

Carmine Crecchio · Maddalena Curci  
Rosaria Mininni · Patrizia Ricciuti · Pacifico Ruggiero

## Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity

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**Abstract** Municipal solid waste (MSW) composts have been frequently used as N and C amendments to improve soil quality and to support plant growth, with the additional benefit of reducing waste disposal costs. However, attention has been paid to the risks of MSW use for the soil environment. The presence of heavy metals in MSW composts can affect some microbiological characteristics of soil such as the structure of the soil microbiota, which are responsible for the transformations making nutrients available to plants. The effects of MSW compost and mineral-N amendments in a 2-year field trial on some physical-chemical properties, some enzyme activities and the genetic diversity of cropped plots (sugar beet-wheat rotation) and uncropped plots were investigated. Variations of pH were not statistically related to MSW compost and mineral-N amendments, or to the presence of the crop. Amendment with MSW compost increased the organic C and total N contents, and dehydrogenase and nitrate reductase activities of soil. In cropped plots amended with MSW compost, dehydrogenase activity was positively correlated with  $\beta$ -glucosidase activity, and both enzyme activities with organic C content. No MSW compost dosage effect was detected. No effects were observed on denaturing gradient gel electrophoresis and amplified rDNA restriction analysis patterns, indicating that no significant change in the bacterial community occurred as a consequence of MSW amendment.

**Keywords** Municipal solid waste compost · Soil quality indicators · Soil enzyme activities · Genetic fingerprints · Soil bacteria community

### Introduction

Agricultural soils are quite often subject to excessive erosion, nutrient run-off, and loss of organic matter and, consequently, a decline in fertility. As the quality of a soil is central to determining the sustainability and productivity of aboveground communities (Doran et al. 1994), maintaining and enhancing the tilth, fertility, and productivity of agricultural soils have had a high priority in the last decades. Meanwhile, greater awareness had recently led to a recognition of the need to improve soil quality and to support plant growth in a sustainable way, which takes into account environmental concerns. One method of reversing the degradation and improving the quality of soils involves the addition of several kinds of wastes such as solid organic waste, sewage sludge, agricultural and industrial wastes, and animal manure (Lalande et al. 1998; Mamo et al. 1999; Pascual et al. 1999b; Albiach et al. 2000; Lalande et al. 2000; Masciandaro et al. 2000). In this way, the additional benefit of reducing waste disposal costs is also obtained. Some of the wastes can be added to the soil without any risk (Lerch et al. 1992), whereas some others can produce toxic and depressant effects on plants and the microbial community (Ayuso et al. 1996).

The use of municipal solid waste (MSW) compost is increasing in many European countries (Allievi et al. 1993; Gigliotti et al. 1997; Convertini et al. 1998; Mamo et al. 1999; Pascual et al. 1999a). MSW compost contains large amounts of organic matter and both organic and inorganic N. The amounts of plant-available N and C from MSW are closely related to the degree of compost maturity, the addition of mineral fertilizers, soil characteristics and environmental parameters. A major concern associated with the use of MSW remains the presence of heavy metals that can be toxic to plants (Carlson et al. 1975), enter the food chain, contaminate water, and affect human health. Furthermore, the addition of MSW compost can affect the main biological characteristics of soil in both the short- and long-term.

C. Crecchio (✉) · M. Curci · R. Mininni · P. Ricciuti · P. Ruggiero  
Dipartimento di Biologia e Chimica Agroforestale ed Ambientale,  
Università di Bari, via Amendola 165/a, 70126 Bari, Italy  
e-mail: crecchio@agr.uniba.it  
Tel.: +39-080-5442854, Fax: +39-080-5442850

Chemical and physical soil parameters have been used to measure soil quality. A range of soil processes have been considered to be the most suitable rapid indicators of changes in soil quality (Visser and Parkinson 1992) or have been measured in long-term experiments (Parr and Papendick 1997). While the chemical characteristics of a soil make a significant contribution to its quality and can, therefore, be measured to define soil quality (Hassink 1997), it is the microbiological and biochemical components of soil which are more sensitive to change in soil quality. Single soil parameters such as total C content or microbial biomass C (Brookes 1995), ratios between two parameters, such as the metabolic quotient (Anderson and Domsch 1993), as well as enzyme activities (Nannipieri et al. 1990) have been proposed as indicators. A possible solution to finding a good soil quality index, sensitive to alterations in the soil quality due to environmental damage or human activity and independent of both seasonal and among-site variations, is to combine the information offered by several parameters (Trasar-Cepeda et al. 1998; Leirós et al. 1999).

