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Effects of organic amendment and tillage on soil microorganisms and microfauna☆

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ABSTRACT

Soil microorganisms (bacteria, fungi) and microfauna (nematodes, protozoa) have been shown to be sensitive to organic amendments, but few experiments have investigated the responses of all these organisms simultaneously and across the soil profile. We investigated the impact of organic amendment and tillage on the soil food web at two depths in a field experiment. Over three growing seasons, field plots received seasonal organic amendment that was either incorporated into the soil (tilled) or not (no-till) as part of a tomato/soybean/corn cropping system. Un-amended, control plots that were either tilled or no-till were also included. We hypothesized that the addition of amendments would have a bottom–up effect on the soil food web, positively influencing the abundance of microorganisms, protozoa, and nematodes, primarily in the surface layers of the soil, but that this effect could be extended into deeper layers via tillage. Organic amendment had positive effects on most measured variables, including organic matter, respiration, protozoan and nematode density, and the abundance of PLFA biomarkers for bacteria and fungi. These effects were more pronounced in the 0–5 cm depth, but most variables increased with amendment in the deeper layer as well, especially with tillage. Denaturing Gradient Gel Electrophoresis (DGGE) of bacterial rDNA fragments indicated that distinct bacterial communities were selected for among tillage and amendment treatments and depths. Nematode faunal indices were not influenced by amendment, however. Increased nematode density in amended soils encompassed all trophic groups of free-living nematodes, with the greatest response among fungal-feeders, particularly with tillage. Increased biomass of microorganisms and decomposer microfauna in amended, tilled soils (0–5 cm depth) corresponded with a decline in the abundance of plant-parasitic nematodes. In control soils (0–5 cm depth), tillage reduced the relative abundance of fungal-feeding nematodes and increased the density of bacterial-feeding nematodes, in particular nematode species contributing to the Enrichment Index. When combined with organic amendment however, tillage was associated with increases in fungal-feeding nematodes and fungal biomarker PLFA. The results of this study suggest that when combined with amendment, tillage enhances the soil food web beyond the effect of amendment alone and is associated with declines in plant-parasitic nematodes.

1. Introduction

The goal of sustainable agricultural practices is to control pests (weeds, parasites, and pathogens) and maintain soil fertility and crop yields, while minimizing or eliminating synthetic chemical inputs. The application of organic amendments to soils (e.g., cover crops, manures, plant biomass) is an integral tool for sustainable agriculture, as evidenced by the increasing interest in optimizing this strategy (Lu et al., 2000). Organic amendment has been shown to enhance soil organic matter and fertility, as well as prevent erosion (Snapp et al., 2005). Less understood, however, are the effects of organic amendments on soil food webs, which contain the biotic assemblages responsible for decomposition and generation of soluble nutrients for plant uptake. Soil food webs also contain parasitic organisms, such as plant-parasitic nematodes, whose densities are influenced by the presence of host plants, the soil environment, and regulation by predators and pathogens (all factors that are potentially influenced by organic amendments). Maximizing the efficacy of organic amendments toward improving soil health requires an

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understanding of how this practice affects the entire soil food web (beneficial and pathogenic/parasitic components), and how these effects are mitigated by other agricultural practices, such as tillage.

The addition of organic amendments to soil contributes to organic matter and has great potential for influencing the structure and function of the soil food web and possibly inducing nematode suppression (Widmer et al., 2002; Oka, 2009). Organic amendments are known to increase the abundance of various components of the soil food web, including the soil microbial community, protozoa, and microbivorous nematodes (Lundquist et al., 1999; Fu et al., 2000; Ferris et al., 2004; Saison et al., 2006; Carrera et al., 2007; Forge et al., 2008). Tillage is frequently integrated within the practice of adding organic amendments to soil, although tillage may have detrimental effects on soil organisms (Lenz and Eisenbeis, 2000; Berkelmans et al., 2003). By itself, tillage is associated with reduced fungal biomass (Beare et al., 1997; Frey et al., 1999; Simmons and Coleman, 2008) and in turn can affect fungal-feeding nematodes (Beare et al., 1992). However, tillage can also be used to incorporate amendments into the soil, and therefore should expand their effect into deeper soil layers. Bacterial-based pathways may show the greatest response when amendments are combined with tillage, while without tillage, fungal-based pathways may predominate (Moore, 1994). While soil microorganisms (bacteria, fungi) and microfauna (nematodes and protozoa) have been shown to be very responsive to soil amendments, few experiments have investigated the responses of all these organisms simultaneously and across the soil profile.

The specific aim of this research was to investigate the impact of soil organic amendments on the decomposer food web in a tomato/soybean/corn cropping system. We hypothesized that the addition of amendments would positively influence the abundance of microorganisms, protozoa, and bacterial- and fungal-feeding nematodes, primarily in the surface layers of the soil, but that this effect could be extended into deeper layers via tillage. Few field studies investigate soil biotic responses at multiple trophic levels (microbes, protozoa, nematodes), but integration of microbial and microfaunal analyses will allow us to comprehensively study the impact of treatments (amendment and tillage) on fungal and bacterial-based decomposition pathways.

2. Materials and methods

2.1. Description of field experiment

In 2005, a microplot field trial was established to investigate the effects of organic amendments with different carbon and nitrogen attributes, as well as the timing of these amendments, on the soil food web and soil suppressiveness towards plant-parasitic nematodes; a subset of those plots was sampled for this research. The field trial was established at the USDA-ARS-Beltsville Agricultural Research Center, Maryland, USA. The soil was classified according to the USDA texture classification scheme as a loamy sand, varying between 63–83% sand, 7–27% silt, and 2–16% clay. Forty microplots were randomized across the area, and treatments were replicated four times. Microplots were 2 m^2 and separated from each other by 1 m on each side. Throughout the course of the experiment, the plots were hand weeded, and the alleyways maintained by mowing. Irrigation was applied as needed by overhead sprinkler. Regardless of treatment, the same cash crop rotation, tomato/soybean/corn, was planted across the field site. Cash crops were managed according to Maryland IPM recommendations for pesticide application. Fertilizer was applied based upon annual soil tests.

