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Effects of mulching and fertilization on soil nutrients, microbial activity and rhizosphere bacterial community structure determined by analysis of TRFLPs of PCR-amplified 16S rRNA genes

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Abstract

Organic mulches such as recycled, ground wood pallets and composted yard waste are widely used in landscapes to suppress weeds, and improve plant health. However, little is known about how mulches affect soil or rhizosphere microbial communities. In a field microcosm study, we compared effects of mulching with composted yard waste, ground wood pallets, or a bare soil control, with or without chemical fertilizer on soil mineral, chemical, biological, and rhizosphere bacterial community properties. Both mulch treatments had significant effects on organic matter content, soil respiration, microbial biomass N, soil pH, cation-exchange capacity, and concentrations of essential plant nutrients. Microbial respiration rate was highest in soils mulched with composted yard wastes (17.2 and 15.3 mg CO_2 kg^{-1} per day for non-fertilized and fertilized plots, respectively) and lowest in bare soil plots (5.0 and 9.4 mg CO_2 kg^{-1} per day for non-fertilized and fertilized plots, respectively). In general, the other parameters were highest in plots mulched with composted yard waste and not affected by fertilization. Bacterial communities in the rhizosphere of cucumber (*Cucumis sativus* L. Straight Light) seedlings grown in the microcosms were analyzed using most probable number (MPN) analysis of culturable heterotrophic fluorescent pseudomonads in King's B medium as well as by analysis of terminal restriction fragment length polymorphisms (TRFLPs) of PCR amplified 16S rRNA genes. Populations of culturable heterotrophic bacteria and fluorescent pseudomonads in the rhizosphere were significantly greater in the composted yard waste plots than the bare soil fertilized mulched plots. TRFLP analysis of PCR amplified bacterial 16S rRNA genes from triplicate root tips grown in each treatment and digested with *Hha*I, *Msp*I, and *Rsa*I revealed that the TRFLP similarity was 0.81–0.91 among triplicate samples and 0.48–0.86 among different treatments. The TRFLP pattern of rhizosphere communities from the bare soil treatment were more similar (54–82%) to plots mulched with ground wood than to plots mulched with compost. Only 48–71% of TRFLP peaks detected in samples from the compost treatment were also detected in the bare soil control. The similarity in TRFLPs between the compost and ground wood pallet treatments was

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56–80%. Although the community profiles showed differences in bacterial diversity, no significant difference in TRFLP-based diversity indices were observed. Unique TRF peaks detected among treatments suggest that specific subcomponents of the microbial communities differed. A higher number of TRFs corresponding with biocontrol organisms such as *Pseudomonas* and *Pantoea* spp. were observed in plots mulched with compost. However, the mulch treatments had more pronounced effects on soil chemical and microbial properties than on TRFLP based bacterial community structure on cucumber roots. Nonetheless, the data show clearly that mulching with compost strongly influenced the structure of the microbial rhizophere community.

Keywords: TRFLP; Phylogeny; 16S rRNA; Compost; FT-ARDRA; *Pseudomonas*; Mulch; Wood mulch; *Pantoea*; Cucumber; Rhizosphere; Pallet wood; Wood waste; Soil

1. Introduction

The rhizosphere, a narrow zone of soil adjacent to, and influenced by, living plant roots (Kennedy, 1999), is a site of high microbial activity in and around roots in soil (Sørensen, 1997) that harbors a great diversity of microorganisms affecting plant growth and health (Boehm et al., 1993; Campbell and Greaves, 1990). The diversity and composition of bacterial taxa in the rhizosphere can be affected by several factors including plant species (Miller et al., 1989), soil type (Hoitink and Boehm, 1999), soil management practices (Rovira et al., 1990), microbial interactions (Hedges and Messens, 1990) and other environmental variables (Kennedy, 1999).

Management practices such as fertilization, crop rotation, application of organic amendments, and tillage may favor some microorganisms over others (Workneh and van Bruggen, 1994). For instance, organic amendments such as composts and manures can reduce the severity of plant diseases by modifying the composition of the bacterial taxa in the rhizosphere, as well as by increasing the general level of microbial activity resulting in increased competition and/or antagonism among microbes and finally, decreased activity of plant pathogens (Cook and Baker, 1983; De Brito Alvarez et al., 1995; Hoitink and Boehm, 1999).

Recently, comparative microbial community analysis utilizing nucleic acid based techniques has been used to gain a better understanding of community structure and function in the edaphic components of soils and the rhizosphere (Duineveld et al., 1998; Kim et al., 1999; Liesack et al., 1997; Miethling et al., 2000; Steer and Harris, 1999). These new methods in microbial ecology allow identification of a greater diversity of microorganisms as compared to a culture-based approach (Clement et al., 1998; Liu et al., 1997; Ludeman et al., 2000; Schwieger and Tebbe, 2000; Schallmach et al., 2000). These nucleic acid-based methods also permit a limited phylogenetic analysis of the microbial community structure (Marsh et al., 2000; Michel et al., 2002).

Fertilization and mulching are considered key soil management practices for ornamental landscapes. Fertilization is the most common cultural practice implemented by professional landscape managers (Braman et al., 1998). Organic mulches are commonly applied to the soil surface to suppress weeds, conserve soil moisture, moderate soil temperatures, and suppress plant diseases (Robinson, 1988; Hoitink and Boehm, 1999). The potential of mulches to improve soil structure, increase organic matter, and establish patterns of nutrient cycling more similar to natural ecosystems has also been recognized (Tukey and Schoff, 1963; Roe, 1998). However, the effects of these management practices on soil microbial communities have not been addressed.

Microbial growth in soils typically is resource limited and growth increases rapidly in response to added carbon sources (Johnson, 1992; Wardle, 1992). The availability and composition of organic matter are key factors affecting microbial biomass, and also community composition (Facelli and Pickett, 1991; Boehm et al., 1997). As microbes utilize high C:N carbon sources, their growth may become N-limited; conversely, decomposition of organic matter with a C:N ratio less than 30:1 may result in carbon limitation of microbial growth (Aber, 1992; Wardle, 1992; Kaye and Hart, 1997). When soil microbes are nitrogen limited, nutrient addition via fertilization may stimulate their growth (Aber, 1992; Johnson, 1992; Wardle, 1992; Vanotti et al., 1995). Therefore, soil management practices such as fertilization and application of organic mulches may

have substantial effects on microbial diversity and abundance.

Materials used as mulches in the landscape industry have changed considerably in recent years as a result of efforts to divert solid wastes from landfills (Glenn, 1999). The quantity of yard wastes generated annually in the USA exceeds 30 million t (McKeever, 1999), much of which is composted for use in landscapes (Glenn, 1999). Pilot scale studies have shown that these composts may suppress several types of diseases caused by soil-borne plant pathogens (Grebus et al., 1994; Tuitert et al., 1998). Commercial sales of wood mulch generated from pallets diverted from landfills have also increased substantially in recent years (Glenn, 1999). Since the composition of fresh wood and these composts differ dramatically, so might their effects on the soil microbial community. The low C:N ratio of composted yard waste resembles high quality forest litter. On the other hand, ground wood, which contains very little nitrogen, has a much higher C:N ratio and its effect on the soil microflora or landscape plants remains unknown.

In this paper, we report our results on the effects of two mulch and two fertilization treatments on soil nutrients, microbial activity and rhizosphere bacterial community structure based on terminal restriction fragment length polymorphisms (TRFLPs) of PCR-amplified 16S rRNA genes.

2. Materials and methods

A field experiment was conducted between November 1997 and September 2000 in 48 microcosms constructed at the Ohio Agricultural Research and Development Center in Wooster, OH on a Canfield silt loam soil (29% sand, 42% silt, and 29% clay). Microcosms were constructed by slicing a narrow (15 cm) 1 m deep trench around the perimeter of 1 m^2 soil plots. The trench was lined with 30 ml PVC landfill liner material that extended 1 m vertically into and 15 cm above the ground, after which the trench was back-filled by returning the native soil to trench exterior to the plots. This isolated the soil environment allowing establishment of replicated, soil environments, without affecting drainage patterns.