Other methods such as the analysis of phospholipid fatty acids and community-level physiological profiles (Zelles et al. 1992; Pajojärvi et al. 1997) and, more recently, molecular methods are being developed for the study of the microbial diversity in soil. Changes in the composition of soil microbiota have been taken as sensitive indicators of soil health and ecosystems (Doran et al. 1994; Doran and Zeiss 2000; Hill et al. 2000).

Genetic fingerprint techniques provide a pattern of the genetic diversity in a microbial community. Polymerase chain reaction (PCR)-amplified ribosomal DNA fragments of the same length but with different sequences can be separated by denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel electrophoresis (TGGE) (Muyzer et al. 1993; Muyzer and Smalla 1998). Fingerprints of soil nucleic acids can also be obtained by amplified rDNA restriction analysis (ARDRA) (Massol-Deya et al. 1995; Smit et al. 1997); amplified 16S-23S rDNA internal spacer analysis (Garcia-Martinez et al. 1999); terminal-restriction fragment length polymorphism (Moeseneder et al. 1999); arbitrarily primed PCR (Kwok-Wong et al. 1996); or polymerase chain reaction-single stranded conformational polymorphism (Clapp 1999). Although these techniques can identify microorganisms within pure cultures (Teske et al. 1996), it is still under debate if their levels of resolution allow the analysis of microbial communities in soil. Recently, some papers reported the response of soil microorganisms to heavy metals, organic pollutants, O<sub>2</sub> availability, and characteristics of soil (Smit et al. 1997; Felske and Akkermans 1998; Kowalchuk et al. 1998; Øvreås and Torsvik 1998; Sandaa et al. 1999; Thompson et al. 1999; Crecchio et al. 2001).

The main objectives of the present study were: (1) to evaluate the effects of a MSW compost amendment in a 2-year field trial on soil physical-chemical characteristics, enzymatic activities and genetic diversity; and (2) to correlate the information arising from different ap-

proaches and contribute to defining the validity of traditional and new indices of soil quality. It has already been demonstrated that the amendment with the MSW compost under investigation positively influenced the grain yield of durum wheat and increased yields of sugar beet roots without affecting sugar content and pulp quality (Convertini et al. 1998, 1999).

## Materials and methods

### Experimental design

A field experiment was started in 1997 on a clay soil (Typic Chromoxerert; clay, 48.3%; silt, 33.4%; sand, 18.3%; pH 8.3) using randomized blocks with three replicates. Sugar beet and durum wheat-rotation cropped plots (CP) were treated with 12 t MSW compost ha<sup>-1</sup> and 24 t MSW compost ha<sup>-1</sup>, corresponding to 120 kg N ha<sup>-1</sup> (CP+MSW1) and 240 kg N ha<sup>-1</sup> (CP+MSW2), respectively; with 120 kg NH<sub>4</sub>NO<sub>3</sub>-N ha<sup>-1</sup> (CP+N), or were untreated (CP); uncropped plots (UP) were also treated with 24 t MSW compost ha<sup>-1</sup> (UP+MSW2), 120 kg NH<sub>4</sub>NO<sub>3</sub>-N ha<sup>-1</sup> (UP+N), or untreated (UP). Plots were amended at 30 cm depth by ploughing 2 months before sugar beet or wheat autumn sowing, harvested in summer and sampled, after harvest, at the end of a 2-year trial.

The compost was produced by SLIA (Castel di Sangro, Italy) through the aerobic transformation of MSW. The following composition of the compost, on a dry weight basis, is based on a 2-year average composition, with coefficients of variation (CV): 20.7 g N kg<sup>-1</sup>, CV 8.6%; 158.0 mg Cu kg<sup>-1</sup>, CV 0%; 381.5 mg Zn kg<sup>-1</sup>, CV 12.8%; 163.8 mg Mn kg<sup>-1</sup>, CV 5.0%; 209.5 mg Pb kg<sup>-1</sup>, CV 9.8%; 112.7 mg Cr kg<sup>-1</sup>, CV 19.6%; 22.1 mg Ni kg<sup>-1</sup>, CV 14.7%; 20.8 mg Co kg<sup>-1</sup>, CV 19.4%; 1.95 mg Cd kg<sup>-1</sup>, CV 1.2%; total organic C (TOC) 28.0 g kg<sup>-1</sup>, CV 5.9%; C/N ratio 29.5, CV 2.7% (Convertini et al. 1998).

