APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2003, p. 1800–1809 Vol. 69, No. 3
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.3.1800–1809.2003

Soil Type Is the Primary Determinant of the Composition of the Total and Active Bacterial Communities in Arable Soils

Martina S. Girvan,* Juliet Bullimore, Jules N. Pretty, A. Mark Osborn, and Andrew S. Ball

Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, England, United Kingdom

Received 5 August 2002/Accepted 25 November 2002

Degradation of agricultural land and the resulting loss of soil biodiversity and productivity are of great concern. Land-use management practices can be used to ameliorate such degradation. The soil bacterial communities at three separate arable farms in eastern England, with different farm management practices, were investigated by using a polyphasic approach combining traditional soil analyses, physiological analysis, and nucleic acid profiling. Organic farming did not necessarily result in elevated organic matter levels; instead, a strong association with increased nitrate availability was apparent. Ordination of the physiological (BIOLOG) data separated the soil bacterial communities into two clusters, determined by soil type. Denaturing gradient gel electrophoresis and terminal restriction fragment length polymorphism analyses of 16S ribosomal DNA identified three bacterial communities largely on the basis of soil type but with discrimination for pea cropping. Five fields from geographically distinct soils, with different cropping regimens, produced highly similar profiles. The active communities (16S rRNA) were further discriminated by farm location and, to some degree, by land-use practices. The results of this investigation indicated that soil type was the key factor determining bacterial community composition in these arable soils. Leguminous crops on particular soil types had a positive effect upon organic matter levels and resulted in small changes in the active bacterial population. The active population was therefore more indicative of short-term management changes.

Despite great progress in overall agricultural productivity in recent decades, land degradation has reduced the productive capacity of soils on nearly 40% of the world's agricultural land (47). These soils suffer physical degradation, such as erosion and compaction; chemical degradation due to acidification, nutrient depletion, pollution from industrial wastes, and overuse of pesticides and fertilizers; and biological degradation by organic matter depletion and loss of biodiversity (6, 12, 13, 52, 54). In light of these threats, there is growing interest in the factors governing soil health, biodiversity, and resilience, as well as in the fundamental relationships between them (15, 29). Soil health has been defined as "the capacity of soil to function as a vital living system to sustain biological productivity, promote environmental quality and maintain plant and animal health" (13). Thus, soil health is inextricably associated with sustainability. The productivity of agricultural systems is known to depend greatly upon the functional processes of soil microbial communities (23, 32, 46). The management of these soils may have a great impact upon the overall health of these communities, with organic and regenerative approaches being widely regarded as conferring a positive effect upon soil biology and thus encouraging a significantly higher level of biological activity due to crop rotations, reduced applications of nutrients, and elimination of pesticides (25, 49, 51, 63).

Management systems, such as tillage regimens, have also been investigated. Curci et al. (11) discovered that enzyme activity was higher in the uppermost 20 cm of soil in plots tilled by shallow ploughing and scarification than in those tilled by deep ploughing. Boddington and Dodd (2) investigated the effect of soil disturbance on the spore density, species richness, and extraradical mycelium lengths of arbuscular mycorrhizal fungi. All factors were reduced in disturbed soil in comparison with undisturbed soil, further indicating that microbial communities are influenced by management practices. Although organic farms have been shown to have slightly higher levels of organic matter and carbon than neighboring conventional farms (14, 37, 53), only limited research has investigated the structures and compositions of microbial communities following a switch to organic farming practices.

Using nucleic acid methodology, clear differences have been observed between communities from different management regimens. A ranking of the complexity of community DNA regimes from grasslands by DNA-DNA hybridization resulted in different complexities, in the order unimproved > semiimproved > improved grassland soil communities (9, 10). The overall levels of management and inorganic nutrient amendment designated the status of agricultural grassland pasture sites, improved being the most managed and unimproved being the least managed. This relationship held true for soils from three separate sites (9, 10). McCaig et al. (39) separated improved and unimproved grassland soils on the basis of their 16S ribosomal DNA (rDNA) clone diversity, with improved soil being less diverse (concurring with the findings of Clegg et al. [10] for the same sites).

Significant changes in soil microbial structure with vegetation change have been detected by use of molecular methods. Nusslein and Tiedje (45) showed that a change in the vegetative cover of a Hawaiian soil from forest to pasture led to a significant change in the composition of the soil bacterial community upon analysis of G+C distributions and corresponding clone group abundances. Peak G+C contents of the forest soil

* Corresponding author. Mailing address: Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, England, United Kingdom. Phone: 01206 873370. Fax: 01206 873416. E-mail: mgirvan@essex.ac.uk.

