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Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure

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Abstract Studies were conducted to evaluate microbial populations and community structures in soils under different management systems in a long-term continuous winter wheat experiment. These soils had been treated with cattle manure for over a century, and P, NP, NPK, or NPK plus lime for over 70 years. Cattle manure application promoted the growth of bacteria, but not fungi, when compared with the control soil. Application of chemical fertilizers enriched the K-strategist bacterial community, while application of manure enriched both r- and K-strategists. DNA recovered was most abundant in the manure-treated soil. Effects on bacterial species richness and evenness following long-term soil treatments were also demonstrated by analyzing bacterial community DNA using amplified ribosomal DNA restriction analysis and repetitive extragenic palindromic-polymerase chain reaction fingerprinting. The richness and evenness of the bacterial community were enhanced by manure treatment and treatments that included N and P, which were positively correlated with soil productivity.

Keywords Animal manure · Microbial community · r/K strategists · ARDRA · REP-PCR

Introduction

Recently, we reported that manure-P is more mobile in soil than chemical fertilizer-P (Parham et al. 2002). Mobility of manure-P may be related to its chemical forms because organic P is reported to be more mobile than inorganic P (Hannapel et al. 1964a, 1964b; Chardon et al. 1997; Parham et al. 2002). Nevertheless, the forms of P in manure may not be the only factor dictating mobility of P in soil. Although cattle manure contains slightly over 50% organic P (Barnett 1994), some manure, such as horse manure, may contain less than 5% organic-P (Peperzak et al. 1959). Once manure is applied to soil, the microflora in soil is capable of converting inorganic P to organic P, such as meso-inositol hexophosphate, which comprises up to 52% of soil organic P (Caldwell and Black 1958). Direct relationship between P movement and microbial activities in soil has been reported by Hannapel et al. (1964a, 1964b) who demonstrated that organic P concentration increased in solutions passed through soil columns amended with plant residue or other organic materials, but was reduced by killing the soil microbes with formaldehyde. Their result was further supported by studies of Abbott and Tucker (1973). Moreover, P accumulation has been reported in soil with repeated short-term (1–2 years) heavy application of animal manure (Carpenter et al. 1998; Reed et al. 1998), but was not apparent in soils following long-term (over a century) manure application (Parham et al. 2002). Based on results reported in literature and obtained from our studies, we hypothesized that high mobility of manure-P was, in part, due to increased microbiological activities induced by manure application (Parham et al. 2002). Mobility of manure-P increases in soil with time following manure application. We demonstrated that long-term application of cattle manure increased microbial biomass C content and dehydrogenase activities, and enhanced activities of enzymes involved in P transformations (Parham et al. 2002).

Microorganisms could be involved in manure-P mobilization or microbial cells might serve as vectors in this process. Thus, not only would the microbial activity be higher in manure-treated soil, but also the microbial population. Furthermore, manure application may promote proliferation of a particular microbial community that increases P mobility in soil. Therefore, the objectives of this study were to determine the effects of long-term animal manure and chemical fertilizer treatments on bacterial and fungal populations and bacterial community structure in soil.

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Materials and methods

Soil samples

Soil samples were obtained from a long-term continuous winter wheat (*Triticum aestivum* L.) experiment located in central Oklahoma, United States. Detailed descriptions of the experimental site and soils can be found in Parham et al. (2002). Briefly, the experiment was initiated on a Kirkland (fine, mixed, thermic Udertic Paleustolls) silt loam with a mean particle-size distribution of 37.5% sand, 40% silt and 22.5% clay. The manure treatment plot was initiated in 1899 and chemical fertilizer treatment plots were initiated in 1929. Six treatment plots (manure, P, NP, NPK, NPK plus lime, and an untreated control) were investigated. Cattle manure from a feedlot had been applied every 4 years since 1899 at 269 kg N ha^{-1} (approximately 13,450 kg manure ha^{-1}). The most recent cattle manure application was conducted in September 1999. The average ratio of N:P of the applied manure was 3.3:1 based on analyses of hundreds of feedlot cattle manure samples in Oklahoma. This suggested that approximately 22.4 kg P ha^{-1} $year^{-1}$ was applied (Parham et al. 2002). Chemical fertilizer plots received annual application rates of 67 kg N, 14.6 kg P and 28 kg K ha^{-1} in the forms of NH_4NO_3, $Ca(H_2PO_4)_2$, or KCl before planting winter wheat in October.