Four treatments were selected for sampling: (1) no organic amendment added to soil, with tillage occurring in May and October of each year (control till); (2) no organic amendment added without tillage (control no-till); (3) a low C/N organic amendment [biosolid, compost, or vetch (*Vicia villosa*) plant material] added to soil in May of each year and a high C/N organic amendment (bailed straw) added to soil in October of each year, both with tillage occurring immediately after amendment (amend till); (4) a low C/N organic amendment (biosolid, compost, or vetch plant material) added to soil in May of each year and a high C/N organic amendment (straw) added to soil in October of each year, both with no tillage (amend no-till). For those treatments that received organic amendment, in year 1 (2005), an alkaline-stabilized biosolid was applied in May [10 Mg dry material ha^{-1}, C/N = 6; see Zasada et al. (2008) for detailed chemical description], and straw (39.5 Mg dry material ha^{-1}, C/N = 40) was applied in October. In year 2 (2006), compost (10 Mg dry material ha^{-1}, C/N = 5) was applied in May, and straw was applied in October as described above. In year 3 (2007), vetch was grown in a field adjacent to the microplots, and was transferred to the appropriate plots in May (0.7 Mg dry material ha^{-1}, C/N = 9). Microplots that received tillage were tilled to a depth of approximately 20 cm with a 0.6-m tractor-mounted rotovator.

Soils were sampled early (21 June) and late (28 August) within a growing season (2007) in which the plots were planted with corn. At each plot, 10 2.5-cm-diameter soil cores were collected randomly at each of two depths (0–5 cm and 5–25 cm), from beneath the plant canopy (i.e., within 20–30 cm of stalk). Where present, loose surface litter was manually scraped away prior to coring. The 10 cores from each depth were bulked and placed in a cooler for transportation to the laboratory. Each sample was hand-mixed and divided into subsamples prior to analyses.

2.2. Soil properties

Soil moisture was determined gravimetrically (110 °C, 48 h). Soil organic matter was measured as loss on ignition (400 °C, 12 h). Bulk density was measured by collection of 10-cm-diameter soil cores from each plot, cutting them into 0–5 cm and 5–25 cm sections, and drying at 104 °C for 48 h before weighing.

2.3. Protozoan density

Total protozoa (active and encysted flagellates, amoebae, and ciliates combined) were estimated by the most-probable-number method (Treonis and Lussenhop, 1997). Ten grams of soil were dispersed in 90 ml sterile 0.9% NaCl by shaking vigorously on a platform shaker for 10 min and then diluted serially (1:10) into 9 ml sterile 0.9% NaCl. One-ml aliquots of each dilution level (10^{-1} to 10^{-6}) were plated onto 24-well cell culture plates, each well of which contained 1 ml of sterile media (0.9% NaCl, 0.5% soil extract). Plates were incubated at 18 °C, and each dilution was observed with a phase contrast, inverted microscope for the presence or absence of ciliates, flagellates, and amoebae during the first, second, and third weeks of incubation.

2.4. Soil respiration

Relative soil respiration rates (i.e., CO_2 evolution) were determined by incubating 25 g soil samples at 22 °C inside of sealed pint-sized glass jars (473 ml). At the end of the incubation period (7 days), the CO_2 concentration of a 25-ml headspace gas sample, withdrawn from each jar through a septum in the lid using a syringe, was determined using an infrared gas analyzer (LI-820, LI-COR, Inc., Lincoln, NE, USA; Zibilske, 1994).

2.5. Nematode density and community structure

Nematodes were extracted from 50 g (0–5 cm depth) and 200 g (5–25 cm depth) of soil using a semi-automatic elutriator (Ingham,

1994), and nematodes were caught on a 400-mesh (38 μm aperture) sieve. Differences in the amount of soil extracted were due to availability. The 400-mesh sieve was backwashed into a container and the contents transferred to a Baermann funnel; nematodes were collected after 72 h. Nematodes were immediately fixed in 5% formalin solution for future enumeration. A dissecting microscope was used to count the total number of nematodes per sample. The sample was centrifuged, and the supernatant removed. The pellet and remaining small amount of formalin solution were spread on a microscope slide and covered with a cover slip (Ferris and Matute, 2003). Two hundred nematodes on each slide (minimum) were identified to genus level when possible using a compound microscope. The exceptions were nematodes in the families Tylenchidae and Rhabditidae. The actual abundance of each taxon was adjusted according to the total number of nematodes in the sample.

Nematodes were assigned to trophic groups based on Yeates et al. (1993). Morphospecies from the family Tylenchidae were considered to be fungal-feeding. Nematode faunal indices were calculated as described by Ferris et al. (2001) and included the Basal Index (BI), Structure Index (SI), Enrichment Index (EI), and Channel Index (CI). These indices provide information regarding the food web structure and decomposition pathways, and each is based on the abundance of nematodes within various trophic groups (bacterial-feeders, fungal-feeders, omnivore/predators), as well as the life history characteristics of the taxa. Nematode taxa that respond fast to nutrient inputs due to high fecundity are considered to be colonizers, and taxa that are long-lived and slow to establish, requiring stable conditions, are considered to be persisters. Using these characteristics, soil nematode taxa have been assigned values along a c-p scale (colonizer-persister; Bongers, 1990) that are used in the calculation of the faunal indices (Ferris et al., 2001).

