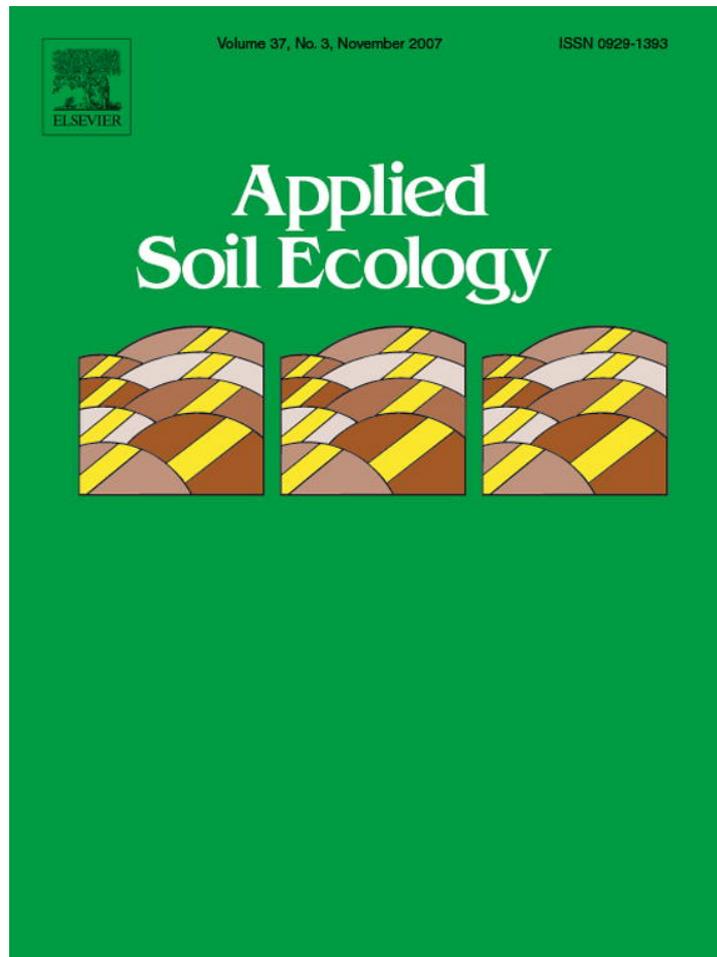


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# Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight

Bo Liu<sup>a</sup>, Cong Tu<sup>a</sup>, Shuijin Hu<sup>a</sup>, Marcia Gumpertz<sup>b</sup>, Jean Beagle Ristaino<sup>a,\*</sup>

<sup>a</sup> Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616, United States

<sup>b</sup> Department of Statistics, North Carolina State University, Raleigh, NC 27695-7616, United States

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## ABSTRACT

The objectives of our research were to evaluate the impact of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors including soil microbial species and functional diversity and their effect on the Basidiomycete plant pathogen *Sclerotium rolfsii*, causal agent of Southern blight. Soils from 10 field locations including conventional, organic and sustainable farms were sampled and assayed for disease suppressiveness in greenhouse assays, and soil quality indicators. Soils from organic and sustainable farms were more suppressive to Southern blight than soils from conventional farms. Soils from organic farms had improved soil chemical factors and higher levels of extractable C and N, higher microbial biomass carbon and nitrogen, and net mineralizable N. In addition, soil microbial respiration was higher in soils from organic than sustainable or conventional farms, indicating that microbial activity was greater in these soils. Populations of fungi and thermophiles were significantly higher in soils from organic and sustainable than conventional fields. The diversity of bacterial functional communities was also greater in soils from organic farms, while species diversity was similar. Soils from organic and sustainable farms had improved soil health as indicated by a number of soil physical, chemical and biological factors and reduced disease.

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## 1. Introduction

Organic production has increased in recent years in many areas of the United States. Organic systems do not use synthetic pesticides and in the long term may be more sustainable than conventional systems. Soils contain enormous numbers of diverse living organisms assembled in complex and varied communities. These organisms play an essential role in the sustainable function of all ecosystems, including recycling of nutrients, regulation of the soil organic matter and soil carbon sequestration, modification of soil physical structure and water regimes, enhancement of the efficiency of nutrient acquisition

and plant health, suppression of undesirable organisms and detoxification of noxious chemicals (Coleman et al., 1978; Kennedy and Smith, 1995). In addition, even though microbial communities are a small fraction of the soil's total organic matter content, they provide a source and sink of nutrients and control soil organic matter mineralization. Changes in microbial communities can be used to predict the effects of ecosystem perturbations by organic and conventional management practices (Bending et al., 2000; Poudel et al., 2002; van Bruggen and Semenov, 2000).

There have been a number of reports that have indicated that organic farming practices have positive effects on soil

\* Corresponding author. Tel.: +1 919 515 3257; fax: +1 919 515 7716.

E-mail address: [jean\\_ristaino@ncsu.edu](mailto:jean_ristaino@ncsu.edu) (J.B. Ristaino).

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microbial populations, processes and activities (Clark et al., 1998; Doran et al., 1996; Drinkwater et al., 1995). In a long-term field trial in which organic and conventional agricultural systems were compared, microbial biomass was higher in soils from organic plots (Gelsomino et al., 2004; Tu et al., 2005; Hu et al., 1997; Liu et al., 2007). Bossio et al. (1998) showed that different farming regimes, including organic, low-input, and conventional, influence soil phospholipid fatty acid (PLFA) profiles. Microorganisms with mono-unsaturated fatty acids increased with organic composts in organic and low-input systems. Soils under no-till and conventional till management were analyzed by PLFA and denaturing gradient electrophoresis (DGGE) profiling, and the results indicated that no-till soils had higher microbial populations and greater diversity of ammonia-oxidizing bacteria (Phillips et al., 2000). Fraser et al. (1994) reported a 10–26% increase in microbial biomass under organic management. The addition of animal or green manures on organic farms provided a significantly greater input of organic carbon, which increased bacterial populations. Mäder et al. (2002) reported results for a 21-year study of agronomic and ecological performance of biodynamic, bioorganic, and conventional farming systems in central Europe. They found enhanced soil fertility and higher biodiversity in organic than conventional plots and concluded this may render organic systems less dependent on external inputs. Moreover, other researchers have shown that incorporation of organic amendments increased soil microbial activity (Elliott and Lynch, 1994), microbial diversity (Girvan et al., 2004; Grayston et al., 2004), densities of bacteria (van Bruggen and Semenov, 2000), fluorescent *Pseudomonas* spp., pathogenic bacteria, fungi, and nematodes (Abawi and Widmer, 2000).