The application of statistical analyses to agricultural experiments was not yet in place at the time that the treatments in this study were initiated. To compensate for the no-replication restriction, the underlying changes in these soils were evaluated based on random, individual samples in combination with sequential samples over a period of time. The plots (30.4×6.5 m) were divided into three subplots (10×6.5 m) with composite samples taken from each subplot. Composite soil samples were taken in October 1998, January 2000 and November 2000, corresponding to 1 year before, 4 months after and 1 year after the last manure application. Sampling and planting times for 2000 were postponed for about 2 weeks due to an unusually hot and dry summer. Samples taken in January were during the wheat growing season, while those taken in October and November were right before planting. Surface soil samples (0–10 cm) were used in this study. Basic soil properties have been reported previously (Parham et al. 2002). Briefly, soil pH ranged from 4.7 to 5.8 and organic C from 6 to 10 g C kg^{-1} soil. Manure application increased soil pH significantly while chemical fertilizer application resulted in slightly lower soil pH when compared to the control soil.

Culturable microbial community

In this study, the culturable microbial community was evaluated by examining total culturable microbial populations and r/K bacterial strategists (De Leij et al. 1993).

Bacterial growth strategists and total bacterial and fungal populations

Bacteria were cultured on 0.1-strength Tryptone soy agar plates (TSA) at 25°C, using a modified procedure described by Zuberer (1994). Plates were examined at low magnification (1.5×) and visible colonies were marked and enumerated on a daily basis for five consecutive days and on day 10. Thus, six counts (classes) were generated per plate. Plates with 20 to 200 colonies were selected for enumeration. When plates became too crowded, the next dilutions were used for enumeration. Total bacterial counts obtained were expressed in colony forming units (CFU) per gram soil. Distribution of bacteria in each class as a percentage of the total counts gave insight into the distribution of r- and K-strategists in each sample. Bacterial colonies appearing within 24 h were designated as r-strategists, and the remaining as K-strategists (De Leij et al. 1993).

Total culturable fungal populations were obtained by culturing on 0.1-strength malt extract agar plates (MEA) at 23°C. Fungal colonies were observed after 72 h incubation with countable plates of the highest dilution being enumerated.

Eco-physiological index

The distribution of the six classes in each sample was expressed using the eco-physiological (EP) index (H′; De Leij et al. 1993), which is a measure of the evenness and richness of groups of microorganisms with similar developmental characteristics. The EP index was derived from the Shannon diversity index (Shannon 1948), which is a measure of community diversity and takes into account both species richness and evenness. The EP indices of soils tested were calculated using the equation

$$H' = \sum \left(P_i * \log_{10} Pi \right)$$

where P_i represented CFU on each day as a proportion of the total CFU in that sample after 10 days incubation. It was proposed that the higher the index, the more evenly distributed the groups within a community, and the "healthier) that community is (Shannon 1948; De Leij et al. 1993).

All plate counts were conducted with four treatment replicates. Results are expressed on a moisture-free basis. Soil moisture was determined after drying at 105°C for 48 h. Distribution curves for colonies formed per day over 10 days were analyzed using principal component analysis (Simonds 1963). Comparisons between spatial and temporal nutritional status in soil samples were made using analyses of variance (ANOVA). Significant difference among treatment means was determined using the least significant difference (LSD) test. Percentage data were normalized using arcsine transformation before analysis (Gomez and Gomez 1984). All results reported are averages of replicated assays and analyses.

Bacterial community DNA analysis

Soil bacterial communities with inclusion of the unculturable community were evaluated by analysis of soil DNA that was extracted from samples taken in January 2000, using an UltraClean Soil DNA Kit (Mo Bio Laboratories, Solana Beach, Calif.). The obtained DNA showed size greater than 23 kb and did not require additional purification before being subject to Polymerase chain reactions (PCR). PCR were performed on an automated thermal cycler (PTC-100, MJ Research, Watertown, Mass.). Replicates and negative controls were included for each reaction. Each reaction was performed under the specified conditions at least three times to ensure reproducibility of the procedure and the obtained products. Unless specified, DNA band(s) separated in agarose or polyacrylamide gels were visualized by staining with ethidium bromide (0.5 μg ml^{-1}) and photographed using a Kodak 1D Scientific Imaging System and a Kodak DC 290 Zoom Digital Camera (New Haven, Conn.).