2.6. Microbial community structure: denaturing gradient gel electrophoresis (DGGE)

Total DNA was extracted from 0.25 g soil samples (June sampling date only) using the MO BIO Powersoil™ DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA) following the manufacturer's instructions. DNA yield and quality were assessed electrophoretically on 0.8% agarose gels. A nested PCR technique was used to amplify a 177 bp segment of the V3 hypervariable region of the bacterial 16S rDNA. The first round of PCR, using primers GM3F and GM4R (Muyzer et al., 1995), was performed by mixing 2 μl DNA template, 2 μl 10 μM GM3F, 2 μl 10 μM GM4R, 6.5 μl nuclease-free water, and 12.5 μl TaKaRa Premix Taq™ (Takara Bio Inc., Otsu, Japan), for a final volume of 25 μl. Reactions were carried out in a Bio-Rad PTC-1148 Minicycler (Bio-Rad Laboratories, Hercules, CA, USA) with an initial denaturing step at 95 °C for 5 min, 30 cycles of denaturing at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1.5 min, and final extension at 72 °C for 10 min. Product from this reaction (2 μl) was used as template for a second round of PCR using the primers EUB341GC and UNIV518 (Muyzer et al., 1993) with the same PCR regime as above, apart from an annealing temperature of 60 °C and an extension time of 1 min per cycle. A GC-clamp was added to the 5′ end of the forward primer EUB341GC (CGCCCGCCGCGCGCG-GCGGGCGGGGCGGGGGCACGGGGGG; Muyzer et al., 1993).

Using the final PCR product, DGGE was performed using the Bio-Rad DCode Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). An 8% (wv^{-1}) polyacrylamide gel with a parallel denaturing gradient between 35 and 65% (where 100% denaturant contains 7 M urea and 40% formamide) was cast using a GM-40 Gradient Maker (CBS Scientific Co., Del Mar, CA, USA) and an MPP-100 Mini Peristaltic Pump (CBS Scientific Co.) set at medium speed. A DGGE lane "marker" was prepared using the nested PCR procedure and stock cultures of *Escherichia coli*, *Agrobacterium tumefaciens*, *Micrococcus luteus*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*. The final PCR products from these five species were combined and loaded onto three lanes of each DGGE gel. Five microliters of marker or 20 μl of final PCR product was mixed with 20 μl 2× gel loading dye (0.05% bromophenol blue, 0.05% xylene cyanol, 70% glycerol) and loaded into the gels. Electrophoresis was performed in 1× TAE at 60 °C for 16.5 h at 75 V. Gels then were stained in approximately 350 ml 1× TAE containing 5 μl ethidium bromide (10 mg ml^{-1}) on a platform shaker at 25 rpm for 30 min, followed by destaining in water (2×, 15 min). Gels were photographed using a Gel Logic 200 photodocumentation system (Kodak/Carestream Health Inc., Rochester, NY, USA). Gel images were normalized and analyzed using Gel Compare II v. 5.10 (Applied Maths, Austin, TX, USA). Each gel image was normalized by identifying bands in the reference lanes, which separated distinctly, spanning the gradient range, and then marking each band relative to the reference positions. Each lane length was adjusted based on reference positions to allow cross gel comparisons. Spectral analysis was conducted using the normalized reference positions, and the brightness and contrast was adjusted accordingly across each gel. Background was subtracted, and least squares filtering applied, resulting in a signal to noise ratio greater than 50 and a Wiener cut off of 0.65%. Bands in each lane were counted and quantified automatically, and summary tables were used for all subsequent analysis.

2.7. Microbial community structure: phospholipid fatty acid profiling (PLFA)

Soils for PLFA analysis were sieved (4 mm) and stored at −20 °C. PLFA analysis was carried out as previously described (Blackwood and Buyer, 2004) with one additional step. After transesterification, the fatty acid methyl esters were dissolved in chloroform and passed through a 100 mg NH_2 SPE column with elution in chloroform. The fatty acid methyl esters were dried under N_2 and chromatographed as described. The PLFAs used as biomarkers for specific microbial groups are listed in Blackwood and Buyer (2004).

2.8. Statistical analyses

Soil bulk density and moisture measurements were used to transform measured variables onto a volumetric basis (i.e., g cm^{-3} of dry soil). Statistical analyses were performed with Statview Version 5.0.01 (SAS Institute, Inc., Cary, NC, USA). Data from both sampling dates initially were analyzed including sampling date as a main effect. Data did not vary significantly by date, however, and there were no interactions between the treatment effects and date of sampling. Therefore, the datasets from each date subsequently were combined for ANOVAs that included only the main effects (tillage × amendment × depth). Data were transformed as needed to meet ANOVA assumptions (log-transformation for total nematode density and protozoa density). Means were compared using Fishers PLSD.

Summary tables of DGGE band patterns were normalized by constructing a matrix of band presence and absence for all sample lanes, creating a binary representation of each band and its respective migration position. Each lane was assigned treatment designations; the coded matrix was then imported into Canoco (Microcomputing Systems, Inc., Ithaca, NY, USA) for canonical correspondence analysis (CCA). The presence/absence matrix was used as the species data set, and six categorical variables (till, no-till, 0–5 cm, 5–25 cm, control, or amended) were assigned as management variables. Field replicates were assigned as co-variables.

Table 1
Response of soil variables to organic amendment and tillage (0–5 cm depth)*.