TABLE 1. Field site information July 2000

Field code	Ordinance Survey Mapping coordinates		Soil series[a]	Soil type	Crop	Management
	x	*y*				
EC2	573325m	326525m	Brown rendzinas 343	Coarse loamy sand over chalk rubble	Lucerne/alfalfa (*Medicago sativa*)	Organic for 1 yr
EC3	573325m	326525m	Brown rendzinas 343	Coarse loamy sand over chalk rubble	Oil seed, rape (*Brassica napus* cv. *oleifera*)	GAP
RMA	648995m	283705m	Typical stagnogley 711	Fine loam	Pea (*Pisum sativum*)	GAP
RMB	648625m	283405m	Typical stagnogley 711	Coarse sandy loam	Sugar beet (*Beta vulgaris altissima*)	Turkey manure (405 kg ha^{-1} yr^{-1}) for 20 yr
RMC	648625m	283405m	Typical stagnogley 711	Coarse sandy loam	Sugar beet	GAP
RCA	639500m	328500m	Typical stagnogley 711	Coarse sandy loam	Winter barley (*Hordeum vulgare*)	GAP
RCC	639500m	328500m	Typical stagnogley 711	Coarse sandy loam	Winter wheat (*Triticum sativum*) undersown with spring barley and mixed clover	Prepared for conversion in 1 yr
RCD	639500m	328500m	Typical stagnogley 711	Coarse sandy loam	Red and white clover (*Trifolium pratense* and *T. repens*) undersown with rye grass (*Lolium perenne*)	Organic for 2 yr

[a] From the Soil Survey of England and Wales (27).

differed from those of the pasture soil, and none of the dominant phylotypes found in the forest soil were detected in the pasture soil. However, these Hawaiian soils are young and relatively geographically isolated (44) and therefore may be more susceptible to change than well-established soils. Indeed, Buckley and Schmidt (5), using 16S rRNA-targeted oligonucleotide probes to quantify the abundance of rRNA from major phylogenetic lineages, observed remarkably similar microbial community structures in soils that shared similar long-term histories of agricultural management practices despite differences in above-ground community compositions and different recent land-use management practices.

In the present research, we investigated soil microbial communities at a number of geographically distant agricultural sites (separated by over 65 km) comprising two distinct soil types at farms located in eastern England. By analyzing the physical, chemical, and biological characteristics of the soils, we sought to identify causal relationships between land-use management practices and soil health. A polyphasic approach was used. In addition to characterizing the soils by traditional analyses, the total and active members of the bacterial communities were described by molecular analysis. Finally, to incorporate metabolic activity, the physiological profiles of the bacterial communities were determined.

MATERIALS AND METHODS

Field sites. The sites selected for study comprised eight fields on three farms in eastern England. Farm RM is situated near the east coast (near Wrentham, Suffolk), farm RC is situated near the northeast coast (near Lessingham, Norfolk), and farm EC is situated near the northwest coast (near Flitcham, Norfolk) (Table 1). The land-use histories of the fields differed by crop type, organic practices versus good agricultural practices (GAP) (42) and, in one case, manure treated versus not manure treated (Table 1).

Sampling procedure. The soils were sampled under crop in late July 2000. A 20- by 20-m square was chosen from an area of the sample site characterized by the homogeneity of the vegetation cover. Within this square, 10 random soil samples were taken from the top 15 cm of the soil by using a 2.5-cm-diameter auger. These samples were pooled to reduce any spatial variability and sieved in the laboratory (2-mm mesh). All samples were subsampled from this bulk soil.

Soil, physical, chemical, and biological analyses. Labile phosphates, nitrates, and sulfates in the soil were measured by ion chromatography with a Dionex Corporation (Sunnyvale, Calif.) chromatograph. The organic matter concentration was measured by loss on ignition with a muffle furnace (1), and total C and N concentrations were measured with a model 2400 CHN analyzer (Perkin-Elmer, Buckinghamshire, United Kingdom). Water-holding capacity was determined by using the 0-bar method of saturation followed by free draining (7). Total soil bacterial counts were measured by using acridine orange staining (26), and viable counts were determined after plating on 0.1× tryptone soy agar (55).

DNA and RNA extraction. DNA and RNA were coextracted (as described by Steffan et al. [59]) from 0.5 g of soil in the presence of 0.5 ml of phosphate buffer (100 mM, pH 7.5) and 0.5 ml of Tris-equilibrated phenol. Mechanical lysis was performed by using 0.5 g of acid-washed, UV-treated glass beads (150 to 212 μm in diameter; Sigma/Aldrich). The soil-bead mixture was vortexed for 30 s by using a Whirlimixer (Fisons Scientific) and placed on ice to prevent enzymatic degradation. The mixture was vortexed two more times for 30 s each time, stored on ice, and centrifuged (Eppendorf) for 10 min at 12,000 × *g* to separate the aqueous layer from the soil-phenol layer. Sequential phenol-chloroform purification was performed by adding 1 volume of phenol and 1 volume of chloroform-isoamyl alcohol (24:1) to the DNA solution, mixing the solution by shaking for 1 min, and centrifuging the mixture for 5 min at 12,000 × *g*. It was at this stage that crude DNA was quantified by agarose gel eletrophoresis to enable equivalent concentrations of DNA to be added as templates to the subsequent PCR. DNA mass ladders (Bioline, London, United Kingdom) were run alongside the environmental DNA. Following analysis with Phoretix 1D Advanced Analysis software (Nonlinear Dynamics Ltd., Newcastle, United Kingdom), calibration was produced from the known standards and the concentrations of the unknowns were calculated from the calibration. Further purification of DNA and RNA for PCR was achieved after agarose gel electrophoresis as follows: the DNA and RNA bands were excised from the gels and purified by using Hybaid recovery DNA purification kit II and a Bio 101 RNaid kit (BIO101, Vista, Calif.), respectively, in accordance with the manufacturers' instructions. DNA and RNA were eluted in nuclease-free sterile water and stored at −20°C.