Repetitive extragenic palindromic-polymerase chain reaction fingerprinting

The nucleotide sequences of the repetitive extragenic palindromic (REP) primers used in this study were IIIICGICGICATCIGGC (REPIR-I) and ICGICTTATCIGGCCTAC (REP2-1; De Bruijn 1992; Stern et al. 1984). These primers are located in the conserved stem of the palindrome in opposite orientations directing outwards from each REP sequence (Versalovic et al. 1991). Each 100-μl PCR mixture contained 50 ng soil DNA, 0.4 μM each primer, 200 μM PCR nucleotide mix (Fisher Bioreagents, Fisher Scientific, Pittsburgh, Pa.), 7 mM $MgCl_2$, 16 µg bovine serum albumin, 10% dimethyl sulfoxide, and 4 units Taq DNA polymerase in PCR buffer (Promega Chemicals, Madison, Wis.). PCR reactions for REP-PCR fingerprinting of soil DNA were performed following a modified procedure of De Bruijn (1992). Briefly, the conditions were 6 min at 95°C, followed by 30 cycles at 94°C for 1 min, 40°C

for 1 min, and 65°C for 8 min, then a final cycle at 65°C for 16 min. The REP-PCR banding patterns were visualized following separation in an 8% polyacrylamide gel in Tris-borate EDTA (TBE).

Amplified ribosomal DNA restriction analysis

Amplified ribosomal DNA restriction analysis (ARDRA) was performed using the almost full-length 16S rDNA (close to 1.6 kb) amplified by PCR using bacterial primers, 5′-AGAGTTTGATC-CTGGCTCAG-3′, and 5′-GGTTACCTTGTTACGAC TT-3′ (Toyota et al. 1999), and template DNAs from soils tested. Each 100 μl PCR mixture contained 50 ng soil DNA, 0.2 μM each primer, 200 μM PCR nucleotide mix (Fisher Bioreagents, Fisher Scientific, Pittsburgh, Pa.), 1.75 mM $MgCl_2$, and 2 units Taq DNA polymerase in PCR buffer (Promega Chemicals, Madison, Wis.). The PCR reactions were conducted as follows: 2 min at 94°C, followed by 35 cycles at 94°C for 1 min, 65°C for 45 s, 72°C for 2 min, then a final cycle at 72°C for 10 min. The obtained 16S rDNA (1.0–1.5 μg) was digested at 37°C overnight with one of the restriction enzymes *Alu*I, *Hae*III, *Hpa*I, or *Rsa*I. (GIBCOBRL, Life Technologies, Grand Island, N.Y.). The ARDRA patterns were then visualized in an 8% polyacrylamide gel in TBE.

Results and discussion

Total culturable microbial populations

With the exception of samples taken in November 2000, culturable bacterial CFU recovered among the soils studied were significantly higher in the century-long manure-treated soil, which was as high as 16 times the control, and over 10 times the other treatments (Fig. 1A). This trend, however, was not apparent in samples taken in November 2000. This could not be due to recent manure application, which was September 1999, between the other two sampling dates. This is possibly due, in part, to the unusual summer climate in that year. Cumulative rainfall from 29 July to 14 October was only 6 mm with the top 5-cm soil temperatures consistently over 30°C from 2 August to 12 September (Oklahoma Climatological Survey 2002). Samples taken 2 weeks after the first rain following the hot and dry summer reflected a flush of microbial growth, evidenced by generally higher bacterial CFU than that of the same season in 1998. In general, samples taken in the fall have considerably lower bacterial population regardless of the sampling year, while those taken in January had considerably higher total culturable bacterial population (Fig. 1A). This is consistent with findings reported by Van Gestel (1992) who found that microbial biomass C was the highest in winter-sampled soils, and the lowest in those sampled in summer. Given the fact that October climate in central Oklahoma is generally warmer with higher rainfall than January (Oklahoma Climatological Survey 2002), wheat growth was likely one of the responsible factors.