The soil treatments were: (1) bare soil control; (2) bare soil plots plus fertilizer; (3) mulching with composted yard waste (C:N ratio of 17:1); (4) composted yard waste plus fertilizer; (5) mulching with recycled ground wood pallets (C:N ratio of 117:1) and (6) mulching with ground wood pallets plus fertilizer. The experiment was designed as a randomized complete block with each of the six treatments, each replicated four times within each of two blocks.

Table 1
Physico-chemical properties of mulches

Properties	Ground wood pallet	Composted yard waste
pH	7.55	7.58
EC (mmhos cm^{-1})	<0.10	0.50
C (g kg^{-1})	447	341
N (g kg^{-1})	3.90	20.30
C:N ratio	114:1	17:1
P (mg kg^{-1})	274	2330
K (mg kg^{-1})	0.10	0.30
Ca (mg kg^{-1})	8077	26472
Mg (mg kg^{-1})	215	1017
Fe (mg kg^{-1})	2272	14183
Bulk density (g l^{-1})	176	212

EC: electrical conductivity.

Mulch treatments were implemented by applying a 5 cm layer to the soil surface in November of 1997. In May of 1998–2000, any residual mulch was removed and replaced with fresh material (see Table 1 for detailed characterization of the mulches). Beginning October 1998 and continuing through May 2000, microcosms receiving the fertilizer treatment (selected randomly) were treated with 18:5:4 NPK applied at a rate 150 kg N ha^{-1} per year. Half of the annual amount of fertilizer was applied in early May, and the other half in early October. Nitrogen was applied in the form of methylene urea (56%), water-soluble urea (27%), and ammonium nitrate (17%). Our fertilization treatment represents a low rate based on extension recommendations for ornamental landscapes, which range from 147 to 294 kg N ha^{-1} per year of actual N (3–6 lb 1000 ft^{-2} per year), with N, P and K applied in ratios ranging from 3:1:1 to 3:1:2 (Smith, 1978).

2.1. Soil chemical properties, nutrient content, and microbial activity

Soil samples were taken from each microcosm on 10 August 2000 to determine treatment effects on organic

matter content, microbial biomass, and soil nitrogen pools. Each sample consisted of five cores (2 cm diameter, 15 cm deep) taken from standardized locations within each microcosm. Prior to coring, mulch was removed from sampling area to prevent contamination of cores with surface organic matter. The five cores from each microcosm were then combined into a single sample, homogenized, and sieved (6 mm). Samples were stored in sealed plastic bags at 4 °C until they were analyzed for organic matter content (loss on ignition; 360 °C for 4 h), mineral nitrogen (NH_4, NO_3, and NO_2) determined colorimetrically following Keeney and Nelson (1982), and dissolved organic nitrogen (amino acids and other low molecular weight organic compounds) determined colorimetrically following Cabrera and Beare (1993). Nitrogen immobilized in microbial biomass N was determined by the chloroform fumigation–extraction method (Brookes et al., 1985; Joergensen and Mueller, 1996). Microbial respiration was determined by colorimetric titration of evolved CO_2 with NaOH traps (Rice et al., 1996).

Soil samples were also taken (as described earlier) from each microcosm on 20 September 2000 to determine the effects of the three seasons of mulching and two seasons of fertilization on soil pH, cation exchange capacity (CEC), available P, exchangeable K, Mg and Ca in the root zone (analysis by Midwest Laboratories Omaha, NE).

2.2. Enumeration of rhizosphere bacteria

Six cucumber (*Cucumis sativus* L. c.v. Straight Light) seeds (germination rate, 90%) were planted in four of the eight replicate microcosms from each treatment (selected randomly) on 11 August 2000. However, because some plants were eaten by rabbits, we were able to harvest only three replicates per treatment. The plots were irrigated daily and one seedling per plot was harvested after 19 days from the B+, B−, M+, M− and C− plots. Seedlings from only one C+ plot were harvestable. However the TRFLP profiles from the roots of seedlings in triplicate plots were nearly identical (see Fig. 2). Uprooted seedlings were shaken gently to remove soil adhering to roots, and then rinsed with deionized water prior to analysis. Root tip sections (1 cm) were homogenized in 300 μl phosphate buffer in a sterilized Ten Broeck homogenizer. Each suspension was then diluted serially in a 96-well microtitre plate (20 μl suspension; 180 μl medium per well) utilizing four replicate wells per root tip for a four-tube most probable number (MPN) analysis. Populations of total culturable bacteria on root tips were determined using 0.1× tryptic soy broth (Difco). After 48 h incubation at 28 °C in the dark, any turbidity (total bacteria) was scored as positive growth. Fluorescent pseudomonads were enumerated using King's B medium (Gould et al., 1985), with any fluorescence observed in a well after 48 h of growth scored as positive for fluorescent pseudomonads.

For MPN analysis, the total number of positive wells was counted from each of four replicate dilutions, with the population sizes of total heterotrophic bacteria and fluorescent pseudomonads estimated using the MPN method (Woomer et al., 1990).

2.3. DNA extraction

Multiple root tips harvested from a single seedling were frozen in liquid N_2 and ground with a mortar and pestle prior to DNA extraction. Total community DNA from the ground root samples was extracted and purified using the UltraClean Soil DNA Isolation Kit (MoBio Laboratories Inc., California, USA). A PCR inhibitor removal solution (UltraClean IRS solution; MoBio Laboratories Inc., California, USA) was added to reduce contamination caused by any organic matter in the cucumber rhizosphere, and to produce PCR-quality DNA. The DNA yield ranged from 10 to 15 μg DNA g^{-1} fresh root tip.

2.4. PCR amplification

TRFLP microbial community fingerprinting (Liu et al., 1997) was used to characterize the bacterial communities in the cucumber rhizospheres. The 16S rRNA genes were amplified using universal bacterial primers: 8F forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1406R (5′-ACGGGCGGTGTGTRC-3′) reverse. The 8F forward primer was labeled with 5-hexachloroflourescein (HEX; Operon Inc., California, USA). Each PCR reaction mixture contained 50 ng DNA template, 2.5 mM $MgCl_2$, 2.5 units *Taq* polymerase (Roche Molecular Biochemicals, Indiana, USA), 1× PCR reaction buffer, 0.2 mM PCR nucleotide mix (Roche Molecular Biochemicals, Indiana, USA), 0.5 μM DNA primers, and 7.5 μg μl^{-1}

bovine serum albumin (Roche Molecular Biochemicals, Indiana, USA) in a final volume of 50 μl. The reaction mixture was incubated at 94 °C for 9 min, and then cycled 30 times through three steps: denaturing (94 °C; 60 s), annealing (58 °C; 45 s), and primer extension (72 °C; 90 s) in a PTC-100 thermal cycler (MJ Research Inc., Massachusetts, USA). Amplification product sizes were verified by electrophoresis in 2.0% agarose and ethidium bromide staining. To obtain sufficient DNA for TRFLP analysis and to minimize PCR bias, amplicons from three PCR runs for each root sample were combined (Clement et al., 1998) and then purified using a PCR purification kit (PCR Clean-up Kit; MoBio Laboratories Inc., California, USA).