### Soil sampling

Composite soil samples (four 7-cm-diameter cores per plot) were randomly collected from the surface layer (0–20 cm) after harvesting, analysed immediately, or stored at +4°C and processed within 1 month.

### Soil chemical and biochemical properties

Particle-size distribution, total N, organic matter and pH, were determined according to the methods of the Italian Society of Soil Science (SISS 1995).

β-glucosidase activity was determined according to Eivazi and Tabatabai (1988) and was expressed as μg *p*-nitrophenol h<sup>-1</sup> g<sup>-1</sup> soil; nitrate reductase activity was determined according to Abdelmagid and Tabatabai (1987) and expressed as μg NO<sub>2</sub><sup>-</sup>-N 24 h<sup>-1</sup> g<sup>-1</sup> soil; dehydrogenase following the method proposed by Tabatabai (1982) and expressed as μg triphenylformazan 24 h<sup>-1</sup> g<sup>-1</sup> soil; urease according to the method of Hofmann (1963) and expressed as mg NH<sub>4</sub><sup>+</sup>-N 3 h<sup>-1</sup> 100 g<sup>-1</sup> soil.

### Extraction and purification of DNA from soil

Total DNA was obtained by modifying a direct lysis method (Tsai and Olson 1991) and a purification protocol (Smalla et al. 1993). Soil samples (1 g) were mixed with 4 ml of 120 mM sodium phosphate buffer, pH 8.0, shaken at 150 r.p.m. for 15 min, and centrifuged for 10 min at 6,000 g. The pellet was washed 3 times, resuspended in 2 ml lysis solution (0.15 M NaCl–0.1 M Na<sub>2</sub>EDTA, pH 8.0) containing 15 mg lysozyme ml<sup>-1</sup> and incubated for 2 h at

37°C with occasional shaking. Then, 1 ml of 0.2 M NaCl-1 M TRIS-HCl, pH 8.0 and 1 ml of 20% sodium dodecyl sulphate were added. After 10 min at room temperature, three cycles of freezing/thawing (-70°C dry ice-ethanol/65°C water bath) were carried out. DNA was then extracted with 0.1 M TRIS-HCl (pH 8.0)-saturated phenol, phenol-chloroform-isoamylalcohol (25:24:1), and chloroform-isoamylalcohol (24:1). The aqueous phase was precipitated overnight at -20°C with an equal volume of isopropanol. The pellet, obtained by centrifugation at 10,000 g for 10 min, was lyophilized and resuspended in 0.5 ml double-distilled water, 0.5 g CsCl was added and the mixture incubated for 3 h at room temperature. The supernatant, obtained by centrifugation at 10,000 g for 20 min, was mixed with 2 ml H<sub>2</sub>O and 1.5 ml isopropanol and incubated for 15 min at room temperature. After centrifugation for 15 min at 14,000 g, the pellet was resuspended in 0.5 ml H<sub>2</sub>O and 0.1 ml of 8 M potassium acetate. After 15 min at room temperature and centrifugation for 15 min at 10,000 g, the supernatant was precipitated with 0.3 ml isopropanol, the pellet washed with 70% ethanol, lyophilized, resuspended in 0.1 ml H<sub>2</sub>O, and purified through a Wizard Clean-up System (Promega). Samples of extracted DNA were analysed on 0.7% agarose gel containing 0.5 µg ethidium bromide ml<sup>-1</sup>. About 10 µg DNA g<sup>-1</sup> soil was extracted with an average size of >20 kb and no degradation smear. PCR amplifiability and digestibility by restriction endonucleases without dilution were checked.