Although the majority of research has shown increased microbial diversity in soils from organic farming systems compared to conventional farming systems, some studies have found different results. Shannon et al. (2002) studied microbial communities in soils managed under organic and conventional regimes, and found conflicting evidence that the size, composition and activity of the soil microbial biomass were attributed to management practice. They found that differences in microbial communities in soils under different management practices were subtle rather than dramatic. Many of the parameters measured, including total carbon and microbial biomass carbon, often showed no consistently significant differences in soils under different management regimes.

Conventional farming systems have been associated with loss of soil fertility, soil erosion, and ground water pollution (Drinkwater et al., 1995). In addition, some conventional agricultural practices inhibit the activity and function of soil microbes. For instance, insecticide applications may promote changes in population biodiversity and dynamics by inhibiting or killing components of the soil microbial community. Fungicide application can cause significant changes to the relative sizes of the bacterial and fungal communities in soil (Sall et al., 2006; Sigler and Turco, 2002). Furthermore, commercial fertilizers used in conventional farming systems interact with microbial communities in soils in a number of ways either promoting growth directly by providing nutrients or indirectly by

stimulating plant growth and enhancing root carbon flow (Buyanovsky et al., 1987). Alternatively, fertilizer inputs may acidify soil limiting microbial growth and activity (O'Donnell et al., 2001).

The objectives of our research were to evaluate the impact of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors including soil microbial species and functional diversity and their effect on Southern blight caused by the Basidiomycete plant pathogen *Sclerotium rolfsii*.

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## 2. Materials and methods

### 2.1. Soil sampling

Soils from 10 farms in North Carolina with a history of organic, sustainable, or conventional crop production were sampled in August 2001, May 2002 and May 2003 (Table 1). Three of the farms were certified organic and did not use synthetic fertilizers or pesticides. They were located in Cedar Grove, NC (organic farm 1), Bear Creek, NC (organic farm 2), and Ivanhoe, NC (organic farm 3). Three of the farms sampled were classified as sustainable, meaning that synthetic pesticides were not used, however synthetic fertilizers were used. These farms were located in Graham, NC (sustainable farm 1), Bear Creek, NC (sustainable farm 2), and Clinton, NC (sustainable farm 3). Four conventional farms were sampled. These farms used monoculture, synthetic fertilizers, pesticide and herbicides. These farms were all located in or near Clinton NC (conventional farms 2–4) or in Faison NC (conventional farm 1). Details of the crops grown, pesticides used and soil fertility amendments are shown in Table 1.

Three composite soil samples were collected from each of the 10 farms in the fall of 2001, and late spring of 2002 and 2003. Composite samples were done by sampling approximately 20 kg of soil from each of three contiguous areas at each farm using a 2.5 cm soil auger in a serpentine pattern down each row to a depth of 20 cm and bulking samples. Bulked samples were kept separate by location within each field so three replications were maintained. Composite soil samples were stored in coolers on ice until returning to the lab. For soil dilution plating and Biolog analysis, the soils were transferred to a storage room and stored at 4 °C until the time of analysis; for DGGE analysis, the soils were stored immediately in a –20 °C freezer until the time of analysis. Biolog assays were done within 48–72 h after sampling soils and soil dilutions within a week after sampling.

### 2.2. Soil physical properties

Two undisturbed soil cores were removed from each of the three locations at each farm for bulk density and water release measurements. Undisturbed soil cores collected from each field with soil sampling rings of known volume were weighed and then dried in an oven and reweighed for bulk density assays. Soil porosity was calculated and soil water content was determined using standard procedures (Chancellor, 1976, Mehlich, 1973).

**Table 1 – Cropping history of three organic, three sustainable and four conventional farms in North Carolina**

Grower type (location)	Texture	Years farming	1997	1998	1999	2000	
Organic 1 (Cedar Grove)	Clay loam with sand	Since 1984	Leafy greens	Leafy greens	Tomato	Tomato	
Organic 2 (Bear Creek)	Loamy sandy	Since 1980	Vegetable, flower, vetch	Vegetable, flower, vetch	Vegetable, flower, vetch	Vegetable, flower, vetch	
Organic 3 (Ivanhoe)	Loamy sandy	Since 1989	Squash, rye, crimson clover	Sweet corn, rye, crimson clover, vetch	Sweet corn, rye, crimson clover	Cantelope, watermelon, rye, crimson clover, vetch	
Sustainable 1 (Graham)	Clay with rock	Since 1981	Blackberry, sod	Tomatoes, leeks, crimson clover and oats	Flowers	Flowers, cowpeas, Sudan grass, rye, vetch	
Sustainable 2 <sup>a</sup> (Bear Creek)	Silt loam	Since 1979	Greenhouse tomato	Greenhouse tomato	Greenhouse tomato	Greenhouse tomato	
Sustainable 3 (Clinton)	Loamy sandy	Since 1996	Tomato	Tomato	Tomato	Tomato	
Conventional 1 <sup>b</sup> (Faison)	Loamy sandy	Since 1977	Strawberry	Tomato	Strawberry	Tomato	
Conventional 2 (Clinton 1)	Loamy sandy	Since 1992	Rye	Rye	Rye	Rye	
Conventional 3 (Clinton 2)	Loamy sandy	Since 1988	Tobacco	Tobacco	Tobacco	Tobacco	
Conventional 4 (Clinton 3)	Silt loam	Since 1987	Cucumber, rye	Pepper, rye	Cucumber, rye	Pepper, rye	
Grower	2001	2002	2003	Fertility amendments	Fungicides	Herbicides	Insecticides
Organic 1	Tomato	Leafy greens	Flowers	Bloodmeal, crabshell meals, oats, rye, vetch, and crimson clover cover crops, feathermeal, sulfate of potash	None	None	None
Organic 2	Pepper	Potato	Potato	Rye, crimson clover, Sudan grass, and buckwheat cover crops, composted manure, feathermeal, langbeinite	None	None	
Organic 3	Tomato	Leafy greens	Leafy greens	Duckweed, chicken compost, hairy-vetch cover crop, leaf litter	None	None	
Sustainable 1	Pepper	Assorted flowers	Assorted flowers	Rye/hairy-vetch and soybean cover crops, vermicomposting in transplants, feathermeal, rock phosphate, potassium sulfate	None	None	
Sustainable 2 <sup>a</sup>	Greenhouse tomato	Greenhouse tomato	Greenhouse tomato	Potassium sulfate, potassium nitrate, ammonium nitrate, magnesium sulfate, micronutrients (iron and boron), leaf litter	Solarization <sup>a</sup>	Solarization <sup>a</sup>	Insect feeding nematodes
	Pepper	Tomato	Tomato	10–10–10 NPK	None	None	None
Conventional 1 <sup>b</sup>	Strawberry	Tomato	Strawberry	4–0–8, 6–6–18 NPK, calcium nitrate	Synthetic	Methyl bromide	Unknown
Conventional 2	Pepper	Cucumber	Cucumber	6–6–18 NPK, calcium, rye cover crop	Synthetic	Devernal	Nemacur Orthene
Conventional 3	Pepper	Tobacco	Tobacco	6–6–18 NPK	Synthetic	Devernal	Orthene
Conventional 4	Pepper	Cotton	Corn	6–6–18 NPK, calcium nitrate, turkey litter, rye cover crop	Synthetic	Devernal	Orthene

<sup>a</sup> Soil solarized every year after harvest.