2.5. TRFLP analysis

Purified PCR products were separately digested with restriction endonucleases *Hha*I (for 5 h), *Msp*I (for 3 h), and *Rsa*I (for 3 h) (Roche Molecular Biochemicals, Indiana, USA) to produce a mixture of variable length end-labeled 16S rRNA gene fragments and generate three unique fingerprints of each bacterial community (Michel et al., 2002). The labeled fragments were electrophoretically separated on a polyacrylamide gel (5.5%) in an ABI model 373 automated sequencer (Perkin-Elmer, California, USA). Electrophoresis was done for 18 h with limits of 2500 V and 40 mA. Thereafter, the lengths of flourescently labeled TRFs were determined by comparison with internal standards using Genescan software (Perkin-Elmer, California, USA) with a peak height detection limit of 50 fluoresence units. Unique TRFs from three TRFLP fingerprints derived from *Hha*I, *Msp*I, and *Rsa*I digestions were compared with the TRF sizes predicted using the TRFLP-TAP tool of the Ribosomal Database Project (RDP) (Marsh et al., 2000).

To detect the presence of bacteria implicated in plant disease biocontrol in the root samples, the genomic DNAs of known *Pantoea* and *Pseudomonas* biocontrol strains isolated from Wooster soils (Boehm et al., 1993) were tested using TRFLP analysis. The TRF lengths found in *Hha*I, *Msp*I, and *Rsa*I TRFLPs of these strains were then compared with TRF lengths from TRFLPs of the root samples. To determine the most dominant culturable fluorescent pseudomonads in the root sample, samples from the most dilute King's B MPN wells that showed fluorescence were also PCR-amplified and subjected to TRFLP analysis. The length of each TRF found in these wells were compared with: (1) TRF sizes found on the root tips; (2) those determined for the *Pseudomonas* strains used in this study (Boehm et al., 1993) and (3) with TRF sizes predicted for *Psuedomonas* strains using the TRFLP-TAP tool of the RDP database (Marsh et al., 2000).

2.6. Similarity and diversity of TRFLP patterns

To determine the similarity among TRFLP fingerprints, the TRFLP profile matrix analysis program from the RDP II web site was used (Marsh et al., 2000; Maidak et al., 1999). TRF fragments from the three digestions were combined to yield one dataset (Liu et al., 1997).

A Shannon diversity index (Atlas and Bartha, 1987) based on the size and number of TRFs was calculated as an estimate of bacterial diversity using the following equation:

$$\text{Shannon index } (\hat{H}) = C/N(N \log_{10} N - \sum n_i \log_{10} n_i)$$

where $C = 2.3$, N = sum of peak areas in a given TRFLP, n_i = area of TRF i and i = *number* of TRFs of each TRFLP pattern.

The equitability index, a measure of dominance (Atlas and Bartha, 1997), was calculated based on the size and number of TRFs using the following equation:

$$\text{equitability } (J) = \hat{H}/H_{\max}$$

where $\hat{H}$ = Shannon diversity index, and $H_{\max}$ = theoretical maximal Shannon diversity index assuming that all peak areas are equivalent in area. $H_{\max}$ was calculated as follows:

$$H_{\max} = C/N(N\log_{10}N - \sum n_i \log_{10} n_i)$$

where $C = 2.3$, N = sum of peak areas in a given TRFLP assuming that each peak has an area of 1, and n_i = area of TRF i (1), i = *number* of TRFs of each TRFLP pattern.

2.7. Statistical analyses

Interactions between the fertilizer and mulch treatments were not significant for all soil variables, and could not be tested in the case of rhizosphere variables because there were only three replicates per treatment. Therefore, we analyzed treatment effects on soil properties, rhizosphere bacterial counts, diversity indices, total number of TRFs, and diversity indices soil properties using one-way analysis of variance (ANOVA).

In the statistical model for tests of treatment effects on soil variables, block (d.f. = 1) was treated as a fixed effect factor, thus treatment effects (d.f. = 5) were tested over S.E.M. (d.f. = 41) (see Newman et al., 1997 for a discussion of fixed versus random block effects in ANOVA). followed by Bonferroni's *t*-tests at $P \leq 0.05$ probability level (Zar, 1999). In the case of rhizosphere variables, limited replication precluded inclusion of block in the statistical model, and treatment effects were tested over S.E.M. (d.f. = 12). When ANOVA showed a significant treatment effect ($P \leq 0.05$), means were separated using Bonferroni's *t*-tests ($P \leq 0.05$).

3. Results

3.1. Soil chemical properties, fertility, and biology

The mulch and fertilization treatments had substantial effects on soil chemistry, fertility, and microbial biomass (Table 2). Soils mulched with composted yard waste had significantly higher CEC values and concentrations of organic matter, P, K, Ca, extractable and dissolved N than did soils mulched with ground wood pallets or the bare soil plots (Table 2). Soil pH was acidic in all treatments with values ranging from 5.5 to 6.3. It tended to be highest in microcosms mulched with composted yard waste.

Mulching with composted yard waste increased microbial respiration and microbial N. The rate of microbial respiration was highest in soils mulched with composted yard wastes (17.2 and 15.3 mg CO_2 kg^{-1} per day for non-fertilized and fertilized plots, respectively). It was lowest in the bare soil plots (5.0 and 9.4 mg CO_2 kg^{-1} per day for non-fertilized and fertilized plots, respectively). Microbial N was also highest in plots mulched with composted yard waste (Table 2).

Table 2
Soil chemistry, nutrient contents, and microbial activities under various mulch treatments

Soil properties	Mulch treatments[a]					
	B−	B+	W−	W+	C−	C+
Organic matter ($g\,kg^{-1}$)	49.7 b	48.4 b	57.0 b	58.0 b	72.4 a	75.3 a
Phosphorus (weak bray) ($mg\,kg^{-1}$)	127 ab	138 a	103 b	116 ab	140 a	136 a
Phosphorus (strong bray) ($mg\,kg^{-1}$)	164 a	172 a	151 a	161 a	172 a	171 a
Potassium ($mg\,kg^{-1}$)	359 b	381 b	379 b	402 bc	612 a	626 b
Magnesium ($mg\,kg^{-1}$)	424 ab	400 b	417 ab	437 ab	453 a	432 ab
Calcium ($mg\,kg^{-1}$)	1543 ab	1458 b	1505 ab	1514 ab	1625 a	1612 a
pH	5.48 bc	5.21 c	5.69 ab	5.63 ab	5.84 a	5.79 ab
CEC (meq. $100\,g^{-1}$)	16.7 ab	17.7 a	15.3 abc	16.0 bc	16.5 abc	16.5 abc
Soil respiration (mg CO_2 kg^{-1} per day)	5.0 b	9.4 ab	12.5 ab	11.8 ab	17.2 a	15.3 a
Total extractable N ($mg\,kg^{-1}$)	60.4 b	60.9 b	72.3 b	61.8 b	93.9 a	106.7 a
Microbial biomass N ($mg\,kg^{-1}$)	47.1 c	45.7 c	57.8 bc	47.0 c	71.1 ab	81.7 a
Dissolved organic N ($mg\,kg^{-1}$)	11.4 b	8.1 b	10.9 b	11.2 b	18.3 a	17.8 a
Mineral N ($NH_4^+ + NO_3^-$) ($mg\,kg^{-1}$)	1.90 a	7.03 a	3.58 a	3.57 a	4.48 a	7.23 a

(B−): non-fertilized bare soil; (B+): fertilized bare soil; (W−): non-fertilized ground wood pallet mulch; (W+): fertilized ground wood pallet mulch; (C−): non-fertilized composted yard waste; (C+): fertilized composted yard waste. CEC = cation exchange capacity. Values are means of four treatment replicates.

[a] Values are means composited core samples from triplicate plots. Means in a row followed by different letters are significantly different (ANOVA followed by Bonferroni's *t*-test, $P \leq 0.05$).

Surprisingly, fertilization had little effect on soil parameters. One-way ANOVA indicated that fertilization had a significant effect only on mineral N ($F = 3.01$, d.f. = 5,41, $P = 0.02$). It was substantially higher in the bare soil and composted yard waste plots that had been fertilized than in other treatments (Table 2). However, the magnitude of these treatment effects was not large enough to result in significant differences as indicated by the more conservative Bonferroni's *t*-test.