#### Polymerase chain reaction

Soil DNA was amplified in a PCR Sprint thermocycler (Hybaid) with two universal eubacterial primer sets for 16S rDNA: (1) 968F-1401R (Heuer and Smalla 1997) to obtain products of about 450 bp for DGGE; (2) 8F-1513R (Wang and Wang 1997) to obtain a product of about 1.5-kb fragments for ARDRA; (3) pH<sub>2</sub>-p23SR01 (Massol-Deya et al. 1995). A third set of primers was used for 16S-23S rDNA to obtain a product of about 1.5 kb for ARDRA. The 968F primer was modified with a 40mer GC clamp to separate PCR products in the gradient gel (Muyzer et al. 1993). The numbers in the primer names indicate the position in the 16S rRNA of *Escherichia coli* (Brosius et al. 1978). Each PCR mixture contained 100 ng DNA template, 100 pmol of each of the two primers, 20 nmol of each of the dNTPs, 2.5 U of Taq DNA polymerase, 2.5 mM MgCl<sub>2</sub> in a buffered final volume of 100 µl. Bovine serum albumin (4 µg) was added to avoid inhibition of amplification by organic compounds co-extracted from soil. The PCR conditions were: (1) a hot start of 3 min at 95°C; 35 cycles consisting of 95°C for 10 s, 54°C for 20 s, 72°C for 40 s; a final step of 10 min at 72°C; (2) a hot start of 3 min at 95°C; ten cycles consisting of 95°C for 30 s, 64–54°C for 30 s decreasing the temperature by 1°C every cycle ("touch-down"), 72°C for 2 min; 30 cycles consisting of 95°C for 30 s, 54°C for 30 s, 72°C for 2 min; a final step of 10 min at 72°C; (3) a hot start of 3 min at 95°C; 35 cycles consisting of 95°C for 30 s, 55°C for 30 s, 72°C for 40 s; a final step of 10 min at 72°C. Amplification products were analysed by electrophoresis in 1.0–1.5% agarose gels stained with ethidium bromide.

#### Denaturing gradient gel electrophoresis

DGGE was performed with the Bio-Rad Dcode system. About 30 µl of PCR products was loaded into 6% polyacrylamide gels with 40% (2.8 M urea-16% v/v formamide) to 60% (4.2 M urea-24% formamide) gradients and run for 16 h at 100 V at 60°C. Silver-stained gels (Cairns and Murray 1994) were photographed with a Bio-Rad Gel Doc 2000 documentation system equipped with Quantity One software for comparison and clustering of profiles.

#### Amplified ribosomal DNA restriction analysis

Aliquots (40 µl) of the amplified rDNA products were digested at 37°C for 2 h with 5 U Hpa II. Restricted DNA was analysed by

horizontal electrophoresis with 16×16-cm 2% agarose gels run for 4 h at 150 V, silver stained and photographed with the Bio-Rad Gel Doc system.

#### Statistical analysis

ANOVA (one- and two-way with replicates) was used to determine significant differences among chemical and biochemical parameters. The effects of amendments and culture were evaluated by multiple comparisons among class means, based on values of a least significant difference (LSD) test and on Duncan's multiple range test ( $P < 0.05$ ). The relationship among each parameter was determined by regression equations and single linear correlation coefficients ( $r$ ).

## Results and discussion

As reported in Table 1, organic C and total N were higher in cropped plots (CP samples) than in uncropped controls (UP). Cropped plots amended with MSW compost increased their organic C content; the influence of MSW compost on the total N content was more evident when double the amount of compost was added to soil and was comparable to the effect of the NH<sub>4</sub>NO<sub>3</sub> treatment. The two-way ANOVA indicated highly significant differences for C and N content among MSW compost- and mineral-N-amended cropped and uncropped plots. Duncan's multiple range test (results not shown), confirmed that the differences among all the treatments were statistically significant.

In contrast, the pH of soil samples was constant for the three replicates of each of the seven amended plots. Correlations between variations of pH (data not shown), ranging within 0.1 units, and MSW compost, mineral-N amendments, and crop, were not statistically significant. The buffering capacity of soil apparently overcame possible changes in the pH as a consequence of different amendments.

Table 1 also reports some enzymatic activities of experimental plots. Overall, enzymatic activities were higher in cropped plots (CP samples) than in uncropped controls (UP samples). The highest values were observed for CP soil amended with MSW compost. In this case, as well as for chemical parameters, there was no significant difference after the application of a single or double dose of compost, as indicated by the LSD (data not shown). A more thorough investigation of the results by ANOVA indicated that only the activities of nitrate reductase and dehydrogenase were significantly related to the type of amendment; on the other hand, all enzymatic activities under investigation were statistically different when the effects of the crop were analysed, as also confirmed by the LSD test.