DGGE analysis. PCR of RNA and DNA was performed with 16S rDNA universal bacterial denaturing gradient gel electrophoresis (DGGE) primers (synthesized by Invitrogen Custom Primers, Paisley, United Kingdom) 2 and 3 (43) throughout to amplify the V3 hypervariable region of 16S rRNA genes (65). Additionally, these primers exclude regions of secondary structure which are located downstream and which are known to inhibit reverse transcription (RT) (67); these primers are thus also suitable for use in RT-PCR amplifications. Prior to RT-PCR, RNA was treated with DNase RQ1 (Promega, Southampton,

United Kingdom) in accordance with the manufacturer's instructions. RT then was performed, and a cDNA copy was synthesized from the rRNA by using Superscript II (Gibco BRL, Paisley, United Kingdom) before PCR with primers 2 and 3. To avoid the possibility of contaminating DNA in the RNA extracts used for RT-PCR, control PCRs containing DNase-treated nucleic acid extracts that were not previously subjected to RT were performed. PCR mixtures for the above-described primer set consisted of 5 μl of PCR buffer (Bioline), 2 mM $MgCl_2$, 250 μM each deoxynucleoside triphosphate, 400 nM each primer, 1 U of Biopro DNA polymerase (Bioline), 1 μl of template DNA, and sterile nuclease-free water to a final volume of 50 μl. PCR conditions were 5 min at 95°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, and 1 min at 72°C; and a final extension for 10 min at 72°C.

The PCR products were examined by DGGE. The gels were formed as stated by Muyzer et al. (43), with the exception that an acrylamide gradient of 8 to 12% was used in addition to the 40 to 60% urea–formamide gradient. The gels were poured by using the gradient delivery system supplied with the Bio-Rad DCode system and were run for 5 h at 180 V and a constant temperature of 58°C. The gels were fixed overnight with 10% (vol/vol) ethanol– 0.5% (vol/vol) glacial acetic acid. Staining was performed with a 0.1% (wt/vol) silver nitrate solution, and developing was performed with a solution containing 0.01% (wt/vol) sodium borohydride and 0.4% (vol/vol) formaldehyde. The gels were fixed with 0.75% (wt/vol) sodium carbonate and preserved with a solution containing 25% (vol/vol) ethanol and 10% (vol/vol) glycerol. The gels were scanned by using an Epson GT9600 scanner and analyzed by using the Phoretix 1D Advanced Analysis package.

Terminal restriction fragment length polymorphism (T-RFLP) analysis. PCR was performed with the fluorescence-labeled oligonucleotides 63F (5′-CAGGC CTAACACATGCAAGTC-3′) (38, 48) and 518R (5′-CGTATTACCGCGGCT GCTCG-3′) (34); these were labeled at the 5′ end with the phosphoramidite dyes FAM and HEX (Applied Biosystems, Foster City, Calif.), respectively. These primers generate an amplicon including the V1, V2, and V3 hypervariable regions (65). PCR mixtures consisted of the ingredients described above. PCR conditions were 2 min at 94°C; 30 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C; and a final extension for 10 min at 72°C. Following the removal of unincorporated nucleotides with QIAquick columns (Qiagen), the products were digested at 37°C for 3 h with restriction enzyme *Alu*I or *Cfo*I. These enzymes, together with primer 63F, were previously demonstrated to generate larger numbers of terminal restriction fragments (T-RFs) than other enzyme combinations; primer 518R provides greater discrimination than primer 1389R, which was previously shown to be less discriminatory than primers located in the 5′ end of the gene (48). Restriction digests were then mixed with 2 μl of deionized formamide and 0.5 μl of a ROX-labeled Genescan 500-bp internal size standard (Applied Biosystems), denatured for 5 min by boiling, and immediately transferred to ice. Triplicate samples were loaded onto an ABI310 automated genetic analyzer (Applied Biosystems) for capillary electrophoresis, and the resulting profiles were analyzed with Genescan software. Fragments with a peak height of less than 50 were excluded from subsequent analyses.