3.2. Culturable rhizosphere bacterial populations

Populations of total culturable bacteria in the rhizosphere, as determined on 1/10 strength TSB, generally were highest (8.6 $\log_{10}$ MPN cm^{-1} root tip) in composted yard waste plots that did not receive any fertilization treatment (C−) (Fig. 1). These populations, however, were not significantly different from those in plots mulched with composted yard waste and fertilized (C+) (Fig. 1). The populations of total culturable bacteria were lowest in the bare soil plots (B− and B+) and fertilized plots mulched with ground wood (W+). The numbers of rhizosphere bacteria in these plots ranged from 6.6 to 7.2 $\log_{10}$ MPN cm^{-1} root tip (Fig. 1). These numbers were significantly lower than that found in the composted yard waste plots (C− and C+).

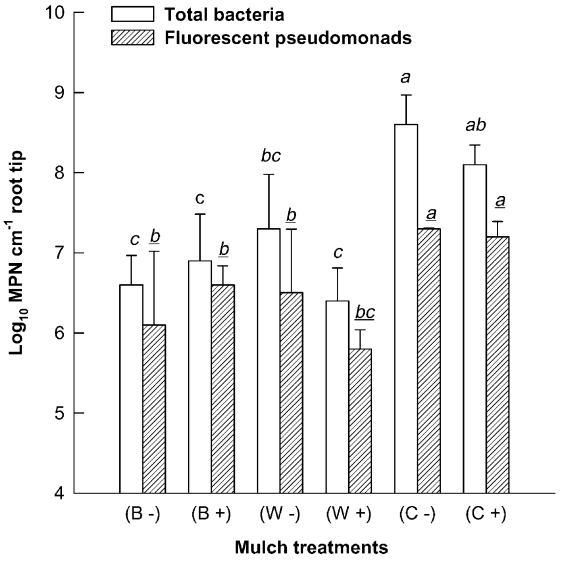

Fig. 1. The $\log_{10}$ MPN counts of total bacteria and fluorescent pseudomonads from rhizospheres of cucumber seedlings grown in non-fertilized bare soil (B−), fertilized bare soil (B+), non-fertilized ground wood pallet mulch (W−), fertilized ground wood pallet mulch (W+), non-fertilized composted yard waste (C−), and fertilized composted yard waste (C+) plots. Bars with different letters (non-italics letters = total bacteria; italics and underscored letters = fluorescent pseudomonads) are significantly different (ANOVA followed by Bonferroni's *t*-test, $P \leq 0.05$).

Rhizosphere populations of fluorescent pseudomonads as determined on King's B medium of were also significantly higher on seedlings grown in the composted yard waste plots (C− and C+), ($\log_{10}$ MPN cm^{-1} root tip were 7.3 and 7.2, respectively) than those in plots mulched with ground wood (W− and W+) or in bare soil plots (B− and B+) (5.8 to 6.6 $\log_{10}$ MPN cm^{-1} root tip) (Fig. 1). The populations of fluorescent pseudomonads across all treatments, however, were an order of magnitude lower than the total bacterial counts (Fig. 1). This was not the case in bare soil plots that were not fertilized (B−), where the $\log_{10}$ MPN cm^{-1} counts of fluorescent pseudomonads were of the same order of magnitude as the total bacterial counts (Fig. 1).

3.3. TRFLP profiles

Each sample yielded 19 to 52 TRFs (Table 3), depending on the restriction enzyme used. The fertilized compost-mulched plots (C+) gave the highest number of TRFs using the *Msp*I restriction enzyme. However, this number was not significantly different compared with the number of TRFs from the other five treatments, due to high variation of TRF numbers between replicates (Table 3).

There were also no significant differences among the six treatments in TRFLP-based Shannon diversity index (Table 3). The Shannon diversity index ranged from 2.4 to 3.3, with the highest values occurring in the plots mulched with compost but not fertilized (C−) (Table 3). However, *Msp*I TRFLPs did reveal that fertilization affected the equitability index of plots mulched with compost. The equitability index was higher for the non-fertilized compost plots (C−) than the fertilized compost plots (C+) (Table 3), as well as the non-fertilized bare soil plots (B).

Samples from each plot were compared to detect the similarities of TRFLP patterns within and between treatments. The TRFLPs patterns for the B−, B+, W−, W+, and C− treatments were very similar, with

Table 3
Number of TRFs and diversity statistics calculated from *Hha*I, *Rsa*I and *Msp*I TRFLP profiles of 16S rRNA genes amplified from cucumber roots

Mulch treatment	Number of TRFs			TRFLP Shannon diversity index			TRFLP equitability index		
	*Hha*I	*Msp*I	*Rsa*I	*Hha*I	*Msp*I	*Rsa*I	*Hha*I	*Msp*I	*Rsa*I
B−	19 ± 5 a	33 ± 9 a	32 ± 10 a	2.5 ± 0.35 a	2.9 ± 0.25 a	3.0 ± 0.11 a	0.83 ± 0.00 a	0.83 ± 0.01 b	0.86 ± 0.03 a
B+	31 ± 10 a	32 ± 9 a	33 ± 6 a	2.9 ± 0.20 a	2.9 ± 0.17 a	3.0 ± 0.10 a	0.86 ± 0.03 ab	0.86 ± 0.02 ab	0.85 ± 0.02 a
W−	31 ± 21 a	26 ± 8 a	31 ± 8 a	2.8 ± 0.49 a	2.8 ± 0.25 a	3.0 ± 0.23 a	0.87 ± 0.03 ab	0.86 ± 0.02 ab	0.87 ± 0.01 a
W+	27 ± 1 a	32 ± 9 a	44 ± 23 a	2.9 ± 0.10 a	3.0 ± 0.23 a	3.3 ± 0.31 a	0.86 ± 0.05 ab	0.86 ± 0.02 ab	0.89 ± 0.03 a
C−	46 ± 14 a	33 ± 4 a	37 ± 13 a	3.4 ± 0.47 a	3.1 ± 0.10 a	3.1 ± 0.25 a	0.88 ± 0.06 a	0.89 ± 0.03 a	0.88 ± 0.01 a
C+	23 ± 2 a	52 ± 18 a	32 ± 9 a	2.4 ± 0.00 a	3.4 ± 0.25 a	3.0 ± 0.11 a	0.75 ± 0.02 b	0.87 ± 0.01 ab	0.85 ± 0.03 a

(B−): non-fertilized bare soil; (B+): fertilized bare soil; (W−): non-fertilized ground wood pallet mulch; (W+): fertilized ground wood pallet mulch; (C−): non-fertilized composted yard waste; and (C+): fertilized composted yard waste. Mean and S.D. of three replicates are shown. Column values followed by different letters are significantly different (ANOVA followed by Bonferroni's *t*-test, $P \leq 0.05$).

nearly complete overlap (Fig. 2). The similarity among the triplicate plots determined using the RDP similarity tool (which compares the presence and absence of individual TRF sizes) was between 0.81 and 0.91. This implies that TRF overlap in root samples from three plots of the same treatment ranged from 81 to 91%.

TRFLP similarity between treatments ranged from 0.48 to 0.82 (Table 4), which was less than that observed within a treatment. The similarity of cucumber root TRFLPs in the bare soil (B− and B+) and ground wood treatments (W− and W+) ranged from 0.54 to 0.82. A somewhat lower similarity range (0.48–0.71) was observed for TRFLP patterns of root samples from plots mulched with compost as compared to the bare soil controls (Table 4). The similarity in TRFLP patterns between plots mulched with compost (C− and C+) and ground wood (W− and W+) ranged from 0.56 to 0.80 (Table 4).