In cropped plots amended with MSW compost, dehydrogenase activity was positively correlated with β-glucosidase activity ( $r = 0.69$ ,  $P < 0.001$ ) and both enzyme activities with organic C ( $r = 0.75$ ,  $P < 0.001$ ;  $r = 0.64$ ,  $P < 0.01$ , respectively), suggesting that the addition of mineralizable organic residues provides substrates for those enzymes and enhances microbial growth (Garcia et

**Table 1** Chemical and biochemical characteristics of soil plots and statistical analysis. All values expressed as means; numbers in parentheses indicate coefficients of variation of three replicates. For least significant difference (LSD) means within each column followed by different letters are significantly different at  $P < 0.05$ .

Plots	Organic C (g kg <sup>-1</sup> soil)	Total N (g kg <sup>-1</sup> soil)	β-Glucosidase (μg <i>p</i> -nitrophenol h <sup>-1</sup> g <sup>-1</sup> soil)	Nitrate reductase (μg NO <sub>2</sub> <sup>-</sup> -N 24 h <sup>-1</sup> g <sup>-1</sup> soil)	Dehydrogenase (μg triphenyl- formazan 24 h <sup>-1</sup> g <sup>-1</sup> soil)	Urease (NH <sub>4</sub> <sup>+</sup> -N 3 h <sup>-1</sup> 100 g <sup>-1</sup> soil)
CP+MSW1	12.5 (0.3)	1.43 (0.7)	157 (12.0)	1.2 (37.4)	63 (11.3)	84 (30.6)
CP+MSW2	12.7 (0.2)	1.51 (0.6)	147 (2.6)	1.4 (7.1)	61 (4.2)	74 (21.5)
CP+N	11.0 (0.2)	1.51 (0.6)	133 (9.0)	1.1 (38.3)	55 (7.6)	59 (3.7)
CP	11.6 (0.8)	1.40 (1.3)	121 (6.6)	0.7 (16.1)	50 (8.0)	80 (37.7)
UP+MSW2	10.6 (0.4)	1.44 (0.7)	114 (10.9)	0.8 (10.9)	47 (16.3)	53 (7.5)
UP+N	10.3 (0.6)	1.40 (0.7)	126 (12.7)	0.9 (12.9)	47 (3.3)	54 (9.1)
UP	9.7 (0.9)	1.36 (1.3)	1.20 (9.1)	0.7 (1.2)	46 (3.3)	47 (4.5)
ANOVA						
Source of variation	<i>F</i>					
Amendments (A)	537.04***	10.75**	n.s.	6.41*	41.41**	n.s.
Crop (C)	2773.68***	196.00***	5.97*	7.72*	17.20**	9.04*
A×C	190.48***	55.75***	n.s.	n.s.	n.s.	n.s.
LSD						
Yes	11.78 a	1.49 a	134.19 a	1.08 a	55.53 a	71.15 a
No	10.19 b	1.40 b	120.24 b	0.82 b	46.33 b	50.97 b

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , n.s. not significant

al. 1998; Albiach et al. 2000). Urease activity was higher in MSW-amended plots (CP+MSW1 and CP+MSW2) than in mineral-N-fertilized plots (CP+N). These data contrast with those reported by Garcia-Gil et al. (2000) dealing with long-term effects of MSW compost applications on soil enzyme activities, who reported inhibition of activities as a consequence of MSW compost treatments. It seems that the presence of heavy metals in the MSW compost does not cause the inhibition of urease, as supposed by Tabatabai (1977). It is reasonable to assume that high amounts of NH<sub>4</sub><sup>+</sup> in mineral-N-amended plots (CP+N) can cause a decrease in the urease activity, as supposed by König et al. (1966). As reported by Nannipieri (1994), the effect of sludges on enzyme activities is very complex and is due to: (1) inhibition of activity by heavy metals or other components, (2) repression or stimulation of enzyme synthesis.

