residue and the method of preparation (Abbasi et al., 2002; Craft and Nelson, 1996; Davis et al., 1994; Hadar and Gorodecki, 1991). The addition of plant residues to soil in general improves soil structure and soil health (Bending et al., 2000; Doran et al., 1996; Garbeva et al., 2004a, b; van Bruggen and Semenov, 2000). Healthy soils have improved plant growth and less disease caused by plant pathogens and pathogenic nematodes (Abawi and Idmer, 2000). For example, damping-off of radish and lesion development caused by *Rhizoctonia solani* was reduced in field experiments when manure was added to soils compared to non-amended controls (Gorodicki and Hadar, 1990; Voland and Epstein, 1994). Similarly, fresh chicken manure has been shown to reduce survival of *Phytophthora cinnamomi* and the incidence of disease on *Lupinus albus* seedlings (Aryantha et al., 2000). However, the impact of animal manures on disease incidence and severity is much less predictable than that of plant composts, and incorporation of manures to soils can sometimes increase the incidence and severity of disease (Aryantha et al., 2000). A limited number of systematic studies have been carried out in field. Bulluck et al. (2002) examined the effects of composted cotton gin trash on the incidence of southern blight of tomatoes. Disease incidence was lower in soils amended with composted cotton gin trash than soils with swine manure, green manure or synthetic fertilizer. Lewis et al. (1992) also found that amendment of field plots with composted sewage sludge significantly reduced the incidence of damping-off of pea caused by *Pythium ultimum*.

The long-term effects of soil management history on microbial communities are still poorly understood. Incorporation of organic amendments has been shown to increase soil microbial activity (Elliott and Lynch, 1994; Liu and Ristaino, 2003), microbial diversity (Girvan et al., 2004; Grayston et al., 2004), bacterial densities (van Bruggen and Semenov, 2000), fluorescent *Pseudomonas* spp., and pathogenic bacteria, fungi, and nematodes (Abawi and Idmer, 2000; Bending et al., 2000; Boehm et al., 1993). Some experiments have compared organic and conventional agricultural systems through examining their effects on soil microbial biomass, microbial activity and substrate utilization and documented a higher microbial biomass in plots with organic amendments (Gelsomino et al., 2004; Hu et al., 1997). Sustained increases in microbial biomass resulting from high organic matter inputs have also been observed in organic and low-input systems (Bossio et al., 1998). Clegg et al. (2003) studied the impact of long-term grassland management regimes with nitrogen-fertilizer application and soil drainage on microbial community structure using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and phospholipid fatty acid (PLFA) profiling. They found that nitrogen fertilizer exerted a significant impact on the total bacterial and actinomycete community structures, whereas soil drainage had a significant impact on the actinomycete and pseudomonad communities. McCaig et al. (1999) compared bacterial communities in grasslands under different management regimes. DGGE profiles demonstrated that there were clear differences among the three grassland types. Research conducted by Palmer and Young (2000) also showed clear effects of soil management regime on rhizobial diversity in soil. Higher diversity of *Rhizobium leguminosarum* was measured in arable soil than in grassland soil. In addition, recent studies demonstrated that plough depth can influence soil bacterial activity (Gelsomino et al., 2004; Lupwayi et al., 1998).

Soil bacteria are critical to the maintenance of soil function in both natural and managed ecosystems because of their involvement in the key processes such as soil structure formation; decomposition of organic matter; toxin removal; and the C and nutrient cycling (Doran et al., 1996; Magdoff and Es, 2000). In addition, some bacteria play key roles in suppressing soil-borne plant diseases and in promoting plant growth (Benhamou and Chet, 1996; Newsham et al., 1995; Garbeva et al., 2004a, b; van Bruggen and Termorshuizen, 2003; Sall et al., 2006). Many soil organisms have overlapping physiologies, and loss in function due to one or a few species can be quickly compensated by other species (Coleman et al., 1994; Kennedy and Smith, 1995). Nevertheless, this does not mean that soil biodiversity is insignificant in terms of mediating nutrient cycling and other soil functions. It is widely believed that soil biodiversity, as displayed by the complexity of the soil food web, is essential for resistance, resilience and recovery of soil functions to environmental perturbation in the short-term (van Bruggen and Semenov, 2000). Much of this research is still very preliminary and it is not known whether functional or species diversity plays a more important role in suppressiveness to soil-borne pathogens.

Many of the sandy coastal plain soils in eastern North Carolina used for vegetable production are low in organic matter and the use of composted animal wastes or plant derived composts and mulches could increase the organic matter content of the soil (Bulluck and Ristaino, 2002). Organic growers in the southeast utilize a variety of soil fertility amendments including animal manures, cover

crops and composted plant debris to improve soil quality. Soil fertility amendments and surface mulch are incorporated each season into soils and yearly applications can change the physical, chemical, and biological characteristics of soils (Tu et al., 2005). However, little is known about the impact of long-term applications of organic matter in the field on soil microbial communities and the development of southern blight of tomato.

We conducted a long-term study examining the impact of tillage and amendments on soil microbial interactions. Our objectives were to determine the impact of tillage and long-term organic and synthetic soil fertility amendments on soil microbial communities and the development of southern blight and yield processing in tomato. Soil physical, chemical and biological factors including specific microbial populations, bacterial functional and species diversity were also assessed to determine their relationship to disease development.

2. Materials and methods

2.1. Experimental design

The research was conducted at the Horticultural Crops Research Station (HCRS) in Clinton, NC in 2001 and 2002. The soil was an Orangeburg sandy loam with 770 g kg^{-1} sand, 170 g kg^{-1} silt and 60 g kg^{-1} clay. The pH was 5.6 and organic matter (OM) was 5.0 g kg^{-1}. The field plots were artificially infested with sclerotia of *S. rolfsii* (1988–1990) and the soil contained sclerotia of the pathogen produced on previous tomato crops (Ristaino et al., 1991).

Soil was amended each spring (1997–2002) in a series of experimental plots to develop contrasting organic and conventional soil fertility treatments. No pesticides were used in any of the plots. The field plots were initially established in 1996 by planting a fall cover crop. The experimental design was a randomized split plot. The frequency of tillage during the growing season differed between the main plot treatments (2 levels) and included either tillage on bare soil or surface mulch application with wheat straw after a single initial tillage. Main plots that receive tillage were tilled weekly (4–5 times per season) until plants were too large for a tractor to clear. In the surface mulched plots, wheat straw was applied 2 weeks after transplanting to the soil surface. The surface mulch was incorporated into plots each fall.

The plots were arranged in four blocks. Soil fertility amendments were applied in the subplots (4 levels) and included either a conventional synthetic fertilizer (10–10–10 applied at 67 kg N ha^{-1}, and 45 kg N ha^{-1} at first flowering), or organic amendments including a composted cotton gin trash (applied at 62,250 kg DW ha^{-1}), composted poultry manure (28,000 kg DW ha^{-1}), or an incorporated rye–vetch green manure. Winter rye was broadcast seeded at a rate of 56 kg ha^{-1} and hairy vetch at a rate of 28 kg ha^{-1} every fall and mowed and incorporated to a depth of 30 cm each spring.