Visual observation of the TRFLP fingerprints from rhizospheres of cucumber seedlings revealed unique peaks among the different mulch treatments (Fig. 2). The unique TRFs from TRFLPs derived from *Hha*I, *Msp*I, and *Rsa*I digestions are reported in Table 5. A 685 bp *Hha*I TRF observed in all the bare soil treatments (B− and B+) was not observed in the mulched treatments (W−, W+, C− and C+), whereas a 510 bp *Hha*I TRF was detected only in plots mulched with compost (C− and C+) only (Fig. 2). Moreover, one *Hha*I unique peak (840 bp) was detected only in plots mulched with ground wood (W− and W+), being absent from the bare soil (B− and B+) and compost-mulched treatments (C− and C+) (Fig. 2).

To identify the microorganisms that correspond to these TRFs, unique peaks in *Hha*I, *Msp*I and *Rsa*I TRFLPs were compared with TRFs of known organisms in the RDP. The unique TRFs from TRFLPs derived from *Hha*I, *Msp*I, and *Rsa*I digestions are reported in Table 5. All three unique peaks (*Hha*I, 685 bp; *Msp*I, 66 and 430 bp; *Rsa*I, 438 bp) in the bare soil plots (B− and B+) corresponded with TRF sizes of *Clostridium* species. In plots mulched with ground wood (W− and W+) and compost (C− and C+), unique TRFs could only be matched with two digestions from the 16S rRNA gene sequence database (Table 5). The 840 bp *Hha*I and 546 bp *Msp*I TRFs unique to the mulched plots (both ground wood and compost) corresponded with TRF sizes of *Mycoplasma* spp. Fragment sizes corresponding to *Rhodocista* spp. (*Hha*I, 510 bp; *Rsa*I, 824) were also found in the non-fertilized, compost-mulched plots (C−) (Table 5).

Differences in the relative abundance of some peaks common to all treatments were also observed. For instance, the relative abundance of 970 and 990 bp *Hha*I TRFs were low in the bare soil treatments (B− and B+) and higher in the mulched treatments (W+, C−, and C+) (Fig. 2). Conversely, the peak area of 70, 205, and 206 bp *Hha* I TRFs were greater in the bare soil controls than in the compost-mulched treatments (Fig. 2). These fragment sizes correspond to a wide variety of species from different phylogenetic groups.

The TRFs found in the various treatments were also compared to TRF sizes of *Pantoea* and *Pseudomonas* biocontrol strains, which revealed differences among treatments in numbers of different strains corresponding to observed TRF sizes (Table 6). *Pseudomonas fluorescens* TRFs were present in all root samples, with the exception of the non-fertilized bare soil control (B−). The rhizosphere from the non-fertilized, bare soil plots (B−) had lower numbers of *Pseudomonas* TRFs than any other treatment. In the non-fertilized, bare soil control (B−), TRFs from only two *Pseudomonas* spp. (*Pseudomonas corrugata* and *P. saccharophila*) were detected, while *Pantoea* spp. TRFs were absent (Table 6).

Interestingly, the *Pseudomonas* and *Pantoea* TRFs detected in the fertilized, bare soil treatment (B+) were similar to those detected in the non-fertilized, compost (C−) treatment. In fertilized plots, the numbers of identified *Pseudomonas* and *Pantoea* TRFs was greatest in the bare soil control, intermediate in the plots mulched with ground wood, and lowest in the plots mulched with compost. In the non-fertilized treatments, the opposite order was observed: *Pseudomonas* and *Pantoea* TRFs were highest in the compost-mulched and lowest in the bare soil treatment (Table 6). Sets of three TRFs corresponding to *Pantoea* spp. were found in the fertilized bare soil and compost treatment (C+) (Table 6). However, these fragment sizes correspond to a wide range of Gram-negative bacteria including *Escherichia coli*, *Salmonella*, and others.

MPN culturing of roots in King's B medium indicated high numbers of fluorescent pseudomonads in the root samples, particularly those from plots mulched with composted yard waste (C− and C+) (Fig. 1). To determine the most dominant culturable

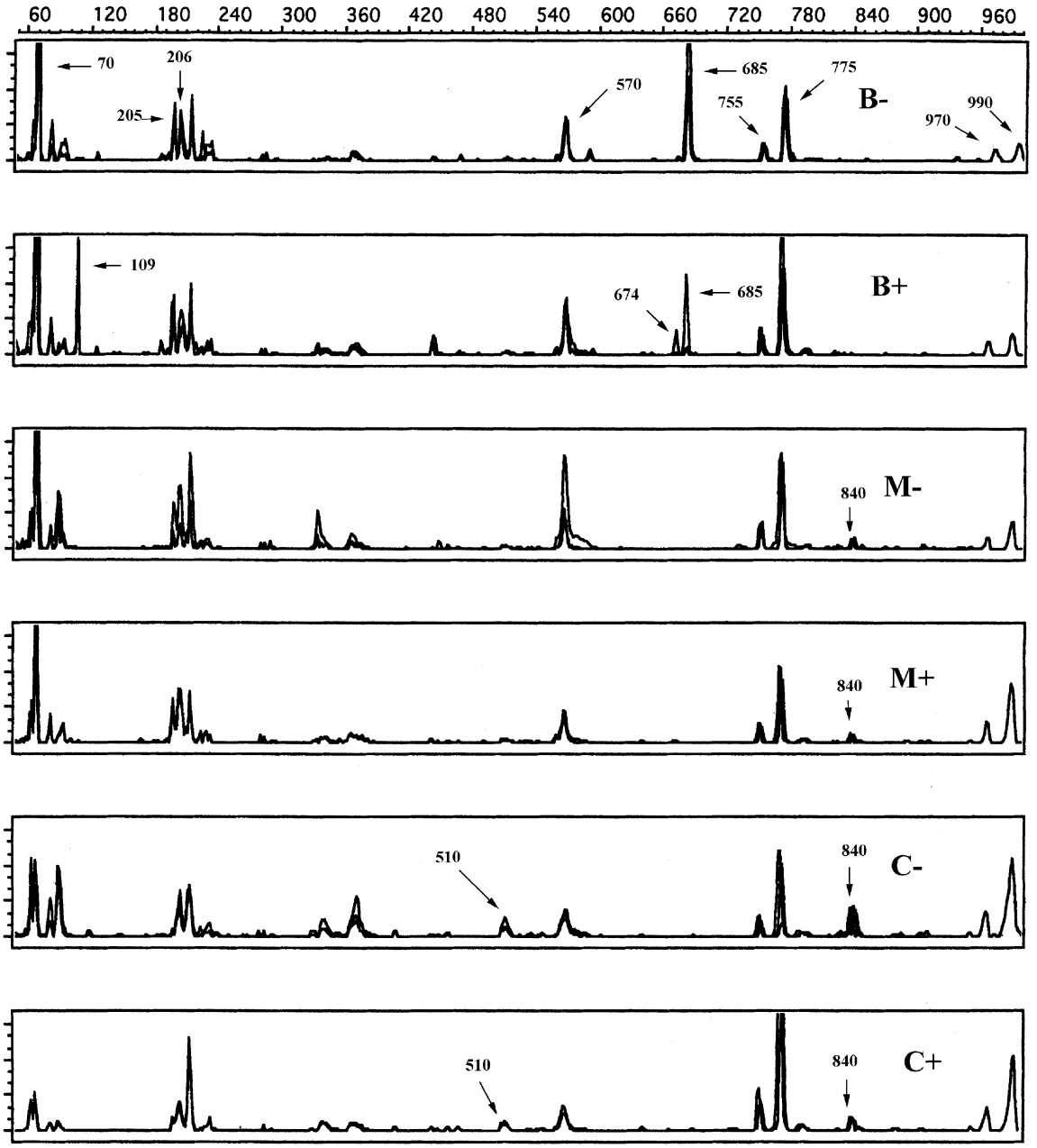

Fig. 2. Electropherograms of the 5′ TRFLP of *Hha*I-digested 16S rRNA genes amplified from rhizospheres of cucumber seedlings grown in non-fertilized bare soil (B−), fertilized bare soil (B+), non-fertilized ground wood pallet mulch (W−), fertilized ground wood pallet mulch (W+), non-fertilized composted yard waste (C−), and fertilized composted yard waste (C+) plots.