In general, the populations of fungi in soils taken in January 2000 were considerably higher than those taken in October 1998 or November 2000, which is consistent with the trend observed for bacterial populations (Fig. 1B). Manure treatment did not result in significantly higher fungal population regardless of the time and year

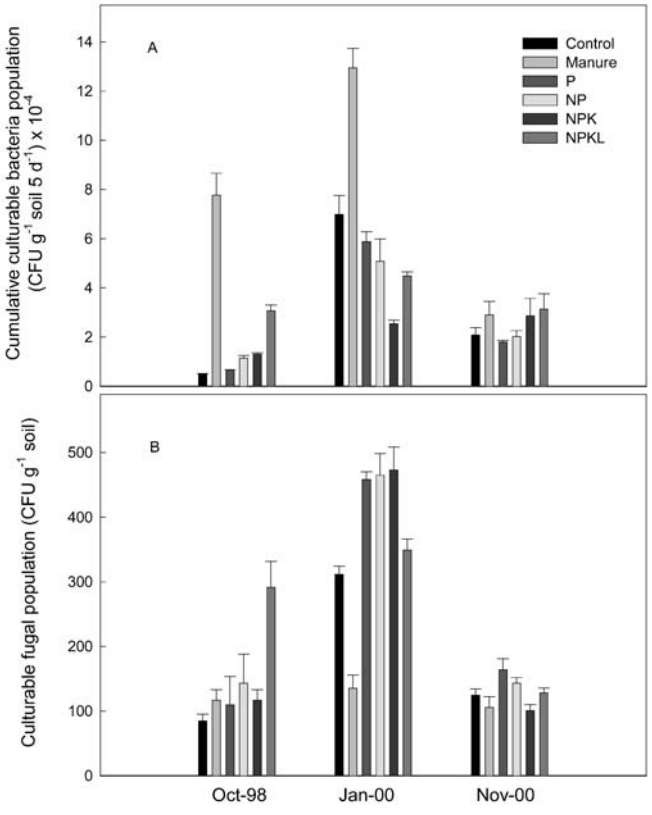

Fig. 1 Cumulative bacterial (**A**) and fungal (**B**) colony forming units (CFU) recovered from soils tested. Bacterial CFU were recovered on 0.1-strength Tryptone soy agar plates (TSA) after 5 days incubation at 23°C. Fungal CFU were enumerated after 72 h of incubation on 0.1-strength malt extract agar plates (MEA) at 23°C. Soil samples were taken at three different times. *Bars* indicate SE

of sampling (Fig. 1B). In fact, for samples taken in January 2000, the lowest fungal CFU occurred in the manure-treated soil, while those of the control soil and chemical fertilizer-treated soils were not significantly different (Fig. 1B). This was partially attributed to limitation of pH values in the manure-treated soils, which were around 5.6, the highest among the treatments evaluated (Parham et al. 2002). Other studies showed that increased soil pH in the acidic range caused a shift toward dominance of the bacterial community, while fungal populations were unaffected (Frostegård et al. 1993; Pennanen 2001). Possibly, the growth of soil bacteria was inhibited at pH 4.6 and facilitated with increasing soil pH (Arao 1999). In both studies, shifting of microbial community toward fungal dominance resulting from decreasing soil pH was attributed to reduced competition from the bacterial community (Frostegård et al. 1993; Arao 1999).

In this study, the dependence of microbial population on soil pH was further evidenced from correlations between bacterial or fungal population and soil pH (Fig. 2). Cumulative bacterial populations recovered in 5 days were significantly correlated to soil pH values in