<sup>b</sup> Soil fumigated with methyl bromide under black plastic every season prior to planting.

### 2.3. Soil chemical properties

Soil chemical properties were assessed by the Soil Testing Laboratory of the North Carolina Department of Agriculture and Consumer Service (NCDA) (Raleigh, NC, USA, <http://www.ncagr.com/agronomi/sthome.htm>). Parameters measured included: extractable acidity, cation exchange capacity, base saturation, soil pH, humic matter, macro- and micro-nutrients including calcium, magnesium, sodium, manganese, zinc, copper, phosphorus and potassium (Mehlich, 1973, 1984; Mehlich et al., 1976).

Soil extractable organic carbon and nitrogen were estimated by the methods of Hu et al. (1997) and net nitrogen mineralization was calculated by the methods of Hart et al. (1994).

### 2.4. Soil microbial and nematode communities and activity

Numbers of culturable bacteria, fluorescent pseudomonad bacteria, enteric bacteria, total fungi, thermophilic microorganisms, *Trichoderma* spp. and oomycetes species including *Phytophthora* spp. and *Pythium* spp. were quantified from each of the three composite samples per farm using dilution plating according to methods described by Bulluck and Ristaino (2002a). Soil dilutions were done in dilute water agar (0.225 g agar + 90 ml water). Soil samples were analyzed for selected soil microorganisms using 10-fold serial dilutions of soil on different selective media (Tryptic-soy agar was used for the characterization of total bacterial populations, yeast-glucose agar for thermophilic microorganisms, King's medium B for *Pseudomonas* spp., Endo agar for enteric bacteria, potato-dextrose agar for total fungi, *Trichoderma* medium E for *Trichoderma* spp. and Masago medium for oomycetes including *Pythium* and *Phytophthora* spp.). Data were expressed as number of colony-forming units (CFUs)/g of dry soil. Three plates of media were used per dilution from each of the three replicated soil samples from each farm for population estimation. Media, light, and temperature conditions are reported in Bulluck and Ristaino (2002a).

Microbial biomass carbon and microbial biomass nitrogen were determined using the chloroform fumigation extraction methods of Vance et al. (1987) and Ross (1992). Soil microbial respiration was estimated using an incubation-alkaline absorption method (Coleman et al., 1978). Total bacterial numbers were estimated by direct counts after staining of appropriate soil dilutions with fluorescein isothiocyanate (FITC) Babiuk and Paul, 1970). Active bacteria and total and active hyphal lengths were determined by staining with fluorescein diacetate (FDA) (Ingham and Klein, 1984; Lundgren, 1981), preparing an agar suspension and determining the length of fluorescent (epifluorescent microscopy) and total (phase contrast microscopy) hyphae present in replicated microscopic fields (Ingham et al., 1990). Biomass was estimated by multiplying bacterial and fungal biovolume by the average bacterial (0.33 g/cm) or hyphal density (0.41 g/cm) (Ingham et al., 1990). Total and active soil bacterial and fungal biomass were determined by Soil Foodweb, LLC (Corvallis, Oregon USA, <http://www.soilfoodweb.com>).

Soil samples (500 cm<sup>3</sup>) were assayed for bacterial feeding nematodes, fungal feeding nematodes, omnivorous nema-

todes, and plant parasitic nematodes by the methods of elutriation (Byrd et al., 1976) and centrifugation (Barker et al., 1996). Nematodes were identified to trophic levels and counted under an inverted microscope at a 40–100 magnification.

### 2.5. Functional diversity using Biolog analysis

Carbon source utilization patterns of soil microbial communities, also called community level physiological profiling (CLPP), were assessed using Biolog 96-well Ecoplates (Biolog, Inc., Hayward, CA, USA) that contained 31 different carbon sources. Bacteria were extracted from 5 g (dry weight) of soil with 45 ml of 1 M K<sub>2</sub>HPO<sub>4</sub> 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 the inoculating suspension was diluted to 10<sup>-5</sup>. Bacterial inoculations were done by transferring 145 µl of the soil dilution on each of the 96 wells on the Ecoplates, and incubating the plates at 26 °C for 4 days. 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 in order to eliminate variation in well color development caused by different cell densities (Garland, 1996; Franklin et al., 2001; Zak et al., 1994). All optical density (OD) readings were adjusted by measuring the OD reading in well 1 (control with water). If a negative number was obtained, it was set to zero.

#### 2.5.1. Statistical analysis of Biolog data

Biolog substrate utilization patterns were analyzed to determine functional diversity (Garland, 1996; Shannon and Weaver, 1963) including substrate richness (the number of substrates utilized), substrate evenness (the distribution of color development between the substrates), and diversity as measured by the Shannon diversity index [ $H' = -\sum Pi \log Pi$ , where  $Pi = (\text{OD reading of well } i) / (\text{sum of all wells})$ ] based on the OD of wells in the Biolog Ecoplates. Diversity, richness, and evenness were compared for each sample by analysis of variance using the Proc GLM procedure of SAS 8.0 (SAS Institute, Inc., Cary, NC, USA). Alpha levels <0.05 were used to denote statistical significance.

### 2.6. Species diversity using DGGE analysis

#### 2.6.1. DNA extraction and PCR amplification

DNA was extracted from soil samples (0.5 g) using a MO BIO kit (MO BIO Laboratories, Inc., CA, USA). Two microliter of DNA was used for PCR amplification. Each 50 µl reaction mixture contained 5 µl of 10× PCR buffer (Invitrogen, Carlsbad, CA, USA), 15 µl of deoxynucleoside-triphosphate mix (2.5 mM each), 2 µl bovine serum albumin (mg/ml), 2 µl of both forward [5'-CCACACTGGGACTGAGACACG-3' (310F, 21 bp)] and reverse primers [5'-GTATTACCGCGGCTGCTGGCA-3' (516R, 21 bp)] (20 µM) (primers amplify bacterial 16s ribosomal DNA) and 0.5 µl *Taq* polymerase (5 U/µl) (Invitrogen). A 40-base GC-clamp was attached to the forward primer for DGGE analysis (Muyzer et al., 1993). 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 a total of 30 cycles, with the extension at 72 °C for 10 min.