BIOLOG analysis. A 100-ml soil suspension was generated from an original 10-g aliquot (dry-weight equivalent) in 0.25× Ringer's solution (BDH, Poole, United Kingdom) (24), thus achieving a 10^{-1} dilution. This solution was vigorously shaken for 10 min on an armed shaker (Stuart Scientific) to dislodge bacteria before dilution to 10^{-2}. Low-speed centrifugation (1,500 × *g* for 10 min) was used to remove soil particles. A 150-μl aliquot of the bacterial suspension was inoculated into each well of a BIOLOG, Inc. (Hayward, Calif.), Eco-Plate. The plate was read with a Dias microplate reader (Dynex Technologies) over a 7-day period at a wavelength of 600 nm. Readings at day 0 were subtracted from subsequent readings to eliminate background color generated from the substrates and the bacterial suspension. In addition, readings generated from the control wells (no substrate; tetrazolium dye only) were also subtracted. The BIOLOG data were analyzed as described by Garland (22). The average well color development (AWCD) value was calculated for each sample at each time point by dividing the sum of the optical density data by 31 (number of substrates). Further analysis was performed on sample data when the AWCD value was approximately 1. The data were normalized by dividing the absorbance values by the AWCD values to reduce bias between samples with different inoculum densities.

General statistical analysis. The Shannon index (H') (56) ($H' = -\Sigma\, p_i \times \ln p_i$) was calculated from the bacterial community profiles, where p_i is the proportion of members that a particular species contributes to the total in the sample. This value was obtained by normalization of the volume data derived from the DGGE gels by using the Phoretix 1D Advanced Analysis package for each phylotype (band) within each community and representation as a proportion of the total volume data for that community. This software eliminates the background and automatically detects peaks when noise levels and minimum peak thresholds are set. Equitability (J) (50) ($J = H'/H'_{max}$) was then calculated for a bacterial community, where H'_{max} is the possible maximum diversity that could be obtained from that community, thus giving a measure of community heterogeneity unbiased by sample size. This principle has been used in other studies of microbial ecology (30, 40). UPGMA (unweighted pair-group method with arithmetic mean) dendrograms were constructed by using the Dice-Sorensen similarity index (50a) with either Phoretix or MultiVariate Statistical Package (MVSP) version 3.12h (GeoMem, Blairgowrie, United Kingdom) (see Fig. 3). Indices were similarly generated from the T-RFLP and substrate utilization data. Statistical significance was determined by analysis of triplicates with analysis of variance and Tukey tests (Microsoft Excel version 7a); a P value of <0.05 was required to establish a significant difference between samples. Alternatively, when duplicate samples were analyzed, all data were cited. Principal-component analysis (PCA) was performed by using Minitab for Windows version 13.20.

RESULTS

Comparisons of the physicochemical statuses of the soils. Variations in physical, chemical, and biological characteristics were observed in all of the fields (Table 2) but with no significant trends for the treated fields (organic farming or manure treatment) versus the GAP-farmed fields. Field RMB, which had been treated with manure for a period in excess of 20 years, did not have significantly elevated organic matter concentrations compared to its counterpart not treated with manure. In addition, there was no significant difference in the numbers of either total or culturable bacteria between the organically and conventionally managed fields (Table 2). However, significantly elevated concentrations were observed for organic matter and nitrate in fields RMA, RCC, and RCD ($P < 0.05$), all of the typical stagnogley soil type and growing leguminous crops (Table 1). EC soils were differentiated from the others by virtue of their underlying structure, as classified by the Soil Survey of England and Wales (27) (EC being brown rendzinas rather than typical stagnogley). Similarly, plotting of loss-on-ignition data against total carbon (Fig. 1) revealed a positive correlation (correlation coefficient, 0.9) ($P < 0.01$), with the exception of EC fields, which fell outside that correlation and showed an increased inorganic carbon content. Overall, the fields were generally grouped by farm, except for RMA, which grouped with RCC and RCD.

Bacterial physiological profiles. A clear differentiation in the PCA ordination of the substrate utilization profiles (Fig. 2, cluster I) was visible. Field RMA was the closest to the EC fields in this ordering. The EC fields were most dissimilar from the other fields, largely due to differences in amino acid utilization; the EC fields exhibited significant decreases in serine, asparagine, and arginine utilization and an increase in threonine utilization in comparison with all other fields ($P < 0.05$). When Shannon indices of diversity constructed from the substrate utilization profiles were examined, EC fields and field RMA exhibited the highest relative substrate utilization diversities (Table 3) ($P < 0.05$).