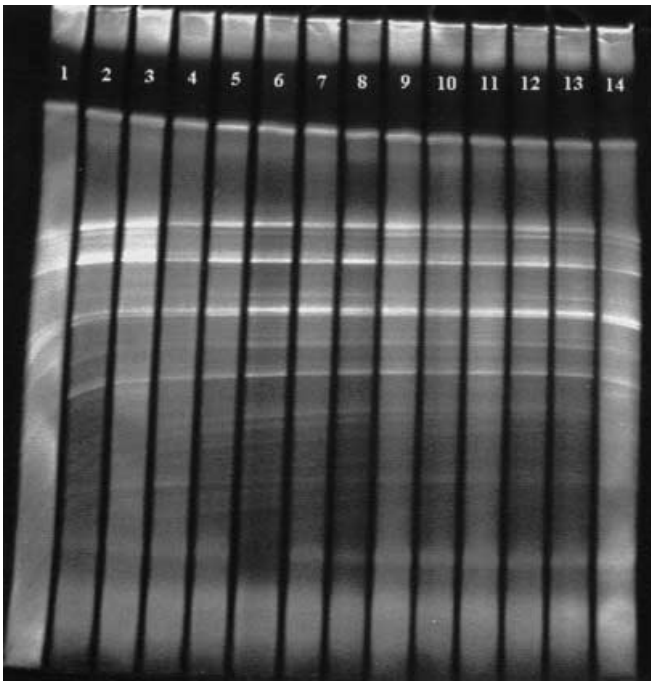
Our results indicate that the C and N contents of the soil plots were affected by amendment with MSW compost. The analysis of other potential indicators of soil quality, such as some key enzymes involved in the C and N cycles and strictly related to microbial mediated transformations that occur in soil (Dick 1994), showed a significant effect of MSW compost amendment only on dehydrogenase and nitrate reductase. Once it was clear that the addition of composted organic residues to an agricultural soil, albeit after a short-term trial, increased the content of nutrients available for plants and some enzymatic activities directly related to biochemical and microbiological transformations, we decided to apply molecular techniques to analyse the structure of the eubacterial community and the changes possibly induced in

CP Cropped plot, UP uncropped plot, MSW1 municipal solid waste compost corresponding to 120 kg N ha<sup>-1</sup>, MSW2 municipal solid waste compost corresponding to 240 kg N ha<sup>-1</sup>, N 120 kg NH<sub>4</sub>NO<sub>3</sub>-N ha<sup>-1</sup>

the soil microbiota by the amendments. PCR-mediated amplification of 16S rRNA or rDNA from a natural habitat provides an elegant way of obtaining information about the composition of microbial communities (Olsen et al. 1986; Øvreås 2000).

Figure 1 shows the DGGE analysis of 500-bp fragments amplified from soil eubacteria 16S rDNA. Two replicates for the seven experimental plots are reported, the third being absolutely identical for all plots as repeatedly checked (data not shown). The 16S rDNA fingerprints show a few strong bands, some bands of lower intensity and an additional number of weak bands resulting in a smear. Consequently, it is not possible to estimate the total number of different 16S rDNA molecules present, despite the high resolution of DGGE, because of the high number of different bacterial genomes present in the soil. No major difference among the patterns could be observed in terms of intensity or presence/absence of single bands. Software analysis showed differences among the lanes of >5% only with a very faint threshold of band intensity and, consequently, high background noise, indicating that, at least as revealed by this approach, no significant change within the bacterial community occurred as a consequence of MSW compost or mineral-N amendment of cropped and uncropped plots.

Microbial diversity was also investigated by digesting amplified rDNA with restriction endonuclease. Figure 2 reports the gel electrophoresis separation of 1,500-bp fragments, covering almost the entire 16S gene, restricted by a four-base enzyme, Hpa II. As for Fig. 1, in order to run DNA samples from all plots in one gel, the third replicate for each sample, previously found to be identical, is