### 2.6.2. DGGE analysis

DGGE was performed with a decode universal mutation detection system (Bio-Rad). Twenty microliters of pooled PCR products from each field sample 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.

### 2.6.3. Statistical analysis for diversity indices

After bands were assigned to the gel tracks, the Shannon–Weaver index ( $H$ ) (Shannon and Weaver, 1963) was calculated with the following equations:  $H = -\sum P_i \log P_i$ , where  $P_i$  is the importance probability of the 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).  $P_i$  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 ( $R$ ) is a simple count of the number of species found in a community. Evenness ( $E$ ) was calculated using the equation  $E = H/\ln R$  (Eichner et al., 1999). Diversity values, richness, and evenness were compared with each sample by analysis of variance using the Proc GLM procedure of SAS 8.0. An alpha level <0.05 was used to denote statistical significance.

### 2.7. Incidence of Southern blight on tomato

Soil from each location in 2002 and 2003 (3 replications by 10 locations) was placed in 10-cm, steam-sterilized plastic pots (5 plants/pot). Soils were immediately infested with *S. rolfisii* (SRCT04 tomato isolate) that was grown for 2 weeks on sterilized oat grains. Six colonized oat grains were buried at a depth of 0.6 cm below the soil surface and 1.25 cm away from the plant stem. Four-week-old tomato seedlings (cultivar Rio Colorado) were transplanted 2 weeks after infestation of soil. Plants were watered from below by filling saucers with 80 ml of water. Disease incidence was measured weekly for 5 weeks.

### 2.8. Statistical analysis

The Statistical Analysis Systems software (PC-SAS 8.0; SAS Institute) was used to analyze data. A generalized linear model procedure (Proc GLM) was used and an analysis of variance was performed for incidence of Southern blight, soil physical, chemical and biological properties. Correlation analyses were performed to relate disease and microbial populations with soil physical, chemical and other biological properties.

## 3. Results

### 3.1. Soil physical properties

There were significant differences in soil bulk density, porosity and water content among soils from the three different

**Table 2 – Soil physical properties in organic, sustainable and conventional farms**

Soil physical property	2001	2002	2003	Average
Bulk density, BD (g/cm <sup>3</sup> )				
Organic	1.07	0.94	1.08	1.03
Sustainable	1.10	0.92	1.07	1.24
Conventional	1.23	1.35	1.43	1.34
	0.0005**	<0.0001**	<0.0001**	
Soil porosity, SP (%)				
Organic	0.61	0.64	0.63	0.63
Sustainable	0.55	0.60	0.56	0.58
Conventional	0.45	0.48	0.46	0.47
	<0.0001**	<0.0001**	<0.0001**	
Soil water content (%)				
Organic	15.53	27.21	18.40	20.38
Sustainable	14.78	20.97	19.18	18.31
Conventional	11.07	9.81	11.03	10.64
	<0.0001**	<0.0001**	<0.0001**	
Humic matter (g/100 cm <sup>3</sup> )				
Organic	1.10	0.80	0.60	0.80
Sustainable	0.70	0.30	0.50	0.50
Conventional	0.90	0.60	0.70	0.60
	<0.0118*	<0.0048*	<0.1868	

\* Significantly different at  $P = 0.05$ .

\*\* Significantly different at  $P = 0.001$ .

farming systems in each year (Table 2). Soil bulk density was significantly higher in soils from conventional than organic or sustainable farms (Table 2). However, soil porosity and soil water content were highest in soils from organic farms (Table 2). In 2 of 3 years soil humic matter was significantly higher in soils from organic than conventional farms (Table 2).