Rates of each soil amendment were standardized to obtain approximately 112 kg plant-available nitrogen per hectare. The inputs of extractable carbon varied in the plots and were published previously (Tu et al., 2005). Synthetic fertilizer consisted of a 10–10–10 (NPK) formulation of NH_4NO_3 (10% plant-available nitrogen), P_2O_5 (10% plant-available phosphorus) and K_2O (10% plant-available potassium). Composted cotton gin trash was obtained from Cotton Ginning and Sales in Goldsboro, NC. The cotton gin trash contained 3.67 kg/ton plant-available nitrogen, 1.04 kg/ton phosphate, 1.77 kg/ton calcium and 0.11 kg/ton sodium, 0.33 kg/ton magnesium, 0.33 kg/ton sulfur and 0.24 kg/ton iron based on wet weight. Composted poultry manure was obtained from North Carolina State University Waste Management Facility, Raleigh, NC. The poultry manure contained 8.66 kg/ton plant-available nitrogen, 8.26 kg/ton phosphate, 9.93 kg/ton calcium and 0.10 kg/ton sodium. The other nutrients available were 0.82 kg/ton magnesium, 1.77 kg/ton sulfur and 0.24 kg/ton iron based on wet weight. Swine manure was used in the animal manure plots in 1997 to 1999, whereas composted poultry manure was used in the plots in 2001 and 2002. Treatments were applied each year except 2000, when a sudan grass cover crop was planted in all plots.

Tomato seeds (cultivar Rio Colorado, Seminis Oxnard, CA) were planted in 200-cell flats containing Metro Mix (W.R. Grace and Co., Cambridge, MA) in April of each year and fertilized biweekly with 1:50 mixture of organic fertilizer solution (Fertrell Fish Emulsion No. 4, Ames Garden Supply, Burlington, VA) and water. Six-week-old seedlings were transplanted 14 d after soil amendment (May 28, 2001, May 31, 2002) into single-row beds (six per experimental unit) that were 7.62 m in length. Within-row spacing was 30 cm and the row width was 1.5 m. The plots were separated at the ends by 3.1 m alleyways. Tomato seedlings were transplanted by hand and fertilized with the organic fertilizer solution. Dead or missing plants were replaced with new healthy plants approximately 1.5 weeks after transplanting. Ten days after transplanting the sides of the beds were mechanically tilled. Straw mulch was placed around each plant on top of the beds on the soil surface in the plots that received mulch.

2.2. Disease incidence, dispersal and yield

Plants in the interior four rows of each 6 row experimental unit were monitored weekly for symptoms of southern blight caused by *S. rolfsii* (June 21, 2001 to August 17, 2001 and June 19, 2002 to August 16, 2002). Disease severity was rated as follows: 0—healthy; 1—chlorosis, may have lesion forming; 2—beginning to wilt, lesion present; 3—severe wilt, dropped leaves, lesion present; 4—dead. Disease incidence was calculated by summing the number of plants with a severity rating

greater than 1 divided by the total number of plants in the plot. Disease incidence data was recorded by spatial location (row and plant number) within each plot. Disease incidence and AUDPC were calculated using disease data from the entire 4 row plots. AUDPC was calculated as $[(Y_{i+1} + Y_i)]/[X_{i+1} - X_i]$, where Y_i = disease incidence at the ith observation, X_i = time (weeks) at the ith observation. The four interior rows were harvested from each experimental unit and the yield of ripe tomatoes was obtained in August of each year.

2.3. Soil sampling

Soil samples were collected in August of each year from each of the plots. Soil cores were collected with a soil probe (15 cm depth) from each of the center four rows of the plots, bulked, placed into plastic bags, held at 4 °C, and assayed within 1 week of sampling for soil physical, chemical and biological parameters including, microbial populations using dilution plating, and bacterial functional diversity using carbon source utilization pattern analysis. Samples for DNA profiling using DGGE were stored at −20 °C until further processing. Surface mulch was removed before sampling to avoid collecting surface organic matter.

2.4. Soil physical parameters

Undisturbed soil cores were collected from the field with soil sampling rings of known volume and soil bulk density, soil porosity and soil moisture were determined (Mehlich, 1973). For gravimetric water content measurements, bulk soil was sieved and sub samples (10.0 g) were weighed into aluminum dishes. These dishes were placed in a 105 ± 1 °C oven for 48 h, and the dry weight was then recorded. Gravimetric soil water content was the difference in soil weights before and after oven drying (Gardner, 1986).

2.5. Soil chemical parameters

Soils were tested by the Soil Testing Laboratory of the North Carolina Department Agriculture (NCDA), Raleigh, NC, USA, according to standardized procedures that can be found online at http://www.ncagr.com/agronomi/sthome.htm. Soil tests included: extractable acidity (EA), cation exchange capacity (CEC), base saturation (BS), soil pH, humic matter (HM), macro- and micronutrients including phosphorus, potassium, calcium, magnesium, sodium, manganese, zinc, and copper (Mehlich, 1973, 1984; Mehlich et al., 1976).

2.6. Soil microbial communities, extractable and microbial biomass carbon and nitrogen and respiration

2.6.1. Soil microbial communities

Numbers of culturable bacteria, fluorescent pseudomonad bacteria, enteric-bacteria, total fungi, thermophilic microorganisms, *Trichoderma* spp. and Oomycetes species were quantified using dilution plating, agar media, incubation times and temperatures described by Bulluck et al. (2002). Soil samples were analyzed for selected soil microorganisms using 10-fold serial dilutions of soil in 2.5% water agar on different selective media (Bulluck et al., 2002). Data were expressed as number of colony-forming units (CFUs) per gram of dry soil.

2.6.2. Soil extractable C and extractable N

Extractable organic C was estimated by equilibrating 20.0 g dry weight equivalent soil with a 50 ml of 0.5 M K_2SO_4 solution (Hu et al., 1997). The concentrations of C in the solutions were then determined using a total organic carbon (TOC) analyzer (TOC-5050A, Shimadzu Corporation, Kyoto, Japan). To estimate extractable N in soil, 10.0 g dry weight equivalent soil samples were shaken with 100 ml of 1 N KCl solution (Hart et al., 1994). The concentrations of NO_3^- and NH_4^+ in the extracts were, respectively, determined with QuikChem® methods 10-107-04-1-A and 10-107-06-2-A on a Lachat flow injection analyzer (Lachat Instruments, Milwaukee, WI, USA).

2.6.3. Soil microbial biomass

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined by the chloroform-fumigation–extraction method (Vance et al., 1987; Ross, 1992). Twenty grams (dry weight equivalent) of soil were fumigated with ethanol-free chloroform for 48 h. Both fumigated and non-fumigated soils were extracted with 50 ml of 0.5 M K_2SO_4 by shaking for 30 min on an end-to-end shaker. The TOC analyzer was used to determine the organic C (C_{org}) in the extracts. The MBC was calculated as follows:

$$\mathrm{MBC} = (C_{org} \text{ in fumigated soil} - C_{org} \text{ in non-fumigated soil})/k_{ec},$$

where $k_{ec} = 0.33$, the factor used here to convert the extracted organic C to MBC (Sparling and West, 1988).

The concentration of N in the extractant was determined on the Lachat flow injection analyzer after digestion using alkaline persulfate oxidation (Cabrera and Beare, 1993). The MBN was calculated using the equation:

$$\mathrm{MBN} = (\text{total } N \text{ in fumigated soil} - \text{total } N \text{ in non-fumigated soil})/k_{en},$$

where k_{en} is 0.45, the factor used to convert the extracted organic N to MBN (Jenkinson, 1988).