	Control, no-till	Control, till	Amend, no-till	Amend, till	Amend†	Tillage†	A × T†
Soil organic matter (g cm^{-3})	0.0215 ± 0.0035b	0.0253 ± 0.0044b	0.0577 ± 0.0160a	0.0623 ± 0.0096a	****	N.S.	N.S.
Respiration (μmol CO_2 day^{-1} cm^{-3})	1.161 ± 0.178b	1.438 ± 0.230ab	2.787 ± 0.420a	2.547 ± 0.356a	***	N.S.	N.S.
Protozoan density (# cm^{-3})	657.058 ± 92.70b	1036.81 ± 206.90ab	6829.66 ± 5306.69ab	13419.32 ± 8080.68a	***	N.S.	N.S.

A × T = interaction term for amendment × tillage. N.S. = not statistically significant, $\alpha = 0.05$.
* Means ± the standard error of the mean (S.E.M.) from both sampling dates ($n = 8$). Within a row, values with different letters are significantly different [Fisher's protected least significant difference (PLSD), $P < 0.05$].
† Significance levels for two-way ANOVA with amendment and tillage as main effects.
*** $P \leq 0.01$.
**** $P \leq 0.001$.

Table 2
Response of soil variables to organic amendment and tillage (5–25 cm depth)*.

	Control, no-till	Control, till	Amend, no-till	Amend, till	Amend†	Tillage†	A × T†
Soil organic matter (g cm^{-3})	0.0079 ± 0.0014ab	0.0066 ± 0.0006b	0.0113 ± 0.0023ab	0.0140 ± 0.004a	**	N.S.	N.S.
Respiration (μmol CO_2 day^{-1} cm^{-3})	0.506 ± 0.064ab	0.414 ± 0.031b	0.745 ± 0.725ab	0.834 ± 0.390a	**	N.S.	N.S.
Protozoan density (# cm^{-3})	493.42 ± 148.97a	340.35 ± 89.14a	508.65 ± 157.48a	606.83 ± 129.49a	N.S.	N.S.	N.S.

A × T = interaction term for amendment × tillage. N.S. = not statistically significant, $\alpha = 0.05$.
* Means ± S.E.M. from both sampling dates ($n = 8$). Within a row, values with different letters are significantly different (Fisher's PLSD, $P < 0.05$).
† Significance levels for two-way ANOVA with amendment and tillage as main effects.
** $P < 0.05$.

3. Results

3.1. Soil properties

Treatments (amendment and tillage) did not affect soil bulk density (three-way ANOVA, $P > 0.05$ for main effects of amendment and tillage). Bulk density was lower for the 0–5 cm depth (mean = 1.52 g cm^{-3} ± 0.016 S.E.M.) than for the 5–25 cm (1.59 g cm^{-3} ± 0.023 S.E.M.) (three-way ANOVA, significant depth effect, $P = 0.013$). Within the 0–5 cm depth, organic matter was higher in amended soils than in controls, regardless of tillage (Table 1). In the 5–25 cm depth, soil organic matter was only higher in amended soils within the tillage treatment (Table 2).

3.2. Soil respiration

In the 0–5 cm depth, soil respiration was higher in amended soils than in controls, but only within the no-till treatment (Table 1). In the 5–25 cm depth, soil respiration also was higher in amended soils, but only within the tillage treatment (Table 2).

3.3. Protozoan density

In the 0–5 cm depth, protozoan density was higher in amended, tilled soils than in control, no-till soils (Table 1). In the 5–25 cm depth, protozoan density did not vary among treatments (Table 2).

3.4. Nematode density and community structure

More nematodes were found in the 0–5 cm depth than in the 5–25 cm depth (Fig. 1; three-way ANOVA, significant depth effect, $P < 0.0001$). Nematode abundance was affected by both amendment and tillage, with increased densities under these treatments versus corresponding controls (Fig. 1; three-way ANOVA, significant amendment, $P < 0.0001$, and tillage, $P = 0.004$, main effects). The effects of tillage on nematode densities were confined to the 0–5 cm depth (Fig. 1).

Within the 0–5 cm depth, several nematode faunal indices were influenced by tillage, but not by amendment (Table 3). The Basal and Channel Indices were higher in no-till soils, and the Enrichment Index was lower (Table 3). The Structure Index was unaffected by treatment (Table 3). In the 5–25 cm layer, only the Structure Index was affected by treatment and was significantly higher for the amended, no-till soils (Table 4).

The relative abundance of nematodes from each trophic group was influenced by tillage and amendment, primarily in the 0–5 cm depth (Tables 3 and 4). Here, the relative abundance of bacterial-feeding nematodes was highest in control, tilled soils than in other treatments (Table 3). This effect was concurrent with a significantly lower relative abundance of fungal-feeding nematodes in these same plots (Table 3). Omnivorous/predaceous nematodes were unaffected by amendment or tillage (Table 3). In amended soils, the relative abundance of plant-parasitic nematodes was lower with tillage than without (Table 3). These results are reflected in the actual densities (# 100 cm^{-3}) of nematodes from various trophic groups (data not shown). For example, plant-parasitic nematodes were significantly reduced in amended, tilled soils (mean = 58.7 ± 23.8 S.E.M.) as compared to amended, no-till soils (mean = 148.0 ± 26.3 S.E.M.) (two-way ANOVA, significant tillage × amendment effect, $P < 0.01$).