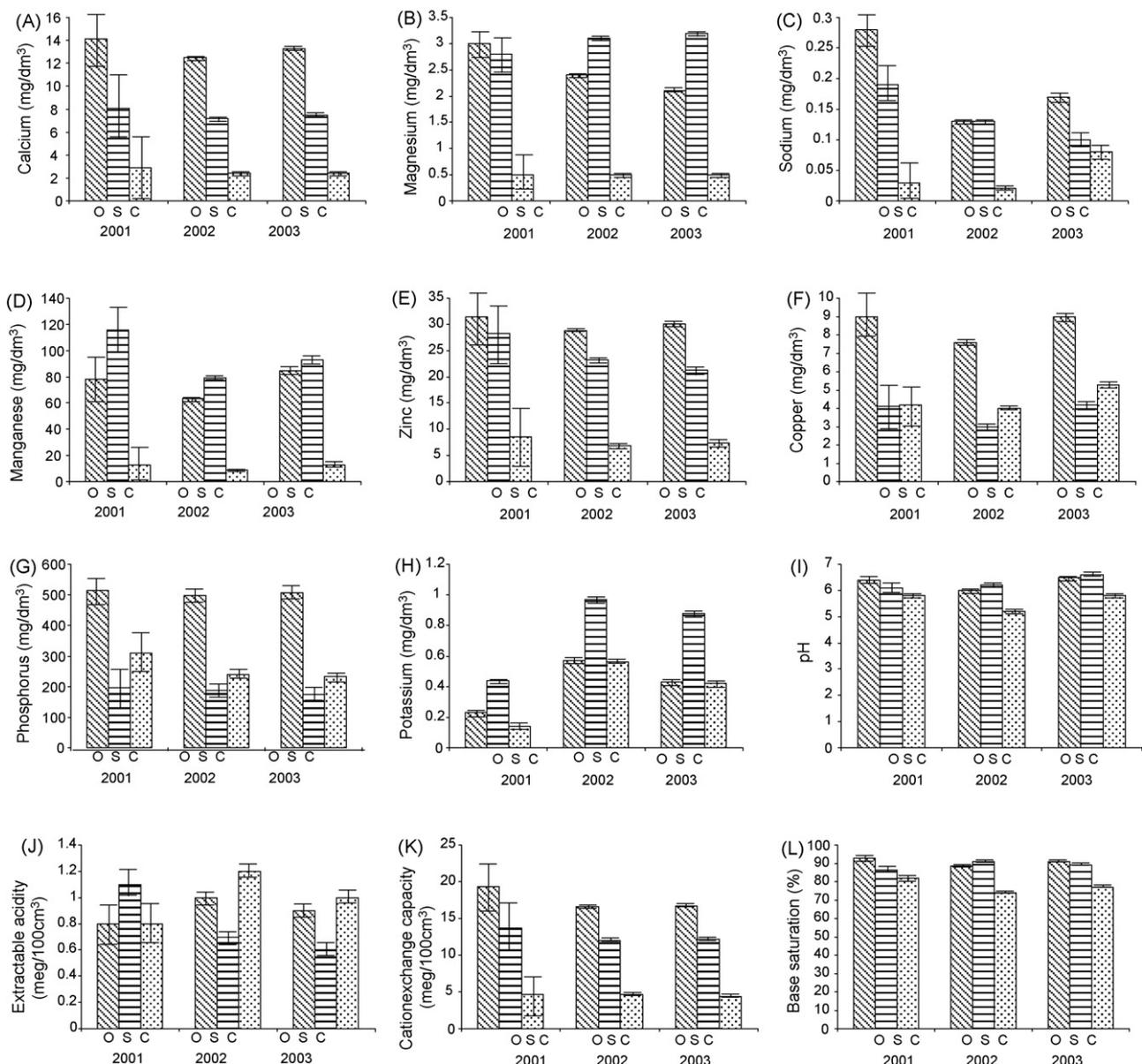
### 3.2. Soil chemical properties

Soils from organic and sustainable farms had significantly higher levels of soil calcium, magnesium, sodium, manganese, and zinc than soils from conventional farms in each year (Fig. 1A–E). Soils from organic farms also had higher levels of soil copper and phosphorus than soils from sustainable and conventional farms (Fig. 1F and G). The level of potassium was

more variable among farms and from year to year (Fig. 1H). Soil pH levels, the cation exchange capacity and base saturation levels were also significantly higher in soils from organic and sustainable than conventional farms (Fig. 1I, K and L). However, the level of extractable acidity was more variable between production systems and year (Fig. 1J).

### 3.3. Soil microbial biomass and extractable carbon and nitrogen and soil respiration

Extractable soil carbon, microbial biomass carbon and nitrogen, net mineralized nitrogen and soil microbial respiration rate were all significantly higher in soils from organic and sustainable farms than in soils from conventional farms for all three years of the study (Table 3). Extractable nitrogen was also



**Fig. 1 – Chemical properties of soils from organic, sustainable and conventional farms in North Carolina in 2001 to 2003 including: (A) calcium; (B) magnesium; (C) sodium; (D) manganese; (E) zinc; (F) copper; (G) phosphorus; (H) potassium; (I) pH; (J) extractable acidity; (K) cation exchange capacity; (L) base saturation. O represents organic farms, S represents sustainable farms and C represents conventional farms.**

**Table 3 – Soil extractable carbon and nitrogen, microbial biomass carbon and nitrogen and net mineralizable nitrogen soil microbial respiration rate in soils from organic, sustainable, and conventional farms in North Carolina for fall 2001, spring 2002, and spring 2003**

Production system	Variable <sup>a</sup>					
	Extractable (mg/kg)		Microbial biomass (mg/kg)		Net mineralized nitrogen (mg/kg)	Microbial respiration (CO <sub>2</sub> mg/kg)
	Carbon	Nitrogen	Carbon	Nitrogen		
Year 2001						
Organic	199.1	34.8	702.5	67.1	27.7	96.7
Sustainable	168.6	109.8	339.3	47.7	22.2	50.2
Conventional	53.0	8.8	138.6	15.3	7.8	11.0
Year 2002						
Organic	137.5	70.9	758.0	123.2	53.5	111.2
Sustainable	93.7	90.8	455.2	62.9	93.4	31.8
Conventional	29.6	52.5	110.1	10.1	28.7	5.2
Year 2003						
Organic	170.3	20.7	879.4	76.3	31.8	123.5
Sustainable	155.1	34.7	536.4	40.0	35.8	34.7
Conventional	66.2	25.6	234.6	20.0	24.9	11.1

<sup>a</sup> All variables were significant at  $P < 0.001$  except extractable N for 2003.

higher in soils from organic and sustainable than conventional farms in 2001 and 2002, but not in 2003.

Total and active fungal and bacterial biomass was also measured in soils from organic, sustainable, and conventional

farms in each year. Total fungal biomass was higher in soils from organic and sustainable than conventional farms in each year (Table 4). However, active fungal biomass and the ratio of active to total fungal biomass were lower in soils from organic

**Table 4 – Total and active fungal and bacterial biomass measurements and biomass ratios in organic, sustainable, and conventional North Carolina field soils sampled in fall 2001, spring 2002, and spring 2003**

Soil microbial biomass	2001	P-value	2002	P-value	2003	P-value	Average
Total fungal biomass ( $\mu\text{g/g}$ ) <sup>a</sup>							
Organic	165.9	0.0557	170.9	<0.0001**	206.7	0.2894	181.17
Sustainable	186.9		128.9		196.2		170.67
Conventional	138.8		79.3		180		132.7
Active fungal biomass ( $\mu\text{g/g}$ ) <sup>b</sup>							
Organic	10.7	0.0006**	5.4	<0.0001**	36.6	0.1485	12.2
Sustainable	32.1		27.1		55		38.07
Conventional	25.3		12.8		55.1		31.07
Active/total fungal biomass ratio <sup>c</sup>							
Organic	0.09	0.0190*	0.04	0.0001**	0.18	0.1011	0.1
Sustainable	0.27		0.37		0.27		0.3
Conventional	0.16		0.15		0.3		0.2
Total bacterial biomass ( $\mu\text{g/g}$ ) <sup>a</sup>							
Organic	110.7	<0.0001**	175.4	<0.0001**	287.3	0.0439*	191.13
Sustainable	121.6		155.2		299.5		192.1
Conventional	152.8		212.9		375.5		247.1
Active bacterial biomass ( $\mu\text{g/g}$ ) <sup>b</sup>							
Organic	41.2	<0.0001**	35.9	<0.0001**	59.4	0.0582	45.5
Sustainable	38.8		63		50		50.6
Conventional	16.7		25.7		44.5		28.97
Active to total bacterial biomass ratio <sup>c</sup>							
Organic	0.37	0.0001**	0.2	0.0001**	0.22	0.0071*	0.26
Sustainable	0.33		0.39		0.19		0.3
Conventional	0.11		0.13		0.12		0.12

<sup>a</sup> Total fungal/bacterial biomass includes the active fraction.

<sup>b</sup> Active fungal/bacterial biomass includes the biomass of organisms that are feeding and reproducing.

<sup>c</sup> Active/total biomass ratio.

\* Significantly different at  $P = 0.05$ .

\*\* Significantly different at  $P = 0.001$ .