2.6.4. Soil microbial respiration

Microbial activity was measured as the heterotrophic respiration in the absence of plant roots by an incubation-alkaline absorption method (Coleman et al., 1978). Sub-samples of sieved soil equivalent to 20.0 g dry weight were adjusted to moisture of about 60% water holding capacity (Alef, 1995), and placed in 1-l Mason jars with a suspended beaker containing 5 ml of 0.5 N NaOH. The jars were

incubated at 25 °C in the dark immediately after sealing. On the day 7 after the incubation, the beaker was replaced with one containing fresh NaOH solution, and the jars were incubated for additional 7 d. The CO_2 trapped in NaOH was titrated with 0.1 N HCl. Microbial respiration was estimated as mg CO_2 kg^{-1} soil d^{-1} by averaging the data.

2.6.5. Net nitrogen mineralization

Net nitrogen mineralization was determined using the method described by Hart et al. (1994). Briefly, soil samples of 10.0 g dry weight equivalent were weighed into Erlenmeyer flasks. The flasks were covered with plastic wrap pierced with a small hole to minimize water loss yet maintain gas exchange, and the soils were incubated for 28 d in the dark at room temperature. Soil moisture was maintained at approximately 60% water holding capacity by monitoring the weight change and adding water weekly during the incubation period. Soil NH_4^+ and NO_3^- were extracted with 1 N KCl at a 1:10 of soil to solution ratio, and their concentrations were determined with the Lachat flow injection analyzer. Net mineralized N in soil was the difference between KCl-extractable inorganic N contents before and after incubation.

2.7. Functional diversity using BIOLOG analysis

Carbon source utilization patterns of soil microbial communities, also called community-level physiological profiles (CLPP), were assessed using BIOLOG 96-well Ecoplates (BIOLOG, Inc., Hayward, CA, USA). The plates contained 31 different carbon sources, consisting of eight carbohydrates, eight carboxylic acids, four polymers, six amino acids, two amines and three miscellaneous substrates, replicated three times on each microplate. Bacteria were extracted from 5 g (dry weight) of soil with 45 ml of 1 M K_2HPO_4 buffer (pH = 7). Soil suspensions were shaken for 30 min at 200 rpm on a reciprocal shaker. After settling for 30 min, 2 ml of inoculating solution were diluted to 10^{-5}. Soil suspensions were transferred (145 μl) to each of the 96 wells on the Ecoplates, and plates were incubated at 26 °C for 4 d. Ecoplates were read at 590 nm on a Microplate E-Max Reader (Bio-Rad, Richmond, USA) at 0, 24, 48, 72 and 96 h. The 96-h data were used for statistical analysis. The average well color development (AWCD) of all 31 carbon sources for each sample was calculated prior to statistical analysis to eliminate variation in well color development caused by different cell densities (Garland, 1996; Zak et al., 1994). All of the optical density (OD) readings were adjusted by measuring the OD reading in well 1 (water control). Negative values were set to zero.

2.8. Bacterial genotype diversity using denaturing gradient gel electrophoresis analysis

2.8.1. DNA extraction and PCR amplification

DNA was extracted from soil samples (0.5 g) using a MO BIO kit (MO BIO Laboratories, Inc. California, USA). Two μl of DNA was used for PCR amplification of bacterial species using universal primers. Each 50 μl reaction mixture contained 5 μl of 10 × PCR buffer (Invitrogen, Carlsbad, California), 15 μl of deoxynucleoside-triphosphate mix (2.5 mM each), 2 μl of bovine serum albumin (1.0 mg/ml), 2 μl of the forward primer [5-CCACACTGGGACTGAGACACG-3 (310F, 21 bp)] and reverse primer [5-GTATTACCGCGGCTGCTGGCA-3 (516R, 21 bp)] (20 μM), and 0.5 μl *Taq* polymerase (5 U/μl) (Invitrogen, Carlsbad, California). A 40-base pair GC-clamp was attached to the forward primer for DGGE analysis (Muyzer et al., 1993; Muyzer, 1999). PCR conditions were 94 °C for 2 min, and then 94 °C for 1 min, 60 °C for 1 min, 72 °C for 3 min for total of 30 cycles, with the extension at 72 °C for 10 min.

2.8.2. DGGE analysis

DGGE was performed with a Decode Universal Mutation Detection System (Bio-Rad, Richmond, USA). Twenty μl of the PCR products from soil samples was loaded onto an 8% acrylamide gel (acrylamide/bis solution, 37.5:1; Bio-Rad) containing a linear chemical gradient ranging from 20% to 70% denaturant [7 M urea and 40% (vol/vol) formamide]. Gels were run for 12 h at 110 V. All of the acrylamide gels were kept at 60 °C in 1 × TAE buffer. The gel was stained with SYBR green I nucleic acid gel stain (1:10,000 dilution; Molecular Probes, Eugene, Oregon) and photographed on a UV transilluminator.

2.9. Statistical analysis

The Statistical Analysis Systems software (PC-SAS 8.2; SAS Institute, Cary, NC) was used to analyze all data. A generalized linear model procedure (Proc GLM) and analysis of variance (ANOVA) were performed for final disease incidence, tomato yield, soil physical, chemical and biological parameters. Correlation analysis was performed on soil physical, chemical and biological data and final incidence of disease. Regression analysis was preformed on final disease incidence and functional and genotype diversity.

BIOLOG substrate utilization patterns were analyzed for functional diversity (Garland, 1996; Shannon and Weaver, 1963) to give substrate richness (the number of substrates utilized), substrate evenness (the distribution of color development between the substrates), and Shannon's diversity index [$H' = -\sum P_i \log P_i$, where P_i = (OD reading of well I)/(Sum of all wells)] based on the OD of wells in the BIOLOG Ecoplates. Diversity indices, richness, and evenness were compared for each sample by ANOVA using the GLM procedure of SAS 8.0 (SAS Institute, Inc., Cary, NC, USA).

Bands from DGGE were assigned to gel tracks using QuantityOne software from Bio-Rad (Bio-Rad, Richmond, USA). The Shannon–Weaver index (H) (Shannon and Weaver, 1963) was used to calculate the diversity of bands in the DGGE gels ($H = -P_i \log P_i$), where P_i is the

importance probability of bands in a track. H was calculated on the basis of the bands in the gel tracks by using the intensities of the bands as judged by peak heights in the densitometric curves using QuantityOne (Bio-Rad, Richmond, USA). P_i was calculated as follows: $P_i = n_i/N$, where n_i is the height of a peak and N is the sum of all peak heights in the densitometric curve. The richness (R) measure of genotype diversity is a simple count of the number of genotypes found in a community. Evenness calculation followed the $E = H/\ln R$ (Eichner et al., 1999). Diversity value, richness, and evenness were compared for each sample by ANOVA using the GLM procedure of SAS 8.0 ($\alpha < 0.05$).