Table 4
Similarity matrix calculated from composited *Msp*I, *Rsa*I and *Hha*I TRFLP profiles of 16S rRNA genes amplified from cucumber rhizospheres grown in plots mulched with compost or ground wood pallets or on bare soil, with (+) and without (−) fertilization

Mulch treatment	B − 1	B − 2	B − 3	B + 1	B + 2	B + 3	W − 1	W − 2	W − 3	W + 1	W + 2	W + 3	C − 1	C − 2	C − 3	C + 1	C + 2	C + 3
B − 1	1.00																	
B − 2	**0.81**	1.00																
B − 3	**0.86**	**0.83**	1.00															
B + 1	0.78	0.77	0.64	1.00														
B + 2	0.63	0.74	0.67	**0.83**	1.00													
B + 3	0.69	0.80	0.64	**0.90**	**0.90**	1.00												
W − 1	0.62	0.58	0.53	0.68	0.62	0.64	1.00											
W − 2	0.60	0.68	0.60	0.67	0.70	0.73	**0.84**	1.00										
W − 3	0.57	0.60	0.54	0.62	0.67	0.62	**0.86**	**0.87**	1.00									
W + 1	0.73	0.72	0.64	0.80	0.74	0.72	0.76	0.73	0.68	1.00								
W + 2	0.71	0.77	0.66	0.77	0.77	0.78	0.86	0.77	0.65	**0.87**	1.00							
W + 3	0.69	0.72	0.66	0.70	0.79	0.82	0.64	0.72	0.67	**0.82**	**0.82**	1.00						
C − 1	0.58	0.63	0.57	0.64	0.70	0.66	0.69	0.69	0.69	0.74	0.68	0.67	1.00					
C − 2	0.53	0.67	0.59	0.58	0.71	0.70	0.61	0.73	0.68	0.65	0.72	0.74	**0.85**	1.00				
C − 3	0.48	0.56	0.58	0.59	0.67	0.59	0.56	0.63	0.66	0.63	0.58	0.63	**0.86**	**0.81**	1.00			
C + 1	0.62	0.63	0.60	0.70	0.68	0.65	0.70	0.68	0.63	0.80	0.69	0.61	0.80	0.68	0.66	1.00		
C + 2	0.55	0.64	0.54	0.62	0.71	0.72	0.63	0.64	0.61	0.69	0.66	0.74	0.75	0.78	0.64	**0.91**		
C + 3	0.61	0.64	0.60	0.70	0.72	0.68	0.67	0.68	0.64	0.77	0.70	0.70	0.79	0.71	0.69	**0.89**	**0.85**	

(B−): non-fertilized bare soil; (B+): fertilized bare soil; (W−): non-fertilized ground wood pallet mulch; (W+): fertilized ground wood pallet mulch; (C−): non-fertilized composted yard waste; and (C+): fertilized composted yard waste. 1: Replicate #1; 2: replicate #2; 3: replicate #3. Bold and underlined values are similarity values of replicate TRFLPs.

Table 5
Unique TRF peaks from *Hha*I, *Rsa*I and *Msp*I TRFLP profiles of 16S rRNA genes amplified from cucumber roots and microorganisms/phylogenetic groups corresponding to observed TRFs from the RDP (TRFLP-TAP)

Treatment[a]	*Hha*I TRFs (bp)	Corresponding organisms	*Msp*I TRFs (bp)	Corresponding organisms	*Rsa*I TRFs (bp)	Corresponding organisms
B−	685	***Clostridium irregularis***	66	***Clostridium sp.***, *Bifidpbacterium asteroids*, *Arthobacter globiformis*	438	***Clostridium sp.***, *Fervidobacterium nodosum*, *Vibrio* sp., *S. runinantium*, *Aeromicrobium fastidiosum*, *Buchnera aphidicola*, *Erhlichia bovis*, *Photobacterium leiognathi*
B+	685	***C. irregularis***	430	***Clostridium kluyveri***, *Alcaligenes eutrophus*, *Bacillus alvei*, *Hyphomonas* spp., *Nitrosolobus multiformis*, *Rhodobacter sulfidophilus*	438	***Clostridium spp.***, *F. nodosum*, *Vibrio* sp., *Selemonas runinantium*, *A. fastidiosum*, *B. aphidicola*, *E. bovis*, *P. leiognathi*
	109	None				
	674	None				
W−	840	***Mycoplasma pulmonis***, *Eubacterium yurii*, *Francisella turalensis*, *Rhodobacter capsulatus*	546	***Mycoplasma sp.***, *Ureaplasma* sp.	311	Many *Bacterodes–Cytophaga/Flexibacter* species, many *γ-Proteobacteria*
W+	840	***M. pulmonis***, *E. yurii*, *F. turalensis*, *R. capsulatus*	546	***Mycoplasma sp.***, *Ureaplasma* sp.	716	None
C−	510	***Rhodocista sp.***, *Erythrobacter logus*, *R. capsulatus*	546	***Mycoplasma sp.***, *Ureaplasma* sp.	824	***Rhodocista sp.***, *Agrobacterium tumefaciens*, *Rhizobium* sp., *Rhodospirillum centrum*, *Magnetospirillum* sp.
	840	***M. pulmonis***, *E. yurii*, *F. turalensis*, *R. capsulatus*	564	*Bacillus/Clostridium* spp.		
C+	510	*Rhodocista* sp., *E. logus*, *R. capsulatus*	546	*Mycoplasma* sp., *Ureaplasma* sp.	156	None
	840	***M. pulmonis***, *E. yurii*, *F. turalensis*, *R. capsulatus*	564	*Bacillus/Clostridium* spp.		

Values in bold are genera consistent with *Pseudomonas* and *Pantoea* biocontrol agents derived from *Hha*I, *Rsa*I, and *Msp*I TRFLP profiles of 165 rRNA genes amplified from cucumber roots.

[a] (B−): non-fertilized bare soil; (B+): fertilized bare soil; (W−): non-fertilized ground wood pallet mulch; (W+): fertilized ground wood pallet mulch; (C−): non-fertilized composted yard waste; and (C+): fertilized composted yard waste.

Table 6
Observed TRFs consistent with *Pseudomonas* and *Pantoea* biocontrol agents derived from *Hha*I, *Rsa*I and *Msp*I TRFLP profiles of 16S rRNA genes amplified from cucumber roots