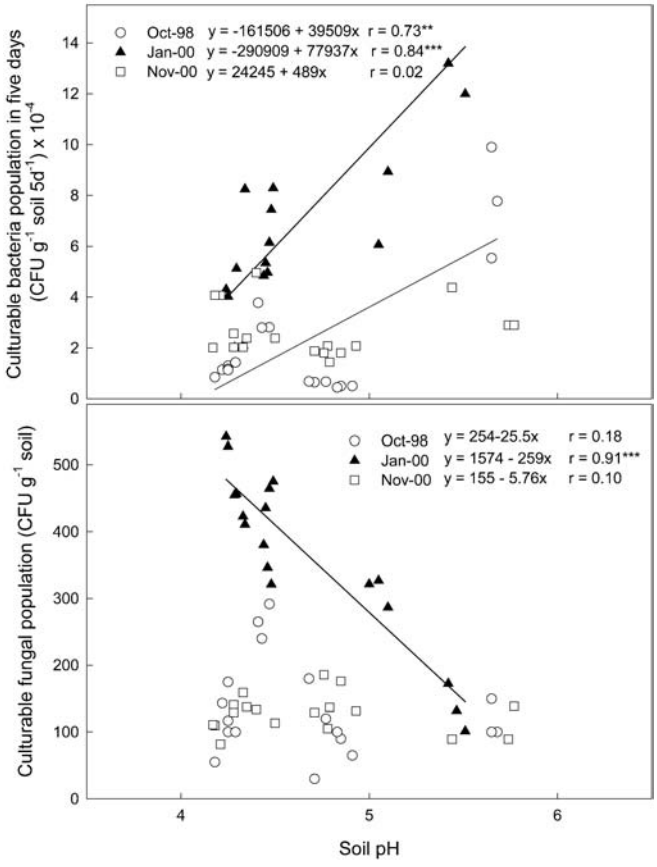

Fig. 2 Correlations between cumulative culturable bacterial and fungal colony forming units and soil pH among samples within each sampling time

samples taken October 1998 and January 2000, but not in those taken in November 2000. Fungal population decreased significantly with increasing soil pH in samples taken in January 2000 (Fig. 2), suggesting fungi in these soils are more competitive than bacteria in the acidic environment. The variations observed in samples taken at different times of the year may be attributed to the most limiting factor at the time of sampling. Drought, heat, and crop cover could alter microbial populations considerably.

Bacterial community structure

Microbial community structure is often evaluated based on detectable differences in terms of growth rate, substrate-use efficiencies, morphology, and C/N content of biomass. These factors, however, differ markedly between fungi and bacteria, and within groups of fungi and bacteria. Moreover, cultivated microorganisms may not reflect the natural microbial community because they represent only 0.001% to 0.3% of total direct cell counts (Amann et al. 1995; Torsvik et al. 1990). Soil DNA analyses overcome some of the limitations but are biased by efficiency of DNA extraction and purification (Kandeler et al. 2000), PCR primer selection and conditions (Von Wintzingerode et al. 1997; Suzuki and Giovannoni 1996; Polz and Cavanaugh 1998), and are incapable of distinguishing DNA from viable and dead cells (Spring et al. 2000). Thus, a combination of cultivation-based and DNA-based methods was employed in this study.

Growth strategists and pattern

The percentage of r-strategists was highest in the control soil, followed by the manure- and P-treated soils, with the lowest in the NPK-treated soil (Table 1). This trend was more evident for CFU recovered after 48 h (Table 1). Bacterial growth patterns from samples taken in January 2000 clearly demonstrated this trend (Fig. 3A). A major portion of bacterial CFU was recovered on day 2 for the control, manure- and P-treated soils, whereas this occurred on day 3 for the other treatments tested. Bacterial CFU recovered on day 1 or day 2 were the highest in manure-treated soil, followed by the P-treated and control soil (Fig. 3A). In addition, the manure-treated soil had two distinct growth peaks, at day 2 and day 5, while other soils experienced one major growth peak. Interestingly, the EP-indices in these soils were not significantly different, except for the NPK treatment for samples taken in January 2000, which had a significantly lower EP index (Fig. 3). This suggests that EP-index was not sensitive to changes in bacterial community. The NPK-treated soil exhibited a lower EP-index and a lower percentage of r-

Table 1 Bacterial strategists in soils under different long-term management practices[a]

Treatment	CFU recovered expressed as percentage of total CFU recovered in 10 days[b]								
	CFU recovered in day 1			CFU recovered in day 2			CFU recovered from days 5 to 10		
	Jan 2000	Nov 2000	Avg.	Jan 2000	Nov 2000	Avg.	Jan 2000	Nov 2000	Avg.
Control	22.01±5.56	16.58±2.59	19.30	81.62±6.06	56.04±5.73	68.8	0.39±0.31	1.02±0.42	0.70
Manure	20.08±5.63	11.18±3.22	15.63	52.54±10.48	34.86± 9.29	43.7	4.23±2.08	7.41±2.39	5.82
P	14.85±5.21	18.91±3.46	16.88	63.47±8.57	51.42±7.17	57.4	3.05±1.95	1.11±0.33	2.08
NP	13.70±8.31	7.85±1.10	10.78	24.89±9.30	31.50±4.24	28.2	2.40±2.34	8.18±0.85	5.29
NPK	6.86±3.05	7.50±2.37	7.18	15.63±5.46	21.56±12.83	18.6	14.71±2.75	3.56±1.17	9.13
NPKL	15.00±3.27	11.03±1.72	13.02	24.81±6.22	34.63±4.38	29.7	4.09±4.95	7.10±2.02	5.60