3. Results

3.1. Disease incidence

Disease was evaluated over time in field plots. Disease onset occurred approximately 24 and 19 d after transplanting in 2001 and 2002, respectively (Fig. 1). Although, the tillage effect was not significant in either year, disease progressed more rapidly and final incidence was higher in both years in plants from tilled than mulched plots (Fig. 1A and C). AUDPC were significantly higher in plants in tilled than mulched soils in one of 2 years (2001). Final disease incidence was 14.7% and 45.2% in tilled and 7.1% and 27.5% in mulched plots in 2001 and 2002, respectively (Table 1).

Soil amendments had a significant effect on disease and AUDPC in one of 2 years. Overall levels of disease were lower in 2001 than 2002 and the incidence of disease and the AUDPC were not significantly reduced in 2001 by soil amendments (Table 1). However, disease incidence was lower in plants from soils amended with cotton gin trash (9.6%) or poultry manure (9.8%) than in plants in soils amended with green manure (10.5%) or synthetic fertilizer (13.7%) in 2001 (Table 1). Disease progressed less rapidly over time in plants in mulched soils amended with cotton gin trash than synthetic fertilizer (Fig. 1B).

In contrast, in 2002, soil amendments had a significant effect ($P < 0.001$) on the incidence of disease (Table 1). Final disease incidence was 15.6% in plants in soils amended with cotton gin trash and 44.7%, 29.6% and 55.6% in plants in soils amended with poultry manure, green manure or synthetic fertilizer, respectively (Table 1). Similar to 2001, disease progressed less rapidly in plants from mulched soils amended with cotton gin trash than the other amendments (Fig. 1D). Final incidence of disease was lowest in soils amended with cotton gin trash compared to soils amended with poultry manure, green manure or synthetic fertilizer. AUDPC's were also significantly lower in soils amended with cotton gin trash than the other soil fertility amendments in 2002 (Table 1). In both years, final disease was lower in soils amended with organic amendments than synthetic fertilizer (Table 1).

3.2. Yield

Tillage did not have a significant effect on fruit yield in either year (Table 1). Soil amendment had a significant effect on fruit yield in 2001 ($p = 0.0001$) and 2002 ($p = 0.0305$). In both years, plants from soils amended with composted cotton gin trash had higher yields than plants from soils amended with green manure or synthetic fertilizer. Overall, tomato yields were lower in 2002 across all treatments since disease incidence was higher than in 2001. Plants from soils amended with green manure had the lowest yields in both years, while plants from soils amended with composted cotton gin trash had the highest yields in both years (Table 1).

3.3. Soil physical parameters

Soil physical properties were affected by tillage and amendments. Soil bulk density was higher in tilled than mulched soils. However, soil porosity and soil water content was higher in mulched than tilled soils in both years (Table 2). Soil bulk density was significantly lower in soils amended with cotton gin trash than soils amended with poultry manure, green manure or synthetic fertilizer (Table 2). In contrast, soil porosity and soil water content were significantly higher in soils amended with cotton gin trash than soils amended with the other fertility amendments (Table 2). Final disease incidence was negatively correlated with soil water content ($p = 0.05$) in both years (Table 3). Bulk density and soil porosity were not significantly correlated with final disease incidence in either year (Table 3).

3.4. Soil chemical parameters

Levels of calcium, magnesium, and CEC were significantly higher in surface-mulched than tilled soils in 2002 (Table 4). None of the other chemical factors were affected by tillage or surface mulch.

Soil pH was significantly higher in soils with organic amendments than synthetic fertilizer. Levels of potassium, calcium, magnesium, extractable acidity, CEC, and humic matter were all significantly higher in soils amended with cotton gin trash than poultry manure, green manure or synthetic fertilizer (Table 4). Levels of manganese and zinc were higher in soils amended with poultry manure than other amendments (Table 4). Levels of copper were lower in soils with cotton gin trash than those with poultry manure, green manure or synthetic fertilizer. Soil amendments did not significantly influence the level of sodium (Table 4).

None of the soil chemical factors were consistently correlated with disease in both years. However, levels of potassium were negatively correlated with the final incidence of disease in 2001 (Table 3). Levels of calcium, CEC and humic matter were negatively correlated with