Similarities in the total and active bacterial communities determined by DGGE. Three replicates were initially produced for each DGGE sample, generating similarities between replicates of >95% (data not shown). For ease of display, however, only duplicates were run on the final gels. When the bacterial community profiles (Fig. 3) generated from the amplified soil DNA (16S rDNA) were examined, the EC fields were again separated from the others, forming a distinct clus-

FIG. 1. Total carbon content (obtained with a CHN analyzer) plotted against organic matter content (determined by loss on ignition [LOI]) (1). Symbols: ●, EC2; ○, EC3; +, RMA; ■, RMB; □, RMC; ◆, RCA; ◇, RCC; and ×, RCD. Groupings were supported by ordering of principal components derived from T-RFLP profiles (see the text and Fig. 7).

ter (cluster I, <10% similar to all other fields) in a UPGMA dendrogram (Dice-Sorensen coefficient of similarity) (Fig. 3). Field RMA formed cluster II (44% similar to the remaining fields). The five other fields from both farm RC and farm RM showed highly similar profiles comprising cluster III (>95% similar to each other). A PCA ordination of the same data (Fig. 4) also confirmed these groupings. No differences were observed with respect to field management.

Analysis of the active community was undertaken by RT-PCR of DNase I-treated nucleic acids. In control PCR experiments in which the RT step was omitted, no PCR products were generated, confirming that the products were derived from RNA and not from contaminating DNA. DGGE profiles constructed by using amplification products from RT-PCR of RNA (16S rRNA) were analyzed (Fig. 5) and showed that the

FIG. 2. PCA ordination of data (axes 1 and 2) generated from the substrate utilization profiles produced from the optical densities measured from the BIOLOG Eco-Plate. Symbols are as defined in the legend to Fig. 1. Groupings were supported by soil physicochemical data.

TABLE 2. Physical, chemical, and biological characteristics of soil from sample sites[a]

Field code	% WHC	pH	%		Concn (mg kg^{-1}) of:			% LOI	No. of bacteria g^{-1}	
			Carbon	Nitrogen	Phosphate	Nitrate	Sulfate		Culturable	Total
EC2	29.84 ± 0.59	7.98 ± 0.06	1.89 ± 0.14	0.18 ± 0.00	25.96 ± 1.49	113.37 ± 2.71	22.96 ± 0.52	2.93 ± 0.02	8.71E+06 ± 9.37E+05	1.06E+09 ± 6.60E+07
EC3	28.32 ± 0.37	7.98 ± 0.06	1.62 ± 0.06	0.17 ± 0.01	40.99 ± 4.00	111.77 ± 9.46	27.90 ± 1.29	2.97 ± 0.01	7.58E+06 ± 9.85E+05	9.51E+08 ± 6.33E+07
RMA	38.11 ± 0.75	7.44 ± 0.06	1.37 ± 0.03	0.18 ± 0.00	34.71 ± 2.84	142.52 ± 9.83	17.31 ± 0.21	3.41 ± 0.04	9.32E+06 ± 2.62E+05	1.22E+09 ± 5.75E+07
RMB	36.74 ± 0.21	7.36 ± 0.02	0.90 ± 0.03	0.14 ± 0.01	97.64 ± 1.50	98.19 ± 8.61	16.75 ± 0.88	2.21 ± 0.08	7.11E+06 ± 2.35E+05	1.24E+09 ± 5.72E+07
RMC	35.26 ± 1.15	7.70 ± 0.00	0.68 ± 0.02	0.11 ± 0.01	39.13 ± 2.74	119.39 ± 5.00	16.66 ± 0.17	2.05 ± 0.01	9.91E+06 ± 2.32E+05	1.42E+09 ± 1.68E+08
RCA	37.02 ± 0.33	6.65 ± 0.01	1.01 ± 0.04	0.14 ± 0.00	48.44 ± 1.53	107.47 ± 5.26	22.89 ± 2.81	2.68 ± 0.03	8.55E+06 ± 1.88E+06	1.87E+09 ± 3.11E+07
RCC	37.47 ± 1.19	6.55 ± 0.01	1.28 ± 0.12	0.17 ± 0.01	102.91 ± 13.20	134.62 ± 2.65	21.12 ± 0.29	3.29 ± 0.01	8.21E+06 ± 7.81E+05	1.63E+09 ± 5.15E+07
RCD	37.35 ± 0.38	6.70 ± 0.01	1.17 ± 0.02	0.16 ± 0.01	65.39 ± 1.45	226.70 ± 3.04	20.77 ± 0.94	3.18 ± 0.09	7.07E+06 ± 4.17E+05	1.83E+09 ± 1.80E+08

[a] Data are reported as means and standard errors of the means for three replicates. WHC, water-holding capacity; LOI, loss on ignition to determine organic matter content.