Species	Representative TRFs[a]			Presence or absence (percent minimum normalized TRF peak area)[b]					
	*Hha*I	*Msp*I	*Rsa*I	B−	B+	W−	W+	C−	C+
Pseudomonas aeruginosa (Ps aerugi3)	370	146	647	−	−	−	−	−	−
Pseudomonas cepacia (25416)	207	143	887	−	−	−	+ (0.35)	−	−
Pseudomonas chlororaphis	206	492	891	+ (0.68)	+ (1.71)	+ (2.89)	+ (2.06)	+ (1.84)	+ (1.86)
Pseudomonas corrugata (84pf)	207	492	476	−	+ (2.16)	+ (3.28)	+ (1.08)	+ (1.42)	+ (1.95)
Pseudomonas fluorescens (WCS)	206	495	888	−	+ (2.16)	+ (3.24)	+ (2.06)	+ (2.20)	+ (1.95)
Pseudomonas fluorescens (2-79)	207	492	888	−	+ (2.16)	+ (3.24)	+ (2.06)	+ (2.58)	+ (0.46)
Pseudomonas fluorescens (D7172)	206	81	650	−	−	−	−	−	−
Pseudomonas mendocina (Ps. Mendoci)	207	494	167	−	−	−	−	−	−
Pseudomonas saccharophila (82)	208	494	887	+ (0.68)	+ (2.45)	+ (0.93)	+ (2.06)	+ (0.99)	+ (0.71)
Pseudomonas syringae (98)	215	457	483	−	−	−	−	−	−
Pantoea agglomerens (278)	375	500	428	−	−	−	−	+ (0.33)	−
P. agglomerens (43875)	375	500	894	−	−	−	−	+ (0.33)	−
Pantoea ananas (DC 130)	374	500	428	−	+ (0.46)	−	−	+ (1.12)	−
Pantoea ananas (DC 147)	373	498	426	−	+ (0.30)	−	−	+ (0.67)	−
Pantoea herbicola pv *gypsophila* (DC 559)	373	500	426	−	+ (0.30)	−	−	+ (0.67)	−
Pantoea herbicola pv *gypsophila* (DC 556)	373	500	426	−	+ (0.30)	−	−	+ (0.67)	−
Pantoea stewartii (SW2)	372	202	428	−	−	−	−	−	−

(B−): non-fertilized bare soil; (B+): fertilized bare soil; (W−): non-fertilized ground wood pallet mulch; (W+): fertilized ground wood pallet mulch; (C−): non-fertilized composted yard waste; and (C+): fertilized composted yard waste.

[a] Observed TRF lengths of *Pseudomonas* and *Pantoea* spp. in TRFLPs generated using *Hha*I, *Msp*I, and *Rsa*I digestions.

[b] "+" Implies that the same three *Hha*I, *Msp*I, and *Rsa*I TRF lengths found in TRFLPs of *Pseudomonas* and *Pantoea* spp. were found in the TRFLP fingerprint of a given sample treatment. "−" Implies that the three *Hha*I, *Msp*I, and *Rsa*I TRF lengths found in TRFLPs of *Pseudomonas* and *Pantoea* spp. were not found in the TRFLP fingerprint of a given sample treatment. Values in parentheses are percent peak area of the *Hha*I, *Rsa*I or *Msp*I TRF with the minimum normalized peak area. Observed TRF lengths are within ±2 bp of TRF lengths of representative TRFs.

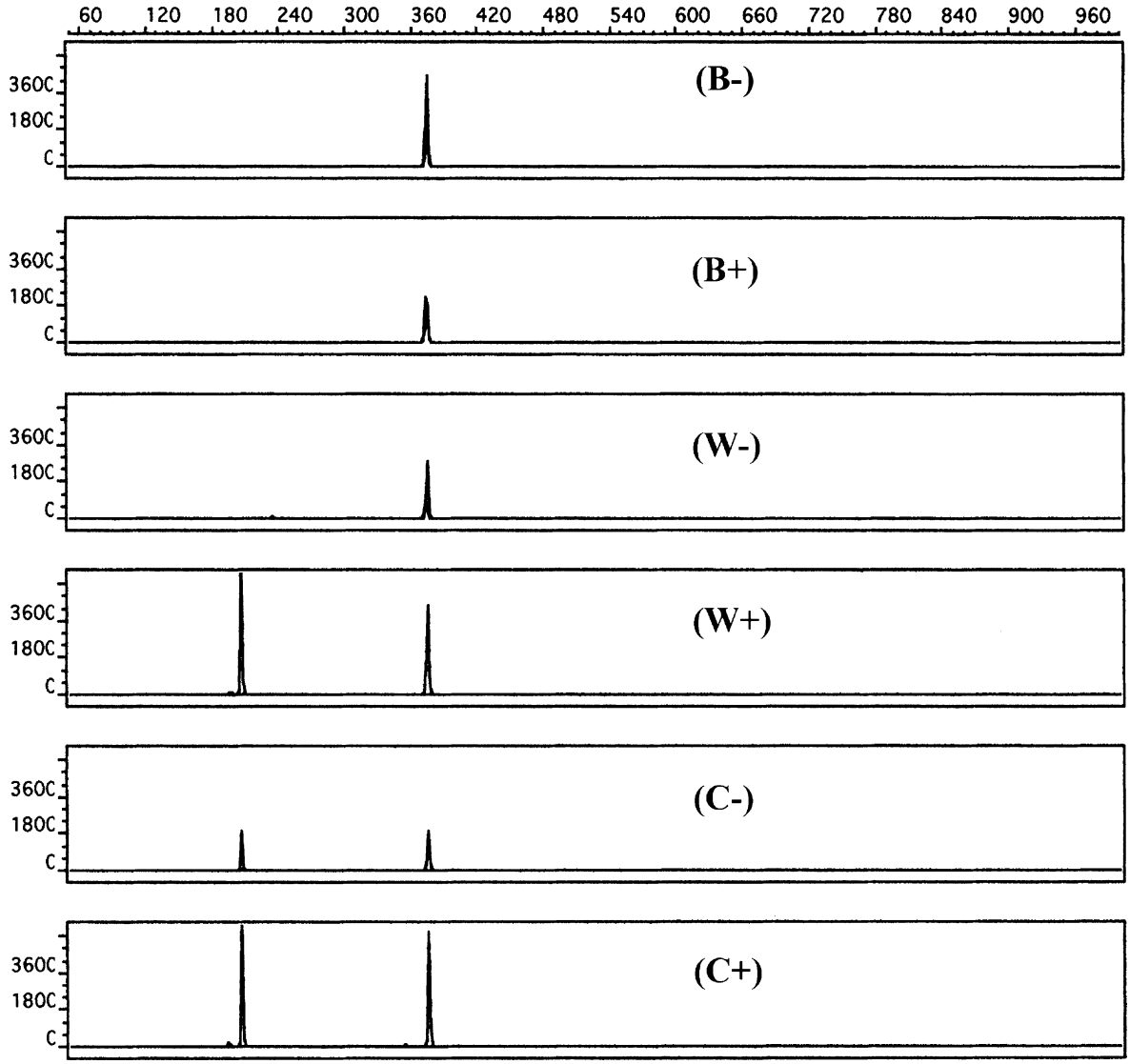

Fig. 3. Electropherograms of the 5′ TRFLP of *Hha*I-digested 16S rRNA genes amplified from the most dilute fluorescent pseudomonads MPN well from cucumber roots grown in non-fertilized bare soil (B−), fertilized bare soil (B+), non-fertilized ground wood pallet mulch (W−), fertilized ground wood pallet mulch (W+), non-fertilized composted yard waste (C−), and fertilized composted yard waste (C+) plots.

fluorescent pseudomonads present, samples from the most dilute King's B MPN wells that showed fluorescence were PCR-amplified and subjected to TRFLP analysis. As an example, Fig. 3 shows a *Hha*I TRFLP pattern obtained from this PCR-amplification. The TRF sizes from three different digestions (*Hha*I, *Msp*I, and *Rsa*I) are shown in Table 7 . Between one and four TRF peaks were found in each TRFLP pattern. The TRF lengths of PCR-amplified culturable fluorescent pseudomonads in the bare soil control (B− and B+) and non-fertilized, ground wood mulch (W−) treatments did not match any of the three TRF lengths predicted for *Pseudomonas* spp. in Table 6. In the W− treatment two of the TRFs (*Msp*I and

Table 7
TRFs for predominant culturable organisms in most dilute King's B MPN well and corresponding *Pseudomonas* TRFs