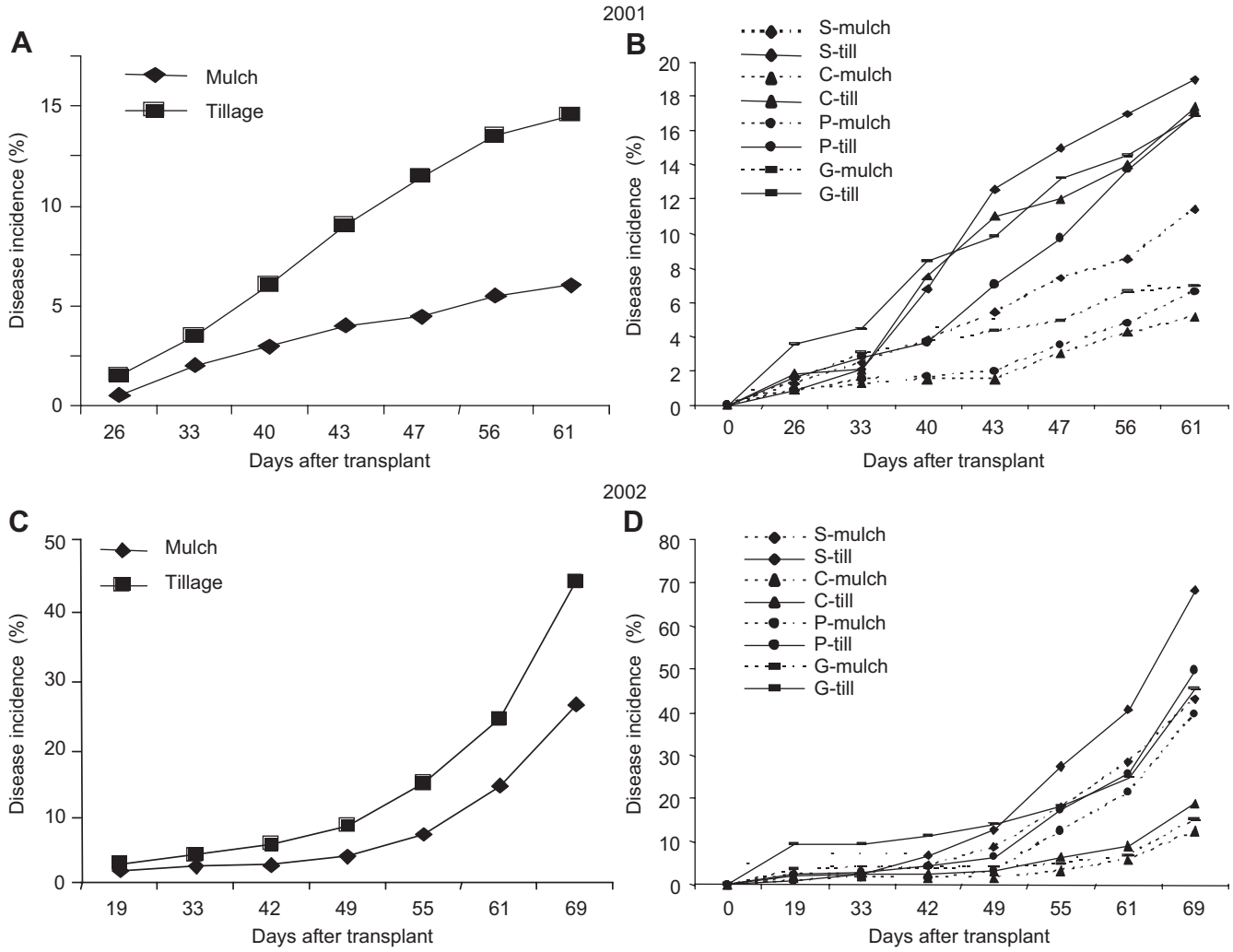

Fig. 1. Disease progress caused by *Sclerotium rolfsii* in tomatoes in soils that were: (A) tilled weekly after transplanting (till) or tilled once and surface mulched (mulch) in 2001; (B) tilled (till) or surface mulched (mulch) soils amended with either organic [cotton gin trash (C), poultry manure (P), rye–vetch green manure (G)] or synthetic fertilizer (S) in 2001; (C) tilled weekly after transplanting (till) or tilled once and surface mulched (mulch) in 2002; (D) tilled (till) of surface mulched (mulch) soils amended with either organic [cotton gin trash (C), poultry manure (P), rye–vetch green manure (G)] or synthetic fertilizer (S) in 2002.

Table 1
Final disease incidence, area under the disease progress curve (AUDPC) and fruit yield (kg/plot) in soils with different tillage and fertility amendments in 2001 and 2002

	2001			2002		
	Final disease incidence (%)	AUDPC	Yield (kg/plot)	Final disease incidence (%)	AUDPC	Yield (kg/plot)
Main plot means						
Tillage	14.7	3.51	13.43	45.2	7.28	8.90
Mulch	7.1	1.94	13.15	27.5	4.20	2.50
Sub plot means—amendments						
Synthetic fertilizer	13.7	3.38	16.78	55.6	8.55	3.58
Cotton gin trash	9.6	2.20	22.48	15.6	2.35	9.25
Poultry manure	9.8	2.57	7.37	44.7	5.95	7.92
Green manure	10.5	2.76	6.53	29.6	6.10	2.06
Sources of variation				*P-values*		
Tillage	0.0004*	0.0071*	0.9364	0.1624	0.1587	0.1593
Amendment	0.3938	0.5030	0.0001*	<0.0001*	<0.0001*	0.0305*
Till × amendment	0.9656	0.9915	0.4017	0.2326	0.2303	0.0491*

*Indicates significantly different at $P<0.05$.

Table 2
Soil physical parameters in soils with different tillage and fertility amendments in 2001 and 2002

	Bulk density (g/ml)		Porosity (%)		Soil water content (g water/g dry soil)	
	2001	2002	2001	2002	2001	2002
Main plot means						
Tillage	1.2	1.30	51.57	52	0.1	0.017
Mulch	1.1	1.20	54.82	55	0.13	0.02
Subplot means—amendments						
Synthetic fertilizer	1.20	1.26	52.46	52.455	0.11	0.014
Cotton gin trash	1.0	1.11	58.11	58.11	0.13	0.026
Poultry manure	1.20	1.29	51.32	51.32	0.11	0.018
Green manure	1.22	1.305	50.76	50.755	0.11	0.015
Sources of variation				*P-values*		
Tillage	0.0338*	0.1486	0.0001*	0.0001*	0.0275*	0.108
Amendment	0.0018*	0.0117*	0.0001*	0.0001*	0.8219	0.0006*
Till × amendment	0.8652	0.7019	0.8213	0.8213	0.9967	0.7603

*Indicates significantly different at $P<0.05$.

Table 3
Correlations between the final incidence of disease and soil physical, chemical, and biological parameters

Soil property	Partial correlation coefficients (r)	
	2001	2002
Bulk density (g/ml)	−0.27	−0.04
Porosity (%)	0.23	0.02
Soil water content (g water/g dry soil)	−0.67*	−0.85*
P (mg/dm^3)	−0.33	−0.53
K (meq/100 cm^3)	−0.70*	−0.56
Ca (meq/100 cm^3)	0.40	−0.61**
Mg (meq/100 cm^3)	−0.25	−0.17
Na (meq/100 cm^3)	ND	ND
PH	0.40	0.02
EA (meq/100 cm^3)	−0.46	−0.06
CEC (meq/100 cm^3)	−0.44	−0.62**
BS (%)	0.22	0.63*
Humic matter (g/100 cm^3)	−0.40	−0.70*
Mn (mg/dm^3)	−0.62**	−0.45
Zn (mg/dm^3)	−0.38	−0.20
Cu (mg/dm^3)	0.08	0.31
Total bacteria (CFUs/g)	−0.65**	0.51
Endobacteria (CFUs/g)	0.10	0.62**
Pseudomonas spp. (CFUs/g)	−0.17	0.38
Thermophiles (CFUs/g)	−0.37	−0.22
Total fungi (CFUs/g)	−0.30	0.06
Oomycetes (CFUs/g)	0.12	−0.25
Trichoderma spp. (CFUs/g)	−0.50	−0.16
Soil respiration (CO_2 mg/kg)	−0.18	0.15
Microbial biomass C (mg/kg)	−0.48	−0.01
Microbial biomass N (mg/kg)	0.13	0.26
Extractable N (mg/kg)	−0.26	0.08
Net N mineralized (mg/kg)	−0.58**	0.03
Extractable C (mg/kg)	−0.26	0.03

EA = extractable acidity; CEC = cation exchange capacity; BS = base saturation.
ND = not determined.
*Final disease incidence was significantly correlated with the parameters at $P<0.05$.
**Final disease incidence were significantly correlated with the parameters at $P<0.01$

final disease incidence in 2002, while BS was positively correlated with final disease incidence in 2002 (Table 3).

3.5. Soil microbial communities, extractable and microbial biomass carbon and nitrogen and respiration

The abundance of total culturable bacteria, enteric bacteria and fluorescent *Pseudomonas* spp. were not affected by soil amendment in any year (Table 5). However, populations of fluorescent *Pseudomonas* species were higher in mulched than tilled soils in one of 2 years (Table 5). Total culturable fungi were higher in mulched than tilled soils in one of 2 years (Table 5). Thermophilic organisms were affected by soil amendment in both years and were significantly higher in soils amended with cotton gin trash than the other soil amendments (Table 5). Oomycete populations were higher in soils amended with cotton gin trash than other soil amendments in one of 2 years (Table 5). *Trichoderma* populations were not affected by tillage or soil fertility treatment.

Soil microbial respiration, microbial biomass carbon, microbial biomass nitrogen, net mineralized nitrogen, and extractable carbon were higher in soils that were surface-mulched than tilled in 2002 (Table 6)(Tu et al., 2005). Soil microbial respiration, microbial biomass carbon, microbial biomass nitrogen, extractable nitrogen, net mineralized nitrogen and extractable carbon were all significantly higher in soils amended with cotton gin trash or poultry manure than synthetic fertility amendments in 2002. Only net mineralizable nitrogen was negatively correlated with final disease incidence in 2001 (Table 3).