TABLE 3. Measures of heterogeneity for the substrate utilization and nucleic acid profiles for the soil communities at the eight sample sites[a]

Field code	Shannon index of diversity for[b]:				Equitability for T-RFLP profiles derived from DNA[c] (n = 3)
	Substrate utilization profiles (n = 3)	DGGE profiles derived from:		T-RFLP profiles derived from DNA (n = 3)	
		DNA (n = 2)	RNA (n = 2)		
EC2	3.29 ± 0.013	2.51 ± 0.011	3.12 ± 0.009	4.28 ± 0.045	0.814 ± 0.003
EC3	3.32 ± 0.012	2.47 ± 0.025	3.07 ± 0.061	3.96 ± 0.051	0.770 ± 0.005
RMA	3.29 ± 0.001	3.07 ± 0.028	2.99 ± 0.047	4.68 ± 0.008	0.897 ± 0.003
RMB	3.11 ± 0.019	2.68 ± 0.023	2.95 ± 0.025	4.29 ± 0.049	0.814 ± 0.002
RMC	3.19 ± 0.030	2.78 ± 0.013	2.94 ± 0.038	4.09 ± 0.112	0.755 ± 0.024
RCA	3.05 ± 0.041	2.73 ± 0.021	3.19 ± 0.025	4.23 ± 0.114	0.794 ± 0.028
RCC	3.14 ± 0.010	2.65 ± 0.074	3.10 ± 0.068	3.99 ± 0.043	0.866 ± 0.006
RCD	3.09 ± 0.021	2.76 ± 0.018	3.46 ± 0.139	3.90 ± 0.050	0.852 ± 0.001

[a] Data are reported as means and standard errors of the means.
[b] Shannon index of diversity (56) for DGGE profiles generated from amplified 16S rDNA and rRNA from soil, for BIOLOG substrate utilization profiles, and for the total number of T-RFLP profiles generated from amplified 16S rDNA.
[c] Equitability (50) for the total number of T-RFLP profiles generated from amplified 16S rDNA.

EC fields were the least similar to the other fields (cluster I, <25% similarity). Field RMA again formed a distinct cluster sharing 45% similarity with the remaining five non-EC fields. However, the five fields (RMB, RMC, RCA, RCC, and RCD) which showed high similarities (>95%) on the basis of rDNA profiles were separated into discrete farm-related clusters. Cluster IIIa comprised fields RMB and RMC (85% similar to each other), and cluster IIIb comprised fields RCA, RCC, and RCD (>90% similar). In addition, small differences separating field RCA from fields RCC and RCD were observed. Treatment differences were also observed in the rRNA profiles; although the same phylotypes (bands) were present in each of the communities (EC2 being similar to EC3 and RMB being similar to RMC), their distributions differed between fields (i.e., the relative abundances differed between EC2 and EC3 and between RMB and RMC).

Similarities in the total bacterial communities determined by T-RFLP analysis. When PCA analysis was performed on the T-RFLP profiles (Fig. 6), the EC fields were again separated from all other fields by both restriction enzyme digests (*Alu*I and *Cfo*I) and forward and reverse primers (Fig. 6, group A). Fields RMA, RCC, and RCD formed another cluster (group B), with the remaining fields in group C. However, when a UPGMA dendrogram was produced from these data