Treatment	Observed TRF lengths (bp)			Putative *Pseudomonas* strains
	*Hha*I	*Msp*I	*Rsa*I	
B−	375	200, 496	430	No match to TRF sizes of known pseudomonads listed in Table 6 and the RDP
B+	375	496	430	No match to TRF sizes of known pseudomonads listed in Table 6 and the RDP
W−	375	200, 496	430	No match to TRF sizes of known pseudomonads listed in Table 6 and the RDP
W+	195, 375	200, 480, **492**, **498**	430, **886**	*P. fluorescens*, *P. putida*, *P. saccharophila*
C−	195, 375	**492**, **498**	430, **886**	*P. fluorescens*, *P. putida*, *P. saccharophila*
C+	195, 375	**492**, **498**	883, **890**	*P. fluorescens*, *P. putida*, *P. saccharophila*, *P. chlororaphis*

(B−): non-fertilized bare soil; (B+): fertilized bare soil; (W−): non-fertilized ground wood pallet mulch; (W+): fertilized ground wood pallet mulch; (C−): non-fertilized composted yard waste; and (C+): fertilized composted yard waste. Values in bold are observed TRF sizes that correspond with TRF sizes of known *Pseudomonas* organisms in Table 5. Non-bold values are observed TRF that did not correspond to TRF sizes of known *Pseudomonas* organisms listed in Table 6 and the RDP. Observed TRF lengths are within ±2 bp of those TRF lengths of known *Pseudomonas* TRFs.

*Rsa*I) found matched the TRF lengths predicted for fluorescent pseudomonads (Table 7). The same was true for plots mulched with compost (C− and C+). These TRF lengths corresponded with those of *P. fluorescens*, *P. putida*, and *P. saccharophila*. The group of three TRFs found in the most dilute MPN well in King's B medium (TRFs H375, M498 and R429 and TRFs H375, M500 and R428) did however match a group of Gram-negative *Proteobacteria* that included *Pantoea*, *Vibrio* strains, and *E. coli* not known to produce fluorescent siderophores. TRFs H375, M498 and R429 were observed in the most dilute MPN well of all treatments, except the fertilized, compost treatment (C+) (Table 7). TRFs H375, M500 and R428 correspond to a set of *Pantoea* strains implicated in biocontrol of plant diseases (Table 6). TRFs within 2 bp of all three of these TRF sizes were observed in the W+ and C− treatments.

4. Discussion

Mulching with recycled ground wood pallets or composted yard waste had substantial effects on soil pH, CEC, organic matter content, nutrient levels, and total microbial biomass (as indicated by soil respiration and microbial N). All of these parameters were highest in plots mulched with composted yard waste as compared to those mulched with ground wood pallets or bare soil plots. Fertilization had little effect on any of these parameters. Despite the dramatic effects of the composted yard waste treatment on soil chemical and biological properties, TRFLP fingerprints of the 16S rRNA genes from rhizospheres of cucumber grown in soils with different mulch and fertilization treatments were surprisingly similar (Fig. 2). The lack of differences in the diversity indicated by TRFLP among plots with different mulch treatments (Table 3), is consistent with previous reports by Boehm et al. (1993) on the effect of organic matter decomposition level on bacterial species diversity and composition. They reported that differences in organic matter levels among different potting mixes (compost-amended dark peat mix, slightly decomposed light peat mix, and decomposed peat mix), appeared to have little impact on the diversity of rhizosphere communities. It is possible that the activity, and less so the diversity of the cucumber rhizosphere communities was influenced by the mulch treatments. In the present study, total microbial biomass (as indicated by soil respiration and microbial N) was significantly higher in rhizospheres sampled from plots mulched with compost than in the bare soil and wood mulched plots (Table 2). Similarly, the population of culturable rhizosphere total bacteria and fluorescent pseudomonads (Fig. 1) were significantly higher in the composted yard waste treatment that than in the bare soil and wood mulched plots.

While significant differences in TRFLP-based diversity indices (Shannon diversity index and equitability index) were not observed, TRFLP patterns among different treatments were much less similar than among replicate samples within a treatment. Some

unique TRF peaks were detected among treatments (Fig. 2), suggesting that specific subcomponents of the microbial communities indeed differed. Using culturable methods, Boehm et al. (1993 and 1997) showed that microorganisms such *Pseudomonas* and *Pantoea* spp. were the most abundant species present in both the rhizosphere and the surrounding non-rhizosphere in compost-amended substrates. These genera harbor strains that are effective biocontrol agents of soil borne pathogens (Weller, 1988; Boehm et al., 1997; Paulitz et al., 2000). Results of the present study indicated that fertilization or mulching (either with composted yard waste or ground wood pallets) led to growth of *Pseudomonas* and *Pantoea* strains (Table 5) in the rhizosphere of cucumber roots. However, the application of fertilizer to plots mulched with compost or ground wood (M+ and C+) had no additive effects on the diversity of these biocontrol organisms in the cucumber rhizosphere. Rhizospheres from non-fertilized compost-mulched plots (C−) had the most TRFs characteristic of *Pseudomonas* and *Pantoea* strains. Rhizospheres from plots mulched with compost and fertilized (C+) had TRFs from a lower number of these species than did plots mulched with compost that were not fertilized (C−) (Table 6). However, fertilization of bare soil plots (B+) increased the number of TRFs corresponding to these biocontrol (Table 6).

The TRF lengths of dominant culturable fluorescent pseudomonads in the rhizosphere samples from bare soil (B− and B+) and wood mulched (W− and W+) plots (Table 7) did not correspond to those predicted for fluorescent *Pseudomonas* strains using the TRFLP-TAP tool of in the 16S rRNA gene sequence database (Marsh et al., 2000). In addition, none of these TRFs matched the TRF sizes of previously isolated fluorescent *Pseudomonas* strains implicated in biocontrol (Table 6), suggesting that the most dominant culturable fluorescent *Pseudomonas* strains remain unknown species.

Moreover, many TRF sizes found in the rhizosphere samples from the mulch treatments (Fig. 2) did not correspond with the TRFs of organisms predicted by the TRFLP-TAP tool. The identity of organisms generating these TRFs are unknown, which is typical of the high number of novel microbes found in environmental samples using nucleic acid-based methods.

This study demonstrated that TRFLP of 16S rRNA genes generates similar rhizosphere community patterns in different mulch treatments. The similarity matrix analysis was able to show that the variability between replicates is small, with similarity values between 81 and 90%. The similarities were also high when similar treatments (C− and C+; M− and M+; B− and B+) were compared. The relationships between different soil management treatments and bacterial community structure may have been obscured in this analysis because the TRFLP patterns were very complex. The patterns generated from by TRFLP required detailed analyses to understand relationships or differences among treatments used in this study, such as diversity analysis of TRFs; comparison of unique TRFs to microorganisms/phylogenetic groups corresponding to that observed from the RDP; presence and relative abundance of biocontrol agents; and comparison of predominant culturable organisms.

The overall results of these analyses showed that the rhizosphere communities in compost-mulched plots are significantly different from the bare soil and wood-mulched plots. A higher number of TRFs that correspond to biocontrol organisms such as *Pseudomonas* and *Pantoea* spp. were also found in compost-mulched plots. However, differences among treatments were more obvious when the soil chemical properties (nutrients, pH, CEC, and organic matter content) and soil microbial activities (total microbial biomass) were compared, than the TRFLPs of cucumber roots. This could be due to the fact that cucumber seeds were planted out-of-season and harvested when they were young. It may be possible that large differences in community structure were not observed based on TRFLPs because roots had not yet thoroughly colonized the soil. There is evidence that microbial communities vary with different plant growth stages (e.g. Deleij et al., 1994; Di Cello et al., 1997). However, the effect of out-of-season planting on the rhizosphere microbial communities has not been addressed.

Nonetheless, the results of this study demonstrate clearly that mulching with compost strongly influenced the composition of the microbial rhizophere community. These data provide baseline information about the effect of soil management practices on the structure, diversity, and function of the microbial communities in the rhizospheres.

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