3.6. Functional and genotype diversity based on BIOLOG and DGGE

Tillage did not significantly affect the diversity of carbon substrates utilized by bacterial communities or the

Table 4
Chemical parameters in soils as affected by tillage and soil amendments in 2001 and 2002

Amendment	P (mg/dm^3)	K (meq/100 cm^3)	Ca (meq/100 cm^3)	Mg (meq/100 cm^3)	Na (meq/100 cm^3)	pH	EA (meq/100 cm^3)	CEC (meq/100 cm^3)	BS (%)	HM (g/100 cm^3)	Mn (mg/dm^3)	Zn (mg/dm^3)	Cu (mg/dm^3)
2001													
Main plot means													
Tillage	208.88	31.06	68.56	22.63	0.10	6.98	0.25	4.33	94.63	0.49	76.81	268.75	100.94
Mulch	206.81	40.75	67.06	23.44	0.10	7.04	0.25	4.69	94.83	0.45	88.69	299.88	100.81
Subplot means—amendment													
Synthetic fertilizer	221.75	35.13	66.13	20.75	0.10	6.83	0.31	3.63	91.63	0.46	80.25	286.75	94.13
Cotton gin trash	221.5	53.38	66.75	22.75	0.10	6.80	0.43	6.58	93.38	0.5	86.25	282.88	87.13
Poultry manure	211.75	31.38	70.38	22.88	0.10	7.19	0.15	4.17	96.63	0.47	87.13	312.75	119.75
Green manure	176.13	23.75	68	25.75	0.10	7.23	0.12	3.63	97.28	0.43	77.38	254.88	102.5
Sources of variation							*P*-values						
Tillage	0.9188	0.2532	0.1704	0.2676	0.392	0.6347	0.9293	0.3793	0.8925	0.2143	0.3172	0.2503	0.9792
Amendment	0.0025*	<0.0001*	<0.0001*	<0.0001*	0.4155	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.7921	0.0423*	0.0846	0.0876
Till × amendment	0.6904	0.7681	0.0736	0.4787	0.4155	0.0289*	0.0382*	0.4148	0.0995	0.3198	0.8675	0.4782	0.6994
2002													
Main plot means													
Tillage	236.3	0.28	3	1	0.1	7.1	0.29	4.6	93.5	0.29	12.3	10.3	1.6
Mulch	235.9	0.3	3.4	1.22	0.09	7	0.41	5.3	91.9	0.33	14.6	11.7	1.5
Subplot means—amendment													
Synthetic fertilizer	211.1	0.135	2.25	0.825	0.09	6.9	0.37	3.4	90.2	0.28	12.65	10.7	1.7
Cotton gin trash	247.9	0.7	4.4	1.6	0.1	7.2	0.45	7.2	93.9	0.43	13.3	10.5	1.3
Poultry manure	301.55	0.131	3.8	1.11	0.1	7.1	0.33	5.45	94.2	0.3	15.6	13.55	1.7
Green manure	183.7	0.1	2.35	0.9	0.1	7.1	0.28	3.65	92.8	0.26	12.2	9.45	1.7
Sources of variation							*P*-values						
Tillage	0.9827	0.4934	0.0184*	0.0078*	0.391	0.1532	0.1334	0.0081*	0.2769	0.3612	0.2924	0.1152	0.2362
Amendment	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.4155	<0.0001*	0.0027*	<0.0001*	<0.0001*	0.0006*	0.0005*	<0.0001*	0.0320*
Till × amendment	0.934	0.8251	0.7447	0.4149	0.4155	0.4941	0.907	0.8586	0.3587	0.9197	0.9533	0.7155	0.8859

EA = extractable acidity; CEC = cation exchange capacity; BS = base saturation; HM = humic matter.
*Indicates significantly different at $P<0.05$.

Table 5
Microbial population densities in soils (CFUs/g dry soil) in soils with different tillage and fertility amendments in 2001 and 2002

Amendment	Total bacteria		Endobacteria		*Pseudomonas* spp.		Thermophiles		Total fungi		*Trichoderma* spp.		Oomycetes	
	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Main plot means														
Colony-forming units per gram of soil	$\times 10^8$	$\times 10^9$	$\times 10^5$	$\times 10^6$	$\times 10^5$	$\times 10^4$	$\times 10^2$	$\times 10^4$	$\times 10^4$	$\times 10^6$	$\times 10^2$	$\times 10^2$	$\times 10^1$	$\times 10^2$
Tillage	5.60	8.50	8.18	5.34	5.53	2.34	6.00	1.65	4.14	1.29	1.93	5.35	8.27	1.18
Mulch	9.00	9.10	5.70	5.95	9.80	2.36	7.50	1.85	6.10	1.07	9.40	16.8	6.90	1.49
Subplot means—amendments														
Colony-forming units per gram of soil	$\times 10^8$	$\times 10^9$	$\times 10^5$	$\times 10^6$	$\times 10^5$	$\times 10^4$	$\times 10^2$	$\times 10^4$	$\times 10^4$	$\times 10^5$	$\times 10^2$	$\times 10^2$	$\times 10^1$	$\times 10^1$
Synthetic fertilizer	6.35	8.35	8.62	6.51	6.39	2.54	4.45	1.64	5.86	9.3	9.31	11.0	8.31	10.8
Cotton gin trash	8.35	11.125	6.85	6.30	6.15	3.24	10.8	3.16	5.67	12.5	2.88	11.2	8.13	22.2
Poultry manure	6.05	7.25	7.33	5.02	7.80	2.01	3.44	1.00	4.72	18.0	3.07	2.52	7.40	6.58
Green manure	8.45	7.85	4.92	4.19	10.4	1.63	8.32	1.19	4.24	7.41	7.43	9.75	6.53	14.0
Sources of variation	*P-values*													
Tillage	0.0695	0.6969	0.317	0.7396	0.0344*	0.9582	0.5427	0.2919	0.0153*	0.7396	0.2616	0.0707	0.3954	0.1992
Amendment	0.7366	0.3492	0.25	0.611	0.6657	0.3715	0.0499*	0.0007***	0.554	0.5286	0.3628	0.9551	0.8581	0.0094**
Till × amendment	0.4954	0.4276	0.4401	0.4884	0.8652	0.8357	0.9765	0.5872	0.3784	0.3047	0.6068	0.3091	0.3093	0.82

*, **, and *** indicate significantly different at $P<0.05$, $P<0.01$, or $P<0.001$ levels, respectively.

Table 6
Soil respiration, microbial biomass carbon and nitrogen, extractable carbon and nitrogen and mineralizable nitrogen in soils with different tillage and fertility amendments in 2001 and 2002.

Year	Soil respiration (CO_2 mg/kg)		MBC (mg/kg)		MBN (mg/kg)		Extractable N (mg/kg)		Net N mineralized (mg/kg)		Extractable C (mg/kg)	
	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002	2001	2002
Tillage	29.7	24.3	168.4	156.5	19.12	15.1	2.35	1.9	7.53	8.6	44.02	43.8
Surface mulch	38.37	43.1	235.48	247.4	21.55	25.6	3.89	4.3	13.54	12.5	55.63	55.8
Synthetic fertilizer	30.81	21.7	172	141.5	18.31	15.95	1.99	1.55	9.91	6.7	46.98	34.6
Cotton gin trash	37.95	50.5	195.9	317.15	19.03	29.05	3.57	6.4	10.26	20.15	47.6	74.9
Poultry manure	35.82	36.45	225.03	195.35	22.52	22.25	3.83	3.15	11.29	8.35	53.81	53.4
Green manure	32.41	26.25	214.92	153.8	21.48	14	3.09	1.35	10.68	6.9	50.91	36.35
	$P>F$											
Tillage	0.9289	0.0371*	0.0240	0.0319*	0.4608	0.0197*	0.3734	0.0637*	0.1071	0.0009*	0.2023	0.0033*
Amendment	0.0001*	<0.0001*	0.0001*	<0.0001*	0.0001*	<0.0001*	0.0001*	<0.0001*	0.0001*	<0.0001*	0.001*	<0.0001*
Till × amendment	0.0111	0.0497*	0.0001	0.4387	0.3723	0.5880	0.8140	0.1879	0.2493	0.3489	0.4438	0.1731

*Indicates significantly different at $P = 0.05$.
MBC represents microbial biomass carbon.
MBN represents microbial biomass nitrogen.

genotype diversity based on DGGE patterns in the soils from the experimental plots (Table 7).