**Table 5 – Free-living, omnivorous and plant parasitic nematode populations in organic, sustainable and conventional farms**

Soil nematode trophic group	2001	P-value	2002	P-value	2003	P-value	Average
Bacterial feeding nematodes (number/500 cm <sup>3</sup> )							
Organic	108.9	0.0043**	ND		1497.8	0.0425 <sup>+</sup>	803.3
Sustainable	291.1		ND		846.6		568.9
Conventional	598.7		ND		1845.0		1221.9
Fungal feeding nematodes (number/500 cm <sup>3</sup> )							
Organic	71.1	0.4881	ND		144.4	0.0014**	107.8
Sustainable	75.6		ND		77.8		76.7
Conventional	102.2		ND		38.3		70.3
Omnivorous nematodes (number/500 cm <sup>3</sup> )							
Organic	46.7	0.0064*	ND		93.3	0.4099	70.0
Sustainable	181.1		ND		186.7		183.9
Conventional	86.5		ND		140.0		113.3
Plant parasitic nematodes (number/500 cm <sup>3</sup> )							
Organic	253.3	0.2531	ND		160.0	0.1794	206.7
Sustainable	220.0		ND		88.9		154.5
Conventional	87.8		ND		216.7		152.2

<sup>+</sup> Significantly different at P = 0.05.

<sup>\*\*</sup> Significantly different at P = 0.001.

than sustainable and conventional farms. In contrast, total bacterial biomass was higher in soils from conventional than sustainable and organic farms for all three years of the study. However, active bacterial biomass and the ratio of active to total bacterial biomass were higher in soils from organic and sustainable than conventional farms.

Soils from conventional farms had higher levels of bacterial feeding nematodes than soils from sustainable or conventional farms (Table 5). Fungal feeding nematodes were more variable among production systems. Soils from organic farms had highest levels of fungal feeding nematodes in only 1 of 2 years of sampling (Table 5). Soils from sustainable farms had highest levels of omnivorous nematodes in both years. Levels of plant parasites were not significantly different among farming systems or years.

### 3.4. Soil microbial communities based on soil dilution plating

The abundance of *Trichoderma* species, total culturable bacteria, fluorescent *Pseudomonas* spp. and enteric bacteria were not significant in any year (data not shown). Total culturable fungi were highest in soils from organic farms (Table 6). Oomycete species were also highest in soils from organic farms all three years, but differences were not significant in 2003. Thermophilic organisms were significantly higher in soils from organic and sustainable, than conventional farms in 2002 and 2003.

### 3.5. Bacterial functional diversity and species diversity

Soil bacterial functional diversity indices based on CLPP were significantly higher in soils from organic and sustainable than conventional farms in 2001, and remained higher in soils from organic than sustainable and conventional farms in 2002 and 2003 (Fig. 2A). In contrast, bacterial species diversity indices based on DGGE were similar among soils from organic,

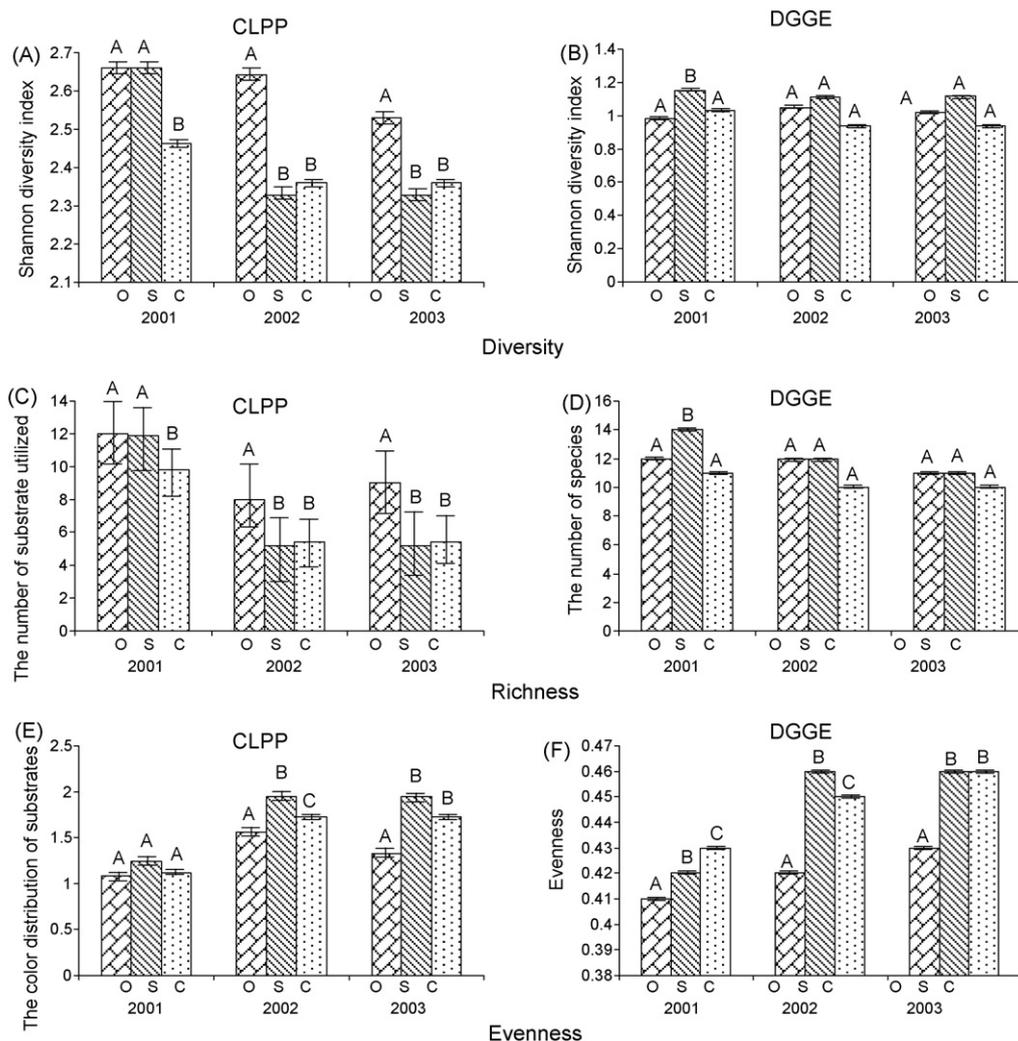
sustainable and conventional farms in each year (Fig. 2B). The richness of bacterial functional communities showed similar trends. Functional richness indices of bacterial communities were higher in soils from organic farms than sustainable or conventional farms over the three years of sampling (Fig. 2C). Bacterial species richness was more similar among farms (Fig. 2D).