In the 5–25 cm depth, the relative abundance of bacterial-feeding nematodes was lower with amendment, but fungal-feeding nematodes were unaffected (Table 4). Within amended soils,

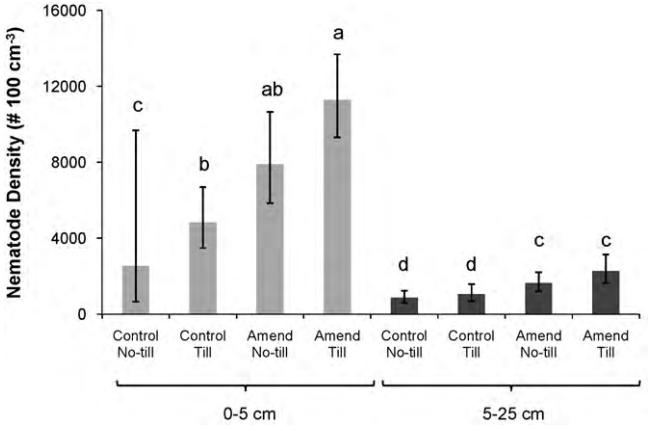

Fig. 1. Nematode density in soils receiving organic amendment and controls, with and without tillage, and at two depths (analyzed by three-way ANOVA, depth effect $P < 0.0001$, amendment $P < 0.0001$, and tillage $P = 0.0035$). Values are back-transformed means ± 95% confidence intervals ($n = 8$). Means with the same letter are not significantly different (Fisher's PLSD, $P < 0.05$).

Table 3
Response of soil nematode communities to organic amendment and tillage (0–5 cm depth)*.

	Control, no-till	Control, till	Amend, no-till	Amend, till	Amend†	Tillage†	A × T†
Basal Index	25.1 ± 2.1b	18.0 ± 1.5c	26.2 ± 1.3a	20.4 ± 1.7bc	N.S.	****	N.S.
Enrichment Index	73.3 ± 2.1b	79.5 ± 1.3a	72.3 ± 1.3b	78.4 ± 1.8a	N.S.	****	N.S.
Structure Index	18.9 ± 4.2a	32.7 ± 8.7a	15.9 ± 3.31a	21.3 ± 3.5a	N.S.	N.S.	N.S.
Channel Index	26.1 ± 4.28a	14.9 ± 1.6b	29.6 ± 2.4ab	21.6 ± 2.6ab	N.S.	**	N.S.
Bacterial-feeders (proportion)	0.52 ± 0.06b	0.68 ± 0.04a	0.45 ± 0.04b	0.55 ± 0.03b	**	***	N.S.
Fungal-feeders (proportion)	0.44 ± 0.05a	0.29 ± 0.04b	0.51 ± 0.04a	0.43 ± 0.03a	**	***	N.S.
Omnivore/predators (proportion)	0.02 ± 0.01a	0.01 ± 0.003a	0.02 ± 0.01a	0.01 ± 0.003a	N.S.	N.S.	N.S.
Plant feeders (proportion)	0.02 ± 0.005a	0.02 ± 0.01ab	0.02 ± 0.005a	0.005 ± 0.002b	N.S.	**	N.S.

A × T = interaction term for amendment × tillage. N.S. = not statistically significant, $\alpha = 0.05$.
* Means ± S.E.M. from both sampling dates ($n = 8$). Within a row, values with different letters are significantly different (Fisher's PLSD, $P < 0.05$).
† Significance levels for two-way ANOVA with amendment and tillage as main effects.
** $P < 0.05$.
*** $P \leq 0.01$.
**** $P \leq 0.001$.

Table 4
Response of soil nematode communities to organic amendment and tillage (5–25 cm depth)*.

	Control, no-till	Control, till	Amend, no-till	Amend, till	Amend†	Tillage†	A × T†
Basal Index	14.8 ± 2.4a	14.2 ± 1.4a	9.9 ± 0.9a	15.5 ± 1.8a	N.S.	N.S.	N.S.
Enrichment Index	82.7 ± 2.7a	83.9 ± 1.4a	86.2 ± 0.8a	83.0 ± 2.0a	N.S.	N.S.	N.S.
Structure Index	48.9 ± 8.5b	45.4 ± 5.2b	72.7 ± 3.9a	34.0 ± 3.7b	N.S.	****	***
Channel Index	9.4 ± 1.5a	9.7 ± 1.9a	10.1 ± 1.0a	13.0 ± 2.2a	N.S.	N.S.	N.S.
Bacterial-feeders (proportion)	0.70 ± 0.03a	0.71 ± 0.04a	0.59 ± 0.04b	0.67 ± 0.04ab	**	N.S.	N.S.
Fungal-feeders (proportion)	0.18 ± 0.02a	0.20 ± 0.04a	0.21 ± 0.02a	0.27 ± 0.03a	N.S.	N.S.	N.S.
Omnivore/predators (proportion)	0.08 ± 0.02b	0.05 ± 0.03b	0.16 ± 0.03a	0.03 ± 0.01b	N.S.	****	**
Plant feeders (proportion)	0.04 ± 0.01a	0.03 ± 0.01a	0.04 ± 0.01a	0.02 ± 0.01a	N.S.	N.S.	N.S.

A × T = interaction term for amendment × tillage. N.S. = not statistically significant, $\alpha = 0.05$.
* Means ± S.E.M. from both sampling dates ($n = 8$). Within a row, values with different letters are significantly different (Fisher's PLSD, $P < 0.05$).
† Significance levels for two-way ANOVA with amendment and tillage as main effects.
** $P < 0.05$.
*** $P \leq 0.01$.
**** $P \leq 0.001$.

omnivorous/predaceous nematodes were affected by tillage in this depth, with highest representation in amended, no-till plots (Table 4). The relative abundance of plant-parasitic nematodes did not differ among treatments in the 5–25 cm depth (Table 4).