Soil amendment had a significant effect on both the diversity and richness of carbon substrates utilized (Table 7). Carbon source utilization patterns were more diverse and more different substrates were utilized in soils amended with cotton gin trash than soils amended with poultry manure, green manure or synthetic fertilizer. Bacterial genotypes recovered by DGGE were also more diverse in soils amended with cotton gin trash than soils amended with poultry manure, green manure or synthetic fertilizer. However, soil amendment did not affect genotype richness of bacterial communities.

The AWCD in the wells of the Ecoplates with L-arginine, L-asparagine, D-mannitol, L-serine, and *N*-acetyl-D-glucosamine were higher from soils amended with cotton gin trash than the other soil fertility amendments, which indicated that specific bacterial functional groups existed

Table 7
Bacterial community diversity, richness and evenness as indicated from community level physiological profiling and denaturing gradient gel electrophoresis in soils with different tillage and fertility amendments in 2002

	CLPP[a]			DGGE[b]		
	Diversity	Richness	Evenness	Diversity	Richness	Evenness
Main plot means						
Tillage	2.48	6.75	1.9	1.28	18.5	0.44
Mulch	2.6	10.44	1.37	1.31	19.8	0.44
Subplot means—amendments						
Synthetic fertilizer	2.385	5.75	1.525	1.275	18.5	0.44
Cotton gin trash	2.84	15.875	1.17	1.31	19.5	0.45
Poultry manure	2.715	9.25	1.99	1.255	19.5	0.435
Green manure	2.205	3.5	1.835	1.305	19	0.425
Sources of variation			*P-values*			
Tillage	0.5792	0.2437	0.231	0.055	0.53	0.89
Amendment	0.0025*	0.0035*	0.4277	0.053*	0.21	0.94
Tillage × amendment	0.4241	0.4376	0.9356	0.069	0.11	0.85
Cotton gin trash vs. other amendments	0.0046*	0.0008*	0.1465	0.051*	0.1	0.147

*Indicates significantly different at $P = 0.05$.
[a]Community-level physiological profiling (CLPP) was used as an indicator of functional diversity based on the carbon source utilization patterns (96 h data) using BIOLOG analysis.
[b]Genotype diversity of bacterial communities was used as an indicator of bacterial diversity based on the banding patterns using DGGE analysis.

in soils amended with cotton gin trash. There was no significant difference for evenness of carbon substrates used or evenness of genotypes recovered by DGGE either in soil with tillage, surface mulch, or in soils with the other fertility amendments (Table 7).

Regression analysis was performed and scatter plots were created to examine the relationship between incidence of disease and bacterial functional diversity. The *p*-value ($p = 0.0459$) for the slope of the regression line was lower than 0.05, which indicated that the incidence of southern blight and bacterial functional diversity was significantly negatively correlated. Disease incidence tended to decrease with an increase in soil bacterial functional diversity (Fig. 2).

Regression analysis was also performed and scattered plots were created to examine the relationship between incidence of disease and bacterial genotype diversity as measured with DGGE. There was also a significant negative relationship between the incidence of southern blight and DGGE measures of bacterial diversity (Fig. 2). Soils amended with cotton gin trash had higher CLPP and DGGE diversity indices and lower levels of disease than other soils (Fig. 2).

4. Discussion

Results from the present experiment showed that compared to the tilled control, mulching reduces the incidence of southern blight (Fig. 1). In fact, in both years, mulching reduced disease incidence by over 50%. Tillage can result in increases in the incidence of southern blight (Gurkin and Jenkins, 1985). Movement of soil containing sclerotia of the pathogen from deep layers in soil to the surface where they can contact the plant stem is promoted with tillage (Jenkins and Averre, 1986).

Soil physical factors can impact the incidence of southern blight (Hoitink et al., 1997; Munnecke et al., 1982). In our study, soils amended with cotton gin trash had higher soil water contents than soils with other amendments. In addition, soil amended with cotton gin trash had lower bulk densities and higher levels of soil porosity than soils with the other fertility amendments. The final incidence of southern blight was negatively correlated with soil water content in both years (Table 3). Sclerotia of *S. rolfsii* respond to cyclical changes in soil water (Punja, 1985). Sclerotia generally germinate near the soil surface and to a greater extent after cycles of drying and rewetting. Soils amended with cotton gin trash retained higher soil water contents and these conditions were probably less conducive for germination of sclerotia and infection of plants.

Soil chemical parameters were assessed to determine if changes in soil chemical parameters were related to disease incidence. Higher levels of extractable acidity, CEC, BS, phosphates, potassium, magnesium, manganese, zinc and humic matter enhance plant growth and plant resistance to disease (Doran et al., 1996). Many soil chemical factors were affected by the organic soil amendments (Table 4). We found higher levels of calcium, CEC and extractable acidity in soils amended with cotton gin trash (Table 4). Disease incidence was negatively correlated with levels of calcium, CEC, BS and humic matter. Others have shown increased levels of calcium in plant tissue following application of calcium fertilizers such as calcium sulfate or calcium nitrate to soils and that calcium applications can reduce the incidence of southern blight (Punja, 1985). Our data support these findings.