[a] Means ± standard deviation
[b] CFU recovered in day 1 and 2 are fast growers (r-like strategists) and those recovered from days 5 to 10 are slow growers (K-like strategists)

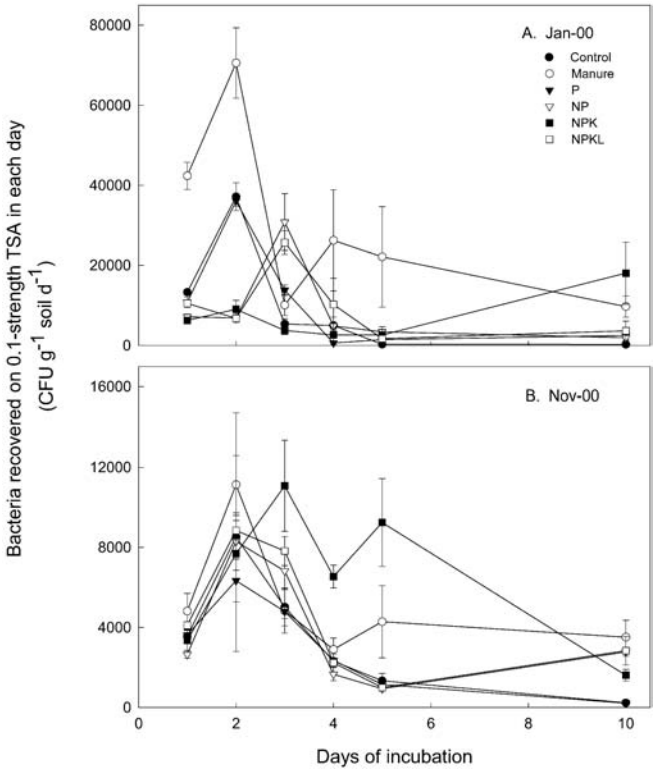

Fig. 3 Culturable bacterial colony forming units recovered each day for up to 10 days on 0.1-strength TSA in samples taken in January (**A**) and November 2000 (**B**), respectively. *Bars* indicate SE. Average eco-physiological (EP) indices (H′) of three subsamples with four measurements each were: (**A**) 0.46bc, 0.57a, 0.50a, 0.50a, 0.42b, and 0.53a for the control, manure-, P-, NP-, NPK-, and NPKL-treated soils, respectively; and (**B**) 0.63a, 0.61a, 0.64a, 0.64a, 0.63a, and 0.59a for these soils. *Different letters* indicate significantly different means at $P < 0.05$ according to least significant difference test

strategists, possibly due to amensalism from a dominant bacterial group in the community. Excessive production of bacterial metabolites was noticed on NPK plates during culture, which might have prevented the formation of additional bacterial colonies on those plates. As is the characteristic of K-strategists (De Leij et al. 1993), the colonies that were formed after 5 days incubation might be resistant to possible toxicity of these metabolites.

The percentage of cumulative bacterial CFU recovered from day 5 to day 10 (slow growers) over the total CFU recovered in 10 days was only 0.7% in the control soil, 2.1% in P-treated soil, and greater than 5% in the other treatments studied (Table 1). Interestingly, soils that showed more than 5% slow growers also had significantly higher wheat yields based on averages of 50 years of yield data (Parham et al. 2002). As discussed earlier, wheat straw was incorporated into soil. Higher yield implies greater production of wheat straw. Thus, the increased proportion of slow growers may be partially attributed to improved soil structure due to incorporation of larger quantities of wheat residues. Proportions of slow growing bacteria decreased in the control soil where fertilizers had not been applied for over 70 years. Chemical fertilizers preserved or increased populations of slow growers. Manure treatment enhanced both fast and slow growers, resulting in a more even distribution of growth strategists and significantly higher bacterial population. It is, however, unexpected that r-strategists were more dominant in the control soil than the chemical fertilizer-treated soils, while the reverse situation was observed for K-strategists.