There were no significant differences in the evenness of bacterial functional communities in 2001 among the three

**Table 6 – Population densities of select soil microorganisms from organic, sustainable, and conventional field soils sampled in fall 2001, spring 2002, and spring 2003**

Microbial group <sup>a</sup>	Number of colony-forming units/g dry soil		
	2001	2002	2003
Type			
Total culturable fungi			
Organic	$9.8 \times 10^4$	$1.7 \times 10^6$	$2.1 \times 10^6$
Sustainable	$4.9 \times 10^4$	$8.1 \times 10^5$	$1.0 \times 10^6$
Conventional	$5.6 \times 10^5$	$8.5 \times 10^5$	$7.4 \times 10^5$
Pr > F	0.0016	0.0663	0.0344
Oomycete species			
Organic	86	370	80
Sustainable	17	180	43
Conventional	37	80	68
Pr > F	0.0003	0.0009	0.3966
Thermophilic species			
Organic	$3.3 \times 10^3$	$2.5 \times 10^4$	$2.3 \times 10^6$
Sustainable	$2.2 \times 10^3$	$8.7 \times 10^3$	$3.7 \times 10^6$
Conventional	$2.4 \times 10^3$	$4.7 \times 10^3$	$5.2 \times 10^5$
Pr > F	0.598	0.0001	0.0001

<sup>a</sup> Dilutions were 10-fold serial dilutions in 0.25% water agar onto the following media: potato-dextrose agar (100 µg/ml streptomycin sulfate) for total culturable fungi; Masago's media for oomycete species; and yeast-glucose agar for thermophilic species.



**Fig. 2 – (A–F) Bacterial functional diversity, richness and evenness indices based on community level physiological profiles (CLPP) and bacterial species diversity based on denaturing gradient gel electrophoresis (DGGE) in soils from organic, sustainable and conventional farms. A different letter indicates there was significant difference at  $P < 0.05$  based on t grouping. O represents organic farms, S represents sustainable farms and C represents conventional farms.**

farming systems (Fig. 2E). Evenness of bacterial functional groups was lower in soils from organic than sustainable and conventional farms in 2002 and 2003. Bacterial species evenness was also lower in soils from organic farms than sustainable and conventional farms during each year (Fig. 2F).

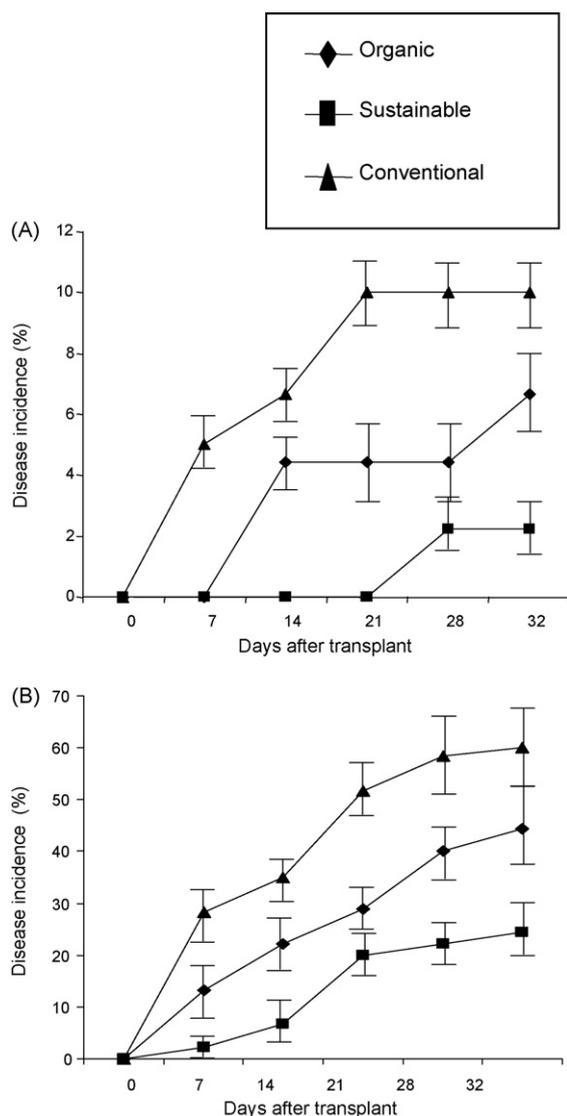
### 3.6. Incidence of Southern blight

The final incidence of Southern blight in the greenhouse bioassays was significantly greater in soils from conventional farms than soils from organic or sustainable farms in each year (Fig. 3). Although, the level of disease in the greenhouse bioassays was higher in 2003 than 2002, clear differences in the progress of disease in soils from the different production systems were evident. Lower levels of disease in each year occurred in soils from the sustainable and organic farms, than the conventional farms.

## 4. Discussion

Soils from organic farms had improved soil quality. Soil physical factors including increased soil water content, soil porosity, and humic matter levels and lower bulk density were evident in soils from organic farms. These growers used a variety of organic fertility amendments but all consistently incorporated fall cover crops unlike the conventional growers. Several other researchers have also shown that organic farming improved the quantity of soils (Drinkwater et al., 1995; Reganold et al., 2001).

Soil chemical factors were also higher in soils from both organic and sustainable farms in contrast to those from conventional farms. Levels of phosphate, calcium, magnesium, pH, cation exchange capacity, base saturation, manganese, zinc, and copper were higher in soils on organic than conventional farms. This agrees with findings from several other studies (Bending et al., 2000; Kennedy and Smith, 1995). We found that soil pH was significantly different in soils from



**Fig. 3 – Disease progress caused by *Sclerotium rolfsii* in soils from organic, sustainable, and conventional farms in North Carolina, 2002–2003. (A) 2002; (B) 2003.**

organic, sustainable and conventional farms and soils from organic farms had highest pH values. However, other researchers have shown that pH was not significantly different between organically and conventionally managed soils (Clark et al., 1998; Mäder et al., 2002; van Diepeningen et al., 2006). The elevated levels of copper and zinc in soils from organic farms may be associated with the use of animal and poultry manures on some of the farms.

Soil microbial respiration rates were higher in soils from organic than sustainable and conventional farms, suggesting that largest activity of soil microorganisms existed in soils from organic farms. Other studies have also shown that biological activity was greater in organically managed than conventionally managed soils (Mäder et al., 2002; van Diepeningen et al., 2006). Microbial biomass carbon, microbial biomass nitrogen, net mineralized nitrogen, and extractable carbon were all significantly higher in soils from organic than sustainable and conventional farms. These results are in

agreement with other studies of soils from California (Poudeh et al., 2002) and the Netherlands (van Diepeningen et al., 2006). Organic farmers apply more organic carbon to their fields to maintain the organic matter in their soils, which may simultaneously increase the microbial populations and microbial respiration rate.