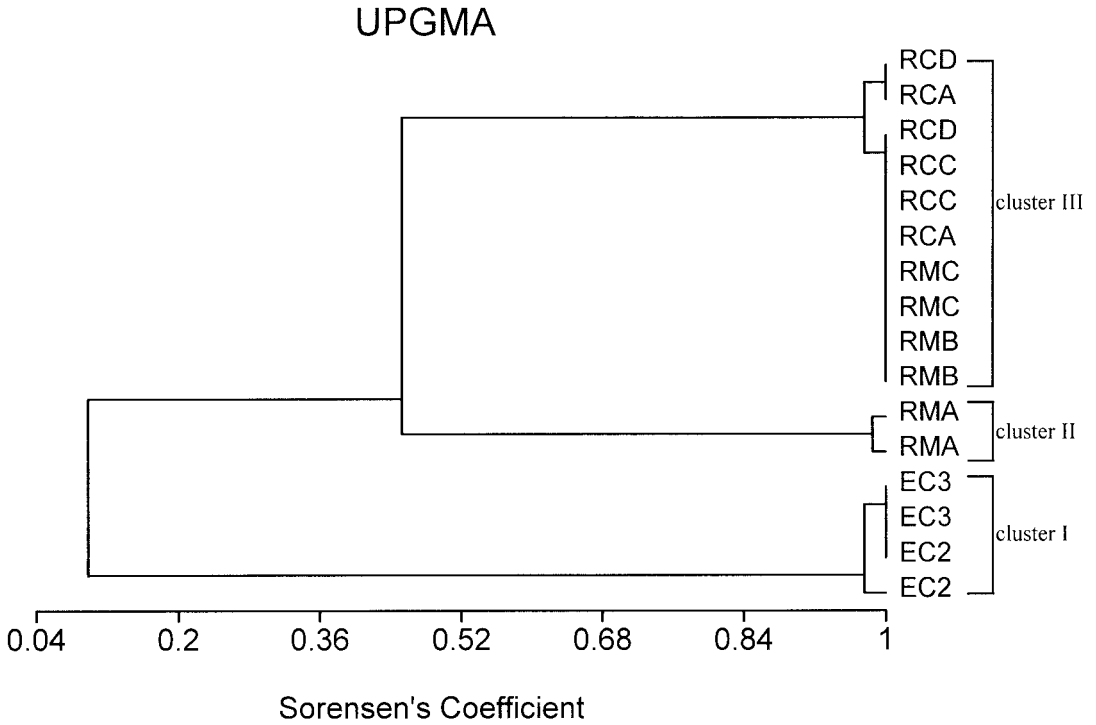

FIG. 3. UPGMA dendrogram constructed from the similarity matching data (Dice-Sorensen index) produced from the DGGE profiles of 16S rDNA amplified from soil and generated by using MVSP version 3.12h. The scale bar represents percent similarity. Duplicate samples were analyzed.

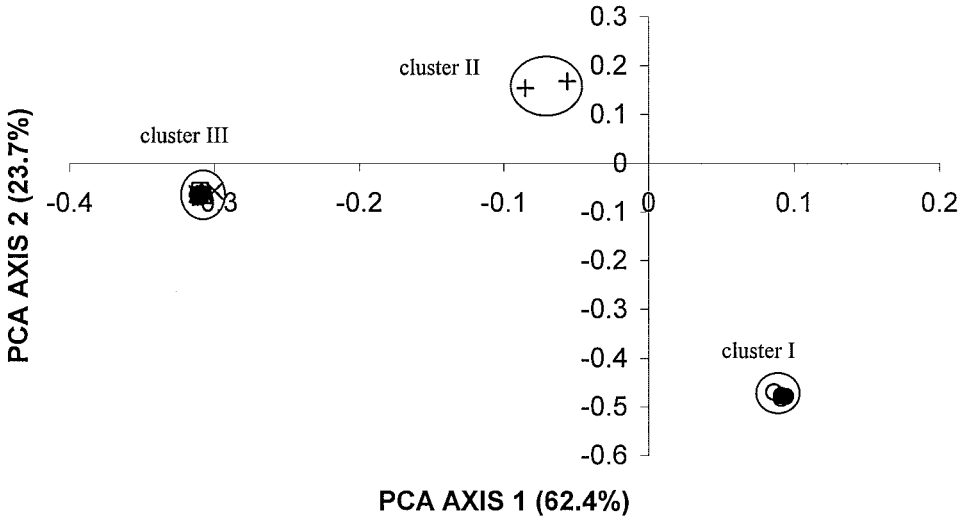

FIG. 4. PCA ordering of data (axes 1 and 2) generated from the DGGE profiles of amplified bacterial 16S rDNA. Symbols are as defined in the legend to Fig. 1. Groupings were supported by cluster analysis of DGGE profiles (Fig. 3).

by using similarity (presence or absence of matching bands) (Fig. 7), the three discrete clusters observed were similar to the groupings provided by DGGE analysis of rDNA profiles (Fig. 3). Five fields (RMB, RMC, RCA, RCC, and RCD) were found to cluster together by T-RFLP analysis. A slight additional separation of the field samples yielded two groupings—RCA, RMB, and RMC in one group and RCC and RCD in another group—but these groupings were not discrete and were not supported by any other data. The variability between replicates was also greater for T-RFLP profiles (75 to 90% similarity) than for the DNA- or RNA-derived DGGE replicates (95 to 100% similarity).

Heterogeneity of bacterial communities. EC2, EC3, and RMA showed the most diverse substrate utilization profiles, and the rDNA profiles also showed the highest relative diversity in RMA soil (Table 3). Field RMA also showed signifi-