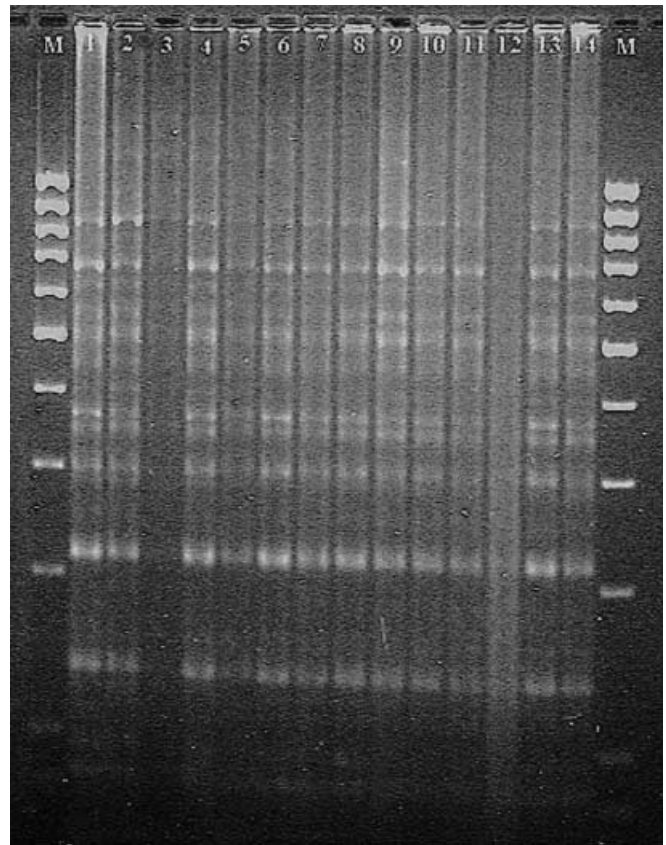


**Fig. 1** 16S rDNA fingerprints of soil plots on a silver-stained denaturing gradient gel electrophoresis gel. See Materials and methods for details. *Lanes 1, 2* cropped plot (CP)+municipal solid waste compost (MSW) corresponding to 120 kg N ha<sup>-1</sup> (MSW1); *lanes 3, 4* CP+MSW corresponding to 240 kg N ha<sup>-1</sup> (MSW2); *lanes 5, 6* CP+120 kg NH<sub>4</sub>NO<sub>3</sub>-N ha<sup>-1</sup> (N); *lanes 7, 8* CP; *lanes 9, 10* uncropped plot (UP)+MSW2; *lanes 11, 12* UP+N; *lanes 13, 14* UP

not shown. About 15 major fragments ranging from 900 to 100 bp were detected, and were almost the same for all plots. As for the DGGE analysis shown in Fig. 1, the very faint few extra bands and/or slight differences in the intensity of the others have, in our opinion, very likely to be ascribed to pitfalls related to PCR-based techniques (Wintzingerode et al. 1997), and are irrelevant to the description of changes in the structure of the soil bacteria community, when considering the overall profile.

Analyses of the genetic variation of the 16S gene are not always sufficient to differentiate between strains within a species, as sequences of 16S rDNA are highly conserved among bacteria. In contrast, genetic fingerprints of the spacer regions between the 16S and 23S rDNA can be used to differentiate organisms at the intraspecific level (Gürtler and Stanisich 1996; De Olivera et al. 1999).

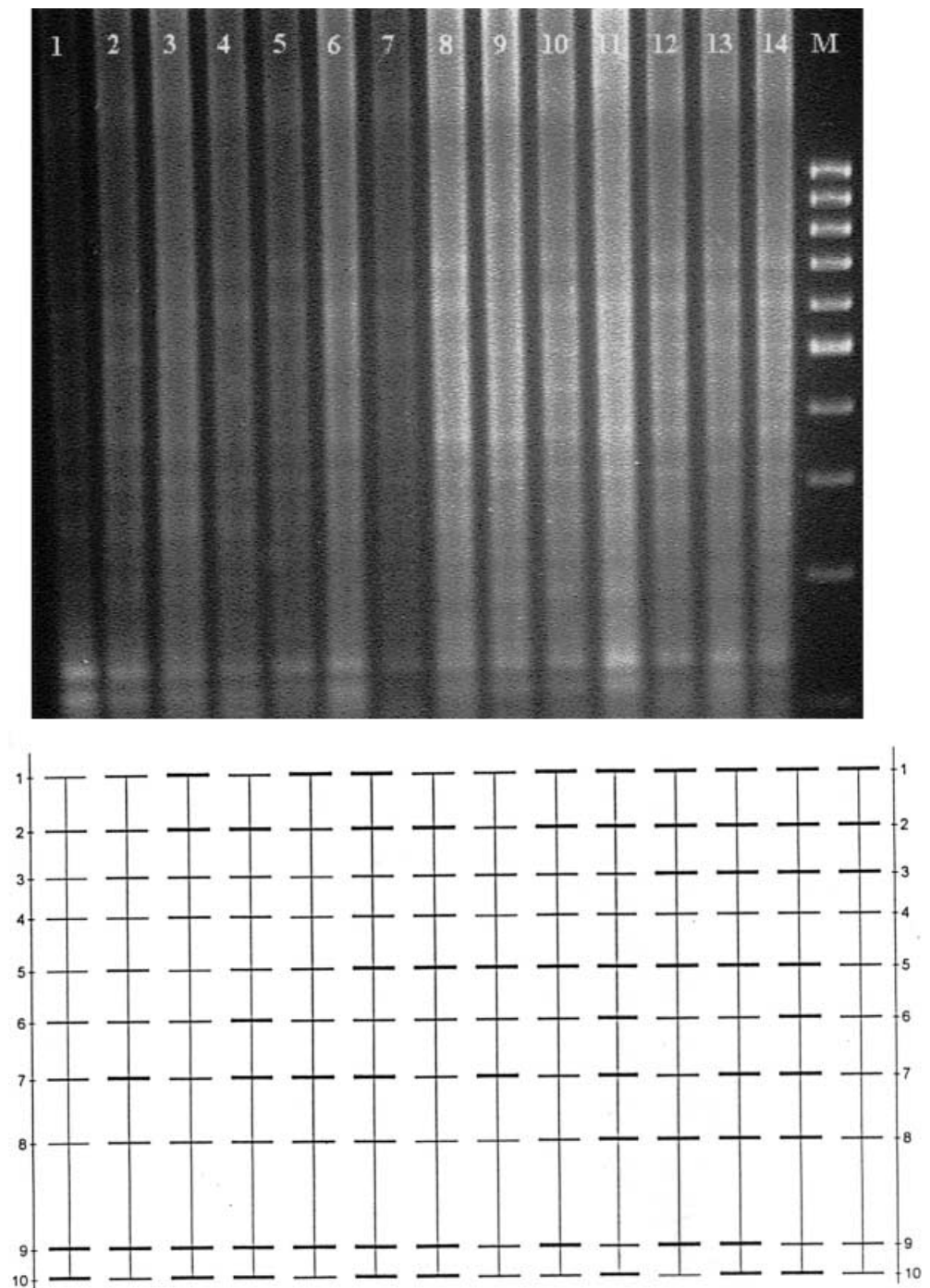
Figure 3 shows the ARDRA profiles of DNA extracted from the experimental plots, amplified for a 1,500-bp 16S-23S region and restricted with Hpa II. Only ten different fragments, ranging from 200 to 600 bp, were detected. Although the analysis of the 16S-23S intergenic region was supposed to be more informative, the lanes of the corresponding gel were smeared, probably because of the presence of several faint quenching bands. The computerized representation of the gel indicated that the major bands were common for all lanes.