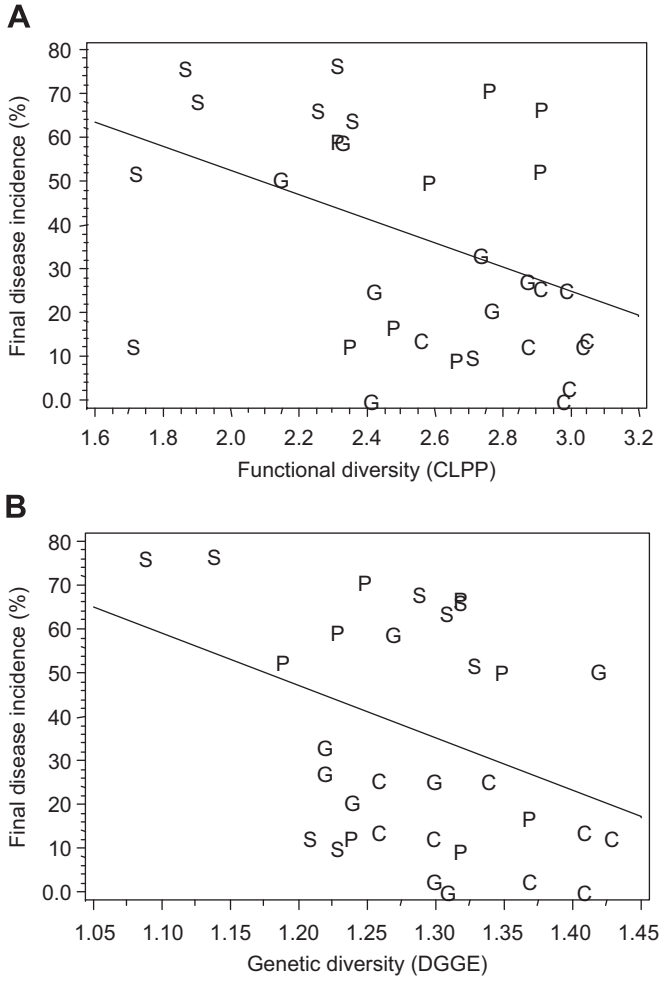

Fig. 2. Scattered plots showing the relationship between: (A) final disease incidence and diversity indices based on community level physiological profiling (CLPP) where final disease = 1.08–0.277 × CLPP with $p = 0.0165$ for the slope and $r^2 = 0.183$; and (B) final disease incidence and diversity indices based on bacterial genotype analysis using denaturing gradient gel electrophoresis (DGGE) where final disease = 1.91–1.2 × DGGE with $p = 0.0370$ for the slope and $r^2 = 0.137$. S = synthetic fertilizer; C = cotton gin trash; P = poultry manure, and G = rye–vetch green manure.

Our data indicate that cotton gin trash reduced the incidence of disease more than other organic soil amendments or synthetic fertilizers in mulched soils. The underlying mechanism is unclear, but the release of ammonia and increased levels of antagonistic microorganisms may play a major role as both N mineralization and microbial biomass were higher in the soils treated with cotton gin trash.

Levels of extractable and mineralizable N were higher in soils amended with organic amendments than synthetic fertilizer (Table 6). Disease incidence was negatively correlated with the net mineralized N in soil in 2001. The development of southern blight is influenced by soil nitrogen (Bakr and Khan, 1981; Punja, 1985). Disease incidence was lower when ammonium fertilizers in the form of urea, ammonium nitrate, and ammonium bicarbonate were applied (Punja and Jenkins, 1984). Ammonia released by ammonium fertilizers may directly inhibit sclerotial germination and retard mycelial growth of *S. rolfsii*. Ammonium fertilizers may indirectly limit disease by altering host susceptibility or increasing populations of antagonistic soil microorganisms (Jenkins and Averre, 1986). Higher nitrogen levels in the soils amended with cotton gin trash may have impacted disease in our study.

Microbial populations were affected by the addition of soil fertility amendments. Microbial activity as indicated by soil microbial respiration was significantly higher in soils with organic amendments than synthetic fertilizers (Table 6, Tu et al., 2005). Both microbial biomass carbon and nitrogen were statistically higher in soils amended with organic amendments. At the end of the second season, levels of microbial biomass carbon, microbial biomass nitrogen, extractable C, extractable N and mineralizable N were highest in soils amended with cotton gin trash than other amendments. Lowest levels of soil microbial respiration, microbial biomass carbon, microbial biomass nitrogen, extractable C, extractable N and mineralizable N were found in soils amended with synthetic fertilizer. Thus, addition of the organic fertility amendments stimulated soil microbial activity.

Soils amended with cotton gin trash had highest levels of thermophilic organisms in both years of the study. The thermophilic organisms detected from these soils consisted mostly of actinomycetes. Neither microbial activity nor populations of thermophiles were significantly correlated with disease incidence. Soils amended with cotton gin trash had higher levels of fluorescent pseudomonads, but there was no direct correlation between increased populations of pseudomonads and reduced disease incidence. Fluorescent pseudomonads are well-known biocontrol microorganisms that can suppress plant pathogens and stimulate plant growth (Weller, 1988; Weller et al., 2002). Populations of *Trichoderma* spp. were not statistically different among treatments. *Trichoderma* spp. and actinomycetes are known biocontrol agents of *S. rolfsii* and other pathogens (Garbeva et al., 2004a, b; Hoitink and Boehm, 1999; Papavizas and Lewis, 1989; Punja, 1985). In our previous study, the germination of sclerotia of *S. rolfsii* was reduced in soils with organic amendments compared to soils with synthetic fertilizers (Bulluck and Ristaino, 2002).

Maintenance of a diversity of microorganisms in soil is critical to soil health and quality, as a wide range of microorganisms are involved in important biochemical functions in soil. In some situations soil or plant type may be key factors in determining soil microbial diversity (Girvan et al., 2003). Microbial community structure and the time required to return to an equilibrium state after the application of various disturbances or stress could be a useful indicator of potential disease suppressiveness of soils. Management of agricultural soils should be directed toward maximizing the quality of the soil microbial communities in terms of disease suppression (van Bruggen and Semenov, 2000; Workneh and van Bruggen, 1994).

The yield of tomato fruit yield was impacted by soil fertility amendments in our study. Soils amended with cotton gin trash had higher yields than soils amended with poultry manure, green manure or synthetic fertilizer in both years. Cotton gin trash increased yield by altering soil physical properties, increasing soil fertility, increasing beneficial microbial populations and reducing incidence of disease. In contrast, soils amended with green manure had the lowest yields in both years probably due to the low levels of nitrogen and the tie up of nitrogen by microbial populations during decomposition of the rye–vetch green manure.

We were also interested in relating changes in bacterial functional diversity as indicated by carbon substrate utilization patterns and bacterial genotype diversity as indicated by DGGE to disease incidence. While measures of functional and genotype diversity were made only in the second year of the study, there were clear negative trends between disease incidence and both of these measures. Each method of measuring diversity is selective. The CLPP method relied on substrate utilization by culturable populations of bacterial, while the DGGE method relied on detection of nonculturable DNA fragments from soils. Only general conclusions can be drawn. Bacterial functional diversity and richness of the communities were greater in soils amended with cotton gin trash than the other soil amendments. In contrast, the actual richness of bacterial genotypes recovered, as indicated by DGGE, were not significantly different among treatments (Table 7). Results indicate that the structure of the bacterial communities was not that different among soils with the various amendments, but that the substrates used by these communities were more diverse in soils amended with cotton gin trash.

In conclusion, the incidence of southern blight was lower in surfaced-mulched soils than tilled soils. Incidence of southern blight was also lower in soils amended with cotton gin trash than soils amended with poultry manure, rye–vetch green manure or synthetic fertilizer. Microbial activity, microbial biomass carbon, microbial biomass nitrogen, extractable C and extractable N, mineralizable N and population levels of thermophilic microorganisms were higher in soils amended with cotton gin trash than other fertility amendments. Disease incidence was negatively correlated with levels of potassium and calcium in soils, net mineralizable N, CEC, and soil water content. Organic soil amendments stimulated soil microbial activity and affected both soil physical and chemical factors and the incidence of southern blight.

Acknowledgments

This research was funded from the Biologically Based Pest Management Strategies panel of the National Research Initiatives Competitive Grants Program, US Department of Agriculture. We thank Ms. Debbie Glenn, Katrina Buckley, and Josie Bloom for technical assistance, Dr. Cong Tu for collection of the soil microbial activity data (Tu et al., 2005), and Dr. Frank Louws and David Ritchie for editing an earlier version of this manuscript.

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