Based on the concept of r/K strategists, competition and natural selection should favor the K-selection. In this study, fertilizer treatments promoted competition and resulted in higher percentages of K-strategists. However, competition for resources should also be intense in the control soil, but CFU recovered from 5 to 10 days (K-strategists) were less than 1% of the total CFU recovered (Table 1). Presumably, natural selection will maximize resource use efficacy; the problem is to understand how the available resources are partitioned among different groups of organisms. Application of fertilizer promoted growth of microbial communities that were limited by mineral nutrients, which resulted in shifting limiting factors for microbial growth to factors such as organic C. K-strategists are presumably efficient users of environmental resources, and would be more competitive in such an environment. In a manure-treated soil, input of easily available organic C and mineral nutrients, and improvements in soil structure promoted growth of both r-strategists and K-strategists. As a result, the richness, evenness, and diversity of the microbial community in the manure-treated soil were enhanced. This is further evidenced from analyses of the bacterial community DNA extracted from these soils.

DNA fingerprinting

Among the soils tested, DNA recovered was most abundant in the manure-treated soil, which was approximately 10 μg g^{-1} soil (at least fivefold of those in the fertilizer-treated soils and tenfold of that in the control soil; data not shown). ARDRA patterns of almost full-length 16S rDNA were similar in soils under different management practices (Fig. 4). This is surprising because results from culturable microbial community suggested that microbial community structure was affected by soil treatments. Given the fact that numerous discrete bands were visualized in the ARDRA gel, there were many species of bacteria detected. Undetected changes in the bacterial community could be due to bias imposed by DNA extraction and purification (Kandeler et al. 2000), selectivity of PCR primers (Spring et al. 2000), or indicate that dominant microbial species in these soils were similar. Although ARDRA analyses have been used to evaluate bacterial communities (Koki et al. 1999), this technique was not sensitive enough to detect changes in bacterial community under the conditions specified. Upon fumigating two Japanese soils with metam, Koki et al. (1999) found that the ARDRA banding patterns were similar between the fumigated and control soils at 26 and

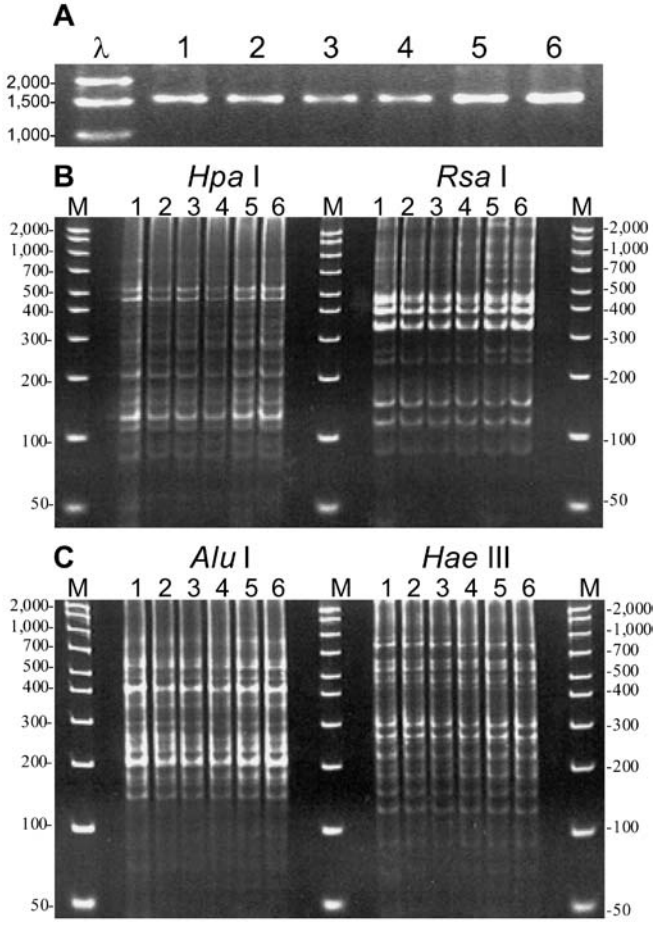

Fig. 4 Amplified ribosomal DNA restriction analysis (ARDRA). **A** A 2%-agarose gel electrophoresis of the almost full-length 16S rDNA (close to 1.6 kb) that was amplified. The template DNAs for lanes 1–6 were from the control soil, and soils treated with manure, P, NP, NPK and NPKL, respectively. There were 50 ng in each band of the marker. **B**, **C** ARDRA patterns of the 16S rDNA shown in **A** using restriction enzymes, *Hpa*I, *Rsa*I, *Alu*I or *Hae*III, respectively, and digested at 37°C overnight. Lane assignments were the same as in **A**. Approximately 5 μg was loaded in each lane