**Fig. 2** Amplified 16S rDNA restriction patterns digested with Hpa II on a silver-stained gel. See Materials and methods for details. *M* Low-range DNA ladder (1,000–80 bp; MBI Fermentas); for description of other lanes, see Fig. 1

The absence of variation among all genetic fingerprints seems to indicate that the amendments with MSW compost or mineral N do not alter, within the experimental trial investigated, the structure of the bacterial community. A dramatic decrease in bacterial diversity or shifts in microbial community structure were observed by molecular approaches in heavy metal-contaminated soils (Smit et al. 1997; Sandaa et al. 1999) as well as in herbicide-treated soils and soil microcosms (Fantroussi et al. 1999; Crecchio et al. 2001). Time of exposure to contaminants and their concentrations, as well as soil characteristics, seem key factors to be considered. In our case, it seems that soil bacteria respond mainly by altering their metabolic activity (i.e. extracellular enzymes). Our results could also be influenced by an apparent insensitivity of PCR-based methods, which utilize bacterial primers targeting the whole bacterial community, which can show fairly high detection limits for specific bacterial groups and easily miss minority groups (Gelsomino et al. 1999). Of course, it cannot be excluded that changes which can be revealed by DNA analysis can be charged to other microbial species (i.e. fungi, Archaeobacteria) not detected by the eubacterial PCR primers here used. Furthermore, heterogeneity among treatments could be detected by ana-

**Fig. 3** Amplified 16S-23S rDNA restriction patterns digested with Hpa II on a silver-stained gel and computerized representation by Bio-Rad Gel Doc documentation system. See Materials and methods for details. For description of lanes, see Figs. 1 and 2



lysing the rRNA fraction, which represents the most active bacteria, more than DNA, which originates from dormant cells, dead cells, and free DNA, as well as from metabolically active microbes. Further research in this direction is needed, as well as long-term trials. Overall, our preliminary results indicate that amendments with MSW compost can improve the quality of soil by increasing its organic matter content and some biochemical properties without altering the structure of the bacterial community. Nevertheless, the risks of inhibiting biogeochemical processes, as well as of con-

tributing to environmental pollution, due to the presence of heavy metals and other pollutants in MSWs, must be constantly monitored and minimized.

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