3.5. Microbial community structure: DGGE

Canonical correspondence analysis (CCA) plots demonstrate the distinct effects treatments had on DGGE profiles of bacterial rDNA fragments, as indicated by the degree of separation between treatment clusters (Fig. 2). The first canonical axis accounted for 38% of the variability in the dataset, whereas the second axis accounted for 34.7%. DGGE profiles were generally very similar between replicates of each depth × tillage × amendment treatment (Fig. 2), suggesting that the treatments consistently selected for specific bacterial ribotypes. As indicated by the relative length of each vector, amendment and depth were correlated more with changes in community structure than tillage. All of the environmental vectors are significant using a Monte Carlo perturbation test ($P < 0.02$).

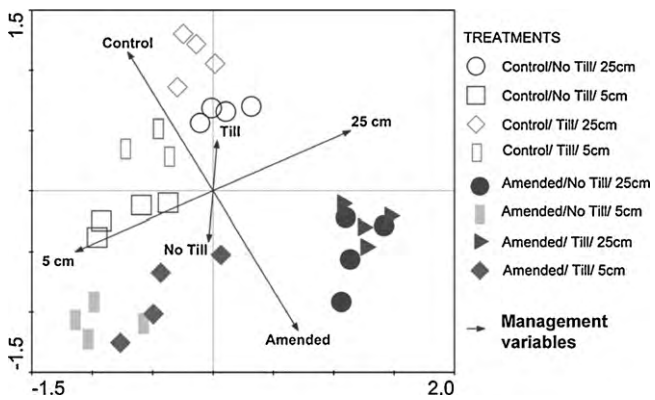

Fig. 2. Results of canonical correspondence analysis (CCA) of banding patterns from DGGE gels of bacterial rDNA fragments. Vectors show the treatments affecting community differences relative to each axis. Similar symbols denote individual replicates within each treatment.

3.6. Microbial community structure: PLFA

Within the 0–5 cm depth, many PLFA biomarker groups (total PLFA, gram negative, eukaryote, protozoa, arbuscular mycorrhiza) were higher in amended soils than in controls (Table 5). Other biomarkers also were higher in amended soils than in controls, but only with tillage (eubacterial, gram positive, and fungal; Table 5). Tillage enhanced the density of most biomarkers in amended soils, as compared to amended, no-till soils (Table 5). Within the control soils, however, tillage had no influence on any of the biomarkers (Table 5). Thus, amendment with tillage generally had the largest effects on PLFA biomarkers, although many biomarkers were increased in the amended treatments even without tillage.

Less dramatic differences were detected between treatments groups in the 5–25 cm depth compared to the 0–5 cm depth, but the general trend was for amended soils with tillage to have significantly higher amounts of biomarker PLFAs than the other treatment groups (Table 6). Actinobacterial biomarker PLFA was unaffected by amendment or tillage, at either depth; the fungal:bacterial biomarker PLFA ratio was similarly unaffected (Tables 5 and 6).

Table 5
Response of PLFA biomarkers to organic amendment and tillage (0–5 cm depth)*.

	Control, no-till	Control, till	Amend, no-till	Amend, till	Amend†	Tillage†	A × T†
Total PLFA (nmoles cm^{-3})	34.68 ± 1.42c	33.17 ± 2.66c	42.25 ± 2.26b	54.83 ± 2.38a	****	**	***
Eubacterial	2.16 ± 0.10b	2.32 ± 0.24b	2.86 ± 0.22b	4.71 ± 0.55a	****	***	**
Gram positive	10.01 ± 0.34c	9.67 ± 0.92c	11.20 ± 0.54b	14.20 ± 0.63a	****	**	***
Gram negative	10.64 ± 0.55b	10.05 ± 0.77b	14.57 ± 1.03b	18.14 ± 0.82a	****	N.S.	**
Actinobacterial	5.79 ± 0.19a	5.43 ± 0.42a	5.27 ± 0.24a	5.91 ± 0.54a	N.S.	N.S.	N.S.
Eukaryote	1.99 ± 0.22c	1.94 ± 0.21c	3.04 ± 0.54b	4.44 ± 0.27a	****	N.S.	**
Protozoa	0.30 ± 0.04b	0.24 ± 0.06b	0.77 ± 0.18a	0.67 ± 0.08a	***	N.S.	N.S.
Fungal	1.58 ± 0.19b	1.44 ± 0.12b	2.09 ± 0.33b	3.48 ± 0.22a	****	**	***
Arbuscular mycorrhiza	1.42 ± 0.08c	1.45 ± 0.13c	3.05 ± 0.22b	3.65 ± 0.24a	****	N.S.	N.S.
Fungal:bacterial ratio	0.75 ± 0.10a	0.67 ± 0.10a	0.76 ± 0.15a	0.80 ± 0.08a	N.S.	N.S.	N.S.

A × T = interaction term for amendment × tillage. N.S. = not statistically significant, $\alpha = 0.05$.
* Means ± S.E.M. from both sampling dates ($n = 8$). Within a row, values with different letters are significantly different (Fisher's PLSD, $P < 0.05$).
† Significance levels for two-way ANOVA with amendment and tillage as main effects.
** $P < 0.05$.
*** $P \leq 0.01$.
**** $P \leq 0.001$.

Table 6
Response of PLFA biomarkers to organic amendment and tillage (5–25 cm depth)*.