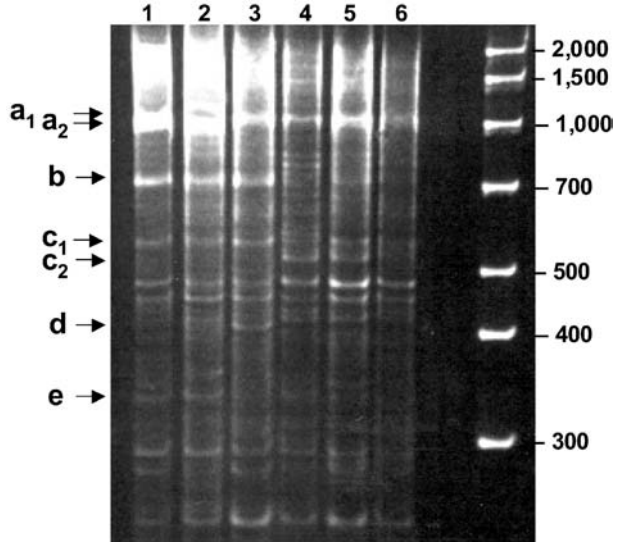

Fig. 5 Rep-PCR fingerprints of different soil bacterial communities. The template DNAs for lanes 1–6 were from the control soil, and soils treated with manure, P, NP, NPK and NPKL, respectively

105 days after fumigation. A low microbial diversity in Cu-contaminated soil was detected using ARDRA (Smit et al. 1997). When different bacterial isolates were examined with ARDRA, Brim et al. (1999) found that most strains showed a close phylogenetic clustering inside the same genus despite their different origins.

Versalovic et al. (1991) demonstrated that REP-like sequences could be utilized as efficient primer binding sites in the PCR to produce fingerprints of different bacterial genomes. This technique does not require microbial cultivation and yields results with very small amounts of template DNA (De Bruijn 1992). Since REP-PCR provides genomic level DNA fingerprinting, it is more sensitive in revealing microbial community shift than analysis of 16S rDNA, such as ARDRA. The generated patterns are specific for bacterial species and strains in the adjacent repeat elements within the limitations of polymerase extension. In this study, REP-PCR fingerprinting produced characteristic patterns and revealed some differences of microbial communities in different soils (Fig. 5). Bands a1 and a2 differed slightly in mobility. Band a1 represents a dominant microbial group in the chemical-fertilizer-treated soils, and band a2 corresponds to dominant groups in the control- and manure-treated soils. Bands b and c represent a predominant microbial group in the control, manure- and P-treated soils (Fig. 5). There are fewer prominent bands in the NP-, NPK-, and NPKL-treated soils, but a greater number of bands were detected (Fig. 5). A greater number of bands were also obtained in the manure-treated soils when compared with control and P-treated soil. These results again suggested that microbial diversity and evenness were enhanced by manure and fertilizer treatment, which coincided with increased wheat yield in these soils (Parham et al. 2002). Although few studies have shown direct linkage between microbial diversity and its ecological functions, organic soils have been shown to harbor higher microbial diversity than sandy soils based on Biolog, ARDRA, REP-PCR and DGGE analyses (Øvreas and Torsvik 1998). This also coincides with high fertility and productivity of organic soils (Diaz-Zorita et al. 2002).

Microbial community structure was altered by long-term soil management practices, as evidenced by REP-PCR and plate counts. In general, cattle manure application promoted the growth of culturable bacteria, but not cultivation-based fungi. Application of chemical fertilizers enriched the K-strategist bacterial community, while application of manure enriched both r- and K-strategists when compared with the control soil. The richness and evenness of the bacterial community were enhanced by fertilization, which was positively correlated with soil productivity.

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