Populations of thermophiles were significantly higher in soils from organic than conventional farms. Thermophilic fungi detected in this study were mainly actinomycetes; which are indigenous soil bacteria which can be responsible for both the degradation of plant debris and inhibition of soil borne diseases (Weller, 1988). Other studies have shown that soils from organic farms (Drinkwater et al., 1995; van Bruggen and Semenov, 2000; Workneh and van Bruggen, 1994) and soils amended with organic fertility amendments (Bulluck et al., 2002c) have higher populations of actinomycetes. Some of these organisms may have been responsible for disease suppressiveness of these soils to Southern blight, but further work would be necessary to confirm this hypothesis.

Our results showed the different farming practices did not significantly influence populations of *Trichoderma* spp. Other studies have shown populations of *Trichoderma* spp. were higher in soils from conventional than organic farms (Weller et al., 2002). *Trichoderma* spp. may be affected to a lesser extent than other soil borne fungi. They can quickly colonized niches left by other organisms following a soil disturbance, such as the application of pesticides or herbicides. In contrast, in previous work, we found that propagule densities of *Trichoderma* species were detected in greater numbers in soils amended with organic than synthetic amendments (Bulluck et al., 2002c). In that study the higher propagule densities of *Trichoderma* spp. were probably related to colonization by the fungus of composts that were incorporated into grower's fields.

Nematodes play a major role in decomposition and nutrient cycling in soil food webs. These organisms are the most abundant multi-cellular organisms in terrestrial and aquatic ecosystems (Bongers and Bongers, 1998). Nematodes vary widely in life-history strategies and functions in agricultural soil (Bongers and Bongers, 1998). Overall nematode abundance tends to be higher in soils under organic management because of the increase in microbial biomass on which the majority of trophic groups feed (Mulder et al., 2003; Neher et al., 2005; Yeates et al., 1993). Generally bacterial feeding nematodes are more abundant under organic management, while fungal feeding nematodes were more abundant in conventionally managed soils (Berkelmans et al., 2003; Clark et al., 1998; Ferris et al., 1996). A higher bacterial and fungal biomass in soil is likely to be responsible for supporting elevated numbers of bacterial and fungal feeding nematodes (Ferris et al., 1996).

We found that bacterial feeding nematodes were highest in soils from conventional farms where bacterial biomass was highest. Some of the conventional fields were located in a region of North Carolina that supports swine production and elevated levels of bacteria in these soils may be a result of application of surface irrigation waters containing bacteria to these fields. In a previous study, we found that populations of bacterial feeding nematodes and fungal feeding nematodes were greater in soils amended with organic amendments

including swine manure, composted cotton gin trash, or ryevetch, than in soils amended with synthetic fertilizer (Bulluck et al., 2002b). Soils amended with swine manure had highest levels of bacteria (Bulluck et al., 2002b). Population dynamics of bacterial feeding nematodes depend on the presence and number of bacteria (Ferris et al., 2001).

We found that populations of fungal-feeding nematodes were higher in soils from organic than sustainable and conventional farms, which is likely a reflection of the higher levels of total fungal biomass and the higher populations of fungi in soils from organic than sustainable and conventional farms. Others have also documented higher fungal populations in organic than conventionally managed soils, whereas bacterial populations were less in organic than conventionally managed soils (Girvan et al., 2004). Berkelmans et al. (2003) suggested that the high level of fungal activity under organic management reported in some studies is an artefact, while Ferris et al. (1996) suggested that the high carbon:nitrogen ratio organic materials commonly added to fields are more likely to select for fungal rather than bacterial-dominated communities. This is probably attributed to the addition of organic fertility amendments such as cover crops, animal manures, feather meal, and plant composts to field soils.

Plant parasitic nematode abundance was more variable and not consistently associated with any particular management system. Plant parasitic nematodes may be more responsive to the host plant than to soil amendments; consequently the crop species may influence nematode community structure more than management practices (Neher et al., 2005). In contrast, Abawi and Widmer (2000) found that plant parasitic nematodes were less in soils amended with different organic substrates, and associated this decrease with the release of ammoniacal nitrogen.

The majority of cases of food-related disease in the US are caused by enteric bacterial pathogens in undercooked meat, eggs, poultry, or contaminated deli meats and are not linked to contaminated produce (Mead et al., 1999). A notable exception is the recent recall of bagged spinach from California due to *Escherichia coli* contamination. Bulluck et al. (2002b) observed higher numbers of enteric bacteria in soils amended with animal manures and other organic amendments than in soils with synthetic fertilizers. However, we did not find a significant change in populations of enteric bacteria in soils in either the long-term organic, sustainable or conventional field soils. Because *E. coli*, *Salmonella* spp., and other enteric bacteria are adapted to an environment with a constant nutrient supply and temperature, their survival rates in soils are minimal and normally do not exceed 10 days (Mead et al., 1999).

The organic farms chosen in this study have practiced organic farming for over 20 years and none were in transition. We found that the functional diversity and richness of bacterial communities was consistently higher in soils from organic than conventional farms. Surprisingly the species diversity and species richness of the bacterial communities were more similar among the three production systems. van Diepeningen et al. (2006) found that the bacterial species diversity and richness steadily increased as years of organic management increased. This diversity increase also increases the resilience of soils leading to improved soil health (van

Bruggen and Semenov, 2000). Our data indicate that although the species diversity of soil bacterial communities was not different among the farming systems, the functional diversity as indicated by carbon substrate utilization patterns was changed by management practices.

Elevated bacterial numbers were not found in organically managed fields in our study or other studies (Girvan et al., 2004). Buckley and Schmidt (2001) failed to detect significant differences in the abundance of microbial group rRNA abundances in five fields despite differences in chemical inputs, tillage, plant composition, and productivity. It is likely that soil type and plant varieties could exert more influence on soil microbial communities than different soil management practices (Felske et al., 1998; Girvan et al., 2003; McCaig et al., 1999; van Diepeningen et al., 2006). This is probably due to the gradual transformation from conventional to organic farming systems. In addition, sometimes, conventional growers adopt measures used in organic management systems such as the incorporation of organic fertility amendments into their soil to increase soil fertility.

There was significantly less Southern blight caused by *S. rolfisii* in soils from sustainable and organic farms than conventional farms. We could not consistently correlate any specific soil physical, chemical, or biological factors with disease suppression in soils from the organic and sustainable farms. Clearly, soils from organic farms had greater microbial activity and specific components of the soil microbial communities may have been suppressive to disease (Garbeva et al., 2004; Weller et al., 2002; Workneh and van Bruggen, 1994; Wiggins and Kinkel, 2005). However, further work will be needed to sort out the exact mechanism of disease suppression in these soils.

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