	Control, no-till	Control, till	Amend, no-till	Amend, till	Amend†	Tillage†	A × T†
Total PLFA (nmoles cm^{-3})	24.43 ± 1.03ab	20.07 ± 1.03c	20.75 ± 1.11bc	26.20 ± 2.70a	N.S.	N.S.	***
Eubacterial	1.32 ± 0.09ab	1.10 ± 0.10b	1.06 ± 0.08b	1.61 ± 0.22a	N.S.	N.S.	***
Gram positive	8.02 ± 0.48a	6.75 ± 0.50a	6.45 ± 0.47a	7.81 ± 0.90a	N.S.	N.S.	**
Gram negative	7.27 ± 0.42ab	6.01 ± 0.27b	6.04 ± 0.37b	8.54 ± 0.81a	N.S.	N.S.	***
Actinobacterial	4.66 ± 0.26a	4.35 ± 0.11a	3.95 ± 0.17a	4.48 ± 0.42a	N.S.	N.S.	N.S.
Eukaryote	0.61 ± 0.08ab	0.48 ± 0.04b	0.43 ± 0.07b	0.88 ± 0.15a	N.S.	N.S.	***
Protozoa◊	0.04 ± 0.04	0.00 ± 0	0.04 ± 0.04	0.20 ± 0.08			
Fungal	0.54 ± 0.06ab	0.45 ± 0.03bc	0.34 ± 0.03c	0.63 ± 0.06a	N.S.	**	****
Arbuscular mycorrhiza	1.16 ± 0.10b	1.01 ± 0.06b	0.94 ± 0.06b	1.46 ± 0.14a	N.S.	N.S.	***
Fungal:bacterial ratio	0.43 ± 0.07a	0.42 ± 0.03a	0.33 ± 0.03a	0.41 ± 0.03a	N.S.	N.S.	N.S.

A × T = interaction term for amendment × tillage. N.S. = not statistically significant, $\alpha = 0.05$.
* Means ± S.E.M. from both sampling dates ($n = 8$). Within a row, values with different letters are significantly different (Fisher's PLSD, $P < 0.05$).
† Significance levels for two-way ANOVA with amendment and tillage as main effects.
** $P < 0.05$.
*** $P \leq 0.01$.
**** $P \leq 0.001$.
◊ ANOVA not performed due to zero-value means for some treatments.

4. Discussion

As expected, amendment had positive effects on most variables, including organic matter, respiration, protozoa and nematode densities, and PLFA biomarkers for bacteria and fungi. This response was enhanced by tillage for most of these variables, particularly in the 5–25 cm depth. While long-term tillage is considered to be detrimental to soil organic matter pools and fungi (Simmons and Coleman, 2008), our results show that when combined with amendment, tillage can have a positive effect on many soil biological properties, including soil fungal biomass. Organic amendments enhance the activity and abundance of decomposer organisms as a direct consequence of qualitative and quantitative improvement in the soil organic matter content. However, in addition to providing substrate for the decomposers, organic amendments also can improve soil water retention (Carter, 2007; Rawls et al., 2003). Thus, amendments contribute to improved soil health through a myriad of direct and indirect effects (Magdoff, 2001; Snapp et al., 2005). The effects of amendment were more pronounced in the surface 0–5 cm depth in our study, but it appears that tillage can extend them into deeper soil layers. It is important to note, however, that all of the organic amendments that were applied in this experiment were produced off-site. Alternative strategies, such as growing cover crops in situ, may modify the differences between depths that we measured in the no-till treatment, as root-derived inputs will occur in deeper layers.

Pest management in sustainable agricultural systems is of particular concern. Enhancement of the decomposer food web through bottom–up approaches (i.e., soil amendments that enhance soil organic matter content; Mikola and Setälä, 1998; Wardle et al., 2001) has been proposed as a mechanism to increase the abundance of predaceous nematodes and other biota that may then exert top–down control over lower trophic levels, including plant-parasitic nematodes (Mankau, 1980; Yeates and Wardle, 1996; Wardle et al., 2001; Ferris et al., 2004). Management of the soil food web to promote the establishment of so-called "suppressive soils" may maintain plant productivity without reliance on nematicides (Stirling, 1991; Widmer et al., 2002). The ability of organic amendments to impact the densities of plant-parasitic nematodes is known to vary among different substrates that can be used for amendment and for different cropping systems (Snapp et al., 2005). Plant-parasitic nematodes may be negatively affected by amendments indirectly, via top–down control, or directly, via allelochemicals that are released from decomposing amendments (Zasada et al., 2007; Oka, 2009). By contrast, amendments have been shown to enhance some plant-parasitic nematode populations in field trials (McSorley et al., 1997), and it is clear that this remains an important, active area of research.

Plant-parasitic nematodes were not a major component of the nematode community that we studied (in general <10% of nematodes identified). However, we did find a significant reduction in the density of plant-parasitic nematodes in amended, tilled soils in the 0–5 cm depth. This is especially noteworthy when the large increase in overall nematode abundance under this treatment is taken into consideration. Omnivorous/predaceous

nematodes, which were found in even smaller proportions than the plant-parasitic nematodes, were unaffected by treatment in this depth. Therefore, we are unable to attribute the decline in plant-parasitic nematodes to top–down control via predator nematodes in this treatment. Omnivorous/predaceous nematodes were negatively impacted by tillage in amended soils in the 5–25 cm depth, but again, this result was not associated with any changes in the density of plant-parasitic nematodes. It is difficult to compare the responses of these two trophic groups of nematodes, given their limited abundances and different life histories (Yeates and Wardle, 1996). Nematode density is also influenced by abiotic factors including changes in the physiochemical environment of the soil, and different species are likely to be impacted differently (Fiscus and Neher, 2002). Furthermore, the density of plant-parasitic nematodes can be influenced by unmeasured changes in root distribution, chemistry, or morphology (Treonis et al., 2007). Thus, it remains challenging to link reductions in plant-parasitic nematode density to predation in field experiments.

Although free-living nematode abundance increased with amendment, none of the nematode faunal indices (Basal, Enrichment, Structure, and Channel) were influenced by amendment at either depth. We predicted that tillage would work synergistically with amendment to positively affect specific taxa of nematodes, resulting in a higher Enrichment Index. This index reflects the abundance of enrichment opportunists (Ferris et al., 2001), i.e., bacterial- and fungal-feeding nematodes that respond rapidly to resource pulses (Rhabditidae, Panagrolaimidae, Diploscapteridae, Aphelenchidae, and Aphelenchoididae). Other studies of nematode community responses have demonstrated that the Enrichment Index increases in plots treated with organic amendments (Ferris and Matute, 2003; Berkelmans et al., 2003; Ferris et al., 2004; Forge et al., 2008; DuPont et al., 2009), but we did not see a similar response to amendment in our study.

The lack of amendment effects contrasts with the significant effects of tillage on the same nematode indices. In un-amended, control soils, tillage decreased the Basal Index and increased the Enrichment Index in the 0–5 cm depth. The Basal Index shift reflects decreases in the abundance of cp-2 bacterial- and fungal-feeding nematodes, while the Enrichment Index reflects increases in nematode groups that respond rapidly to resource inputs (cp-1 bacterial-feeding and cp-2 fungal-feeding nematodes). Thus, these changes in the indices with tillage suggest that cp-2 bacterial-feeders were replaced with cp-1 bacterial-feeding nematodes. Significant effects of tillage were also seen on the relative abundances of fungal- and bacterial-feeding nematodes within control soils (0–5 cm only). With tillage, the relative abundance of bacterial-feeding nematodes increased, while the relative abundance of fungal-feeding nematodes decreased. Tillage had an overall positive effect on nematode abundance in these same control soils. These results suggest that by itself tillage selectively enhances populations of bacterial-feeding nematodes, specifically those with colonizer-persister values of 1 (Rhabditidae, Panagrolaimidae, and Diploscapteridae).

Prior studies have demonstrated neutral or mixed effects of tillage on nematodes (Carter et al., 2009). For example, several studies have found that nematodes were less abundant with tillage (Miura et al., 2008; Parmelee and Alston, 1986), as compared to no-till field plots, while Fu et al. (2000) observed the reverse effect. Furthermore, Parmelee and Alston (1986), sampling from agricultural soils in Georgia (USA) across a 0–20 cm profile, reported decreases in bacterial-feeding nematodes with tillage during the growing season, which is the opposite of our observations. Overall, our results suggest that nematode community structure was affected more by tillage than by amendment, with a shift toward communities with more bacterial-feeding nematodes. When tillage was combined with amendment, however, nematode abundance was very responsive to amendment, with all trophic groups of free-living nematodes increasing in abundance.

Tillage is thought to adversely affect fungi by physically disrupting hyphal networks and/or by affecting soil moisture status, resulting in decreased fungal biomass (Frey et al., 1999; Helgason et al., 2009). However, we did not measure a decline in fungal biomarker PLFA with tillage in the control soils we studied. Furthermore, tillage actually increased fungal PLFA in amended soils at both depths. While we predicted that tillage would favor bacterial-based decomposition pathways with amendment, our results suggest that the fungal pathway has a greater role, particularly in the 5–25 cm depth. Both fungal biomarkers PLFA and fungal-feeding nematode density showed the greatest enhancement with amendment and tillage at this depth.

While the fungal-based decomposition pathway showed significant responses to our treatments, DGGE profiling of bacterial ribotypes demonstrated that amendment and tillage also selected for very specific bacterial communities, of which heterotrophic decomposers are a significant component. Overall, DGGE suggests that the bacterial community was affected by treatments on a very fine scale (species-specific), as has been seen by others (Buckley and Schmidt, 2001). Although not assessed in this study, the effects of these types of changes in bacterial diversity on bacterial-feeding protozoa and nematodes may be significant. The linkages between diversity of these groups are not well understood, due to the limited empirical understanding of the feeding preferences of these bacterial-feeding organisms (i.e., protozoa, nematodes). Laboratory experiments suggest that microbial food sources have different nutritional value to nematodes, in terms of supporting development and reproduction (Djigal et al., 2004; Salinas et al., 2007). It is not known, however, whether bacterial-feeding nematodes in soils can influence the structure of bacterial communities via predation, and in turn, whether nematode diversity is influenced by available prey species. This is a critical need for future research into the functioning of soil food webs.

Distinguishing the impacts of management practices on soil microorganisms and microfauna within soil layers is critical, particularly when practices influence the distribution of soil resources, as organic amendments and tillage do (Angers et al., 1997). In our field plots, depth in the soil profile was a significant factor influencing the density of microorganisms and microfauna, regardless of treatment (amendment or tillage). While the varying distribution of soil organisms between layers frequently has been documented (Sohlenius and Sandor, 1987; Liang et al., 2005), depth is not always considered explicitly in evaluating the impact of agricultural practices. Our data supports depth as an important spatial factor influencing soil ecology in agricultural soils.

5. Conclusions

The results of this study suggest that soil microbial and microfaunal responses to organic amendment tend to be most dramatic near the soil surface (0–5 cm), but can be extended deeper with tillage. When amendment and tillage were combined, both bacterial- and fungal-based decomposition pathways responded positively, and we also measured a decline in plant-parasitic nematodes. Future research evaluating the influence of agricultural management practices on soil micoroorganisms and microfauna should employ sampling schemes that account for stratification of resources by depth. Furthermore, it is important for studies investigating the effects of soil management on soil biota to incorporate multiple trophic layers, which can help to discern the complex relationships among the diverse taxa found in soils.

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