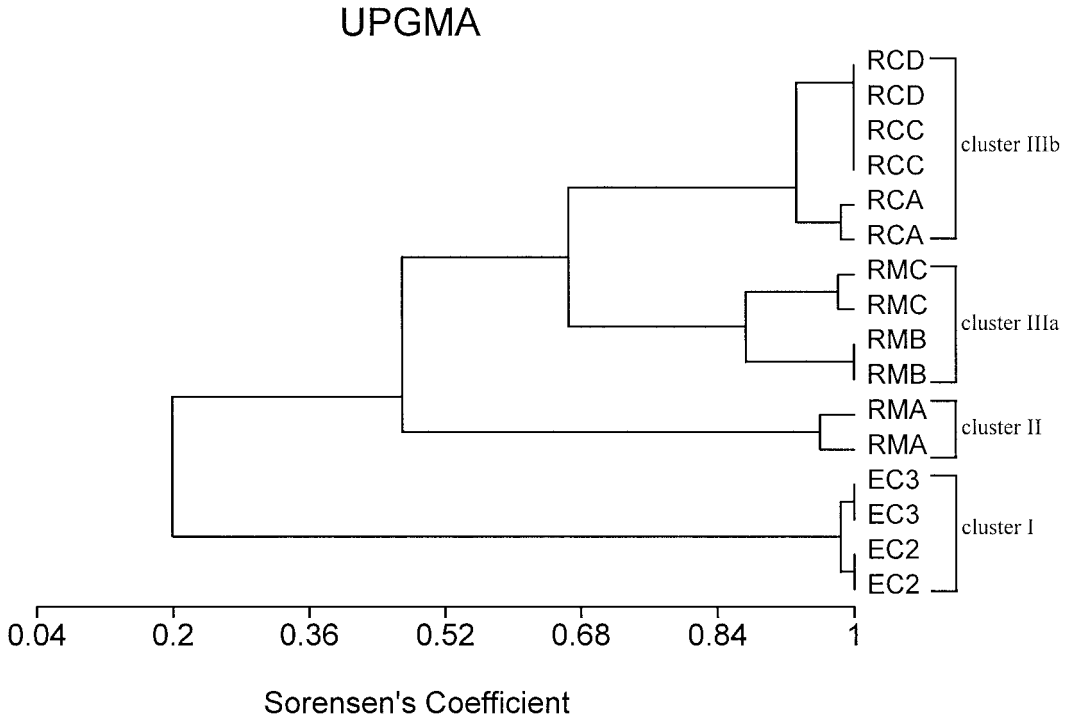

FIG. 5. UPGMA dendrogram constructed from the similarity matching data (Dice-Sorensen index) produced from the DGGE profiles of 16S rRNA amplified from soil and generated by using MVSP version 3.12h. The scale bar represents percent similarity. Duplicate samples were analyzed.

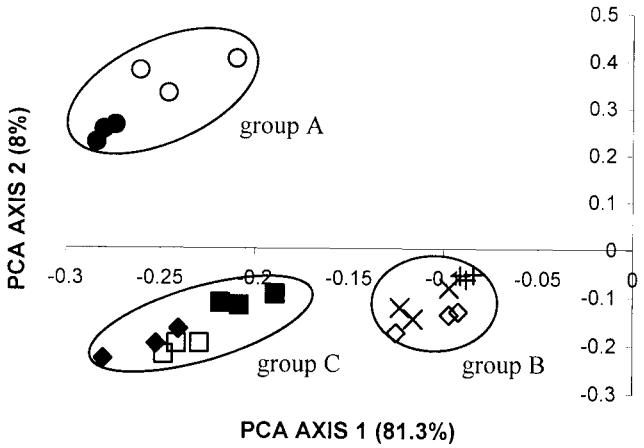

FIG. 6. PCA ordering of data (axes 1 and 2) generated from all T-RFLP fragments (5′ and 3′ T-RF frequencies generated after *Alu*I and *Cfo*I digestion) of 16S rDNA amplified from soil. The mean and standard error number of T-RFs analyzed per sample was 172.4 ± 4.0; the mean and standard error number of T-RFs per primer-enzyme combination was 43.1 ± 1.2. The eight soil samples analyzed in triplicate yielded 24 T-RFLPs. Symbols are as defined in the legend to Fig. 1.

cantly greater diversity, on the basis of T-RFLP analysis, and greater equitability than all other fields, except for fields RCC and RCD ($P = 0.01$) (Table 3). EC2 and EC3 had the least diverse bacterial communities, on the basis of rDNA-derived DGGE profiles, but their rRNA-derived DGGE profiles were more diverse (Table 3). Finally, organic field RCD possessed the highest rRNA sequence diversity, as determined by DGGE, including its nonorganic counterpart, field RCC (also undersown with clover), and the other organic field, EC2. The latter was also found to be significantly more diverse by T-RFLP analysis than EC3, its nonorganic counterpart ($P = 0.05$).

DISCUSSION

In this study, a polyphasic approach incorporating traditional and molecular (PCR-based) techniques was used to examine the effects of different soil types, land-use management practices, and cropping regimens upon the structures of total and active soil microbial communities. Despite recognized inherent biases in PCR-based methods (60, 61), relative comparisons between microbial communities based on such methods have been routinely applied to the study of microbial diversity within complex environments, overcoming biases resulting from the low culturability of the majority of microorganisms found in soils. In support of the application of PCR-based techniques, several reports investigating the effects of target DNA dilution or decreasing numbers of PCR cycles have suggested that such PCR biases may not be such a significant problem when applied to 16S rRNA gene-based community fingerprinting (16, 18, 20, 48). Nevertheless, when such data are used to calculate diversity and equitability indices, these values should be considered relative to those for other samples within a given study and thus should not be considered absolute values. As such, these PCR-based methods would seem to be the most representative methods for comparison of microbial community structure and diversity within environmental systems.

Land-use management has been shown to have an effect on both the physical and the chemical compositions of the soil and on the structure of the microbial community (2, 14, 37, 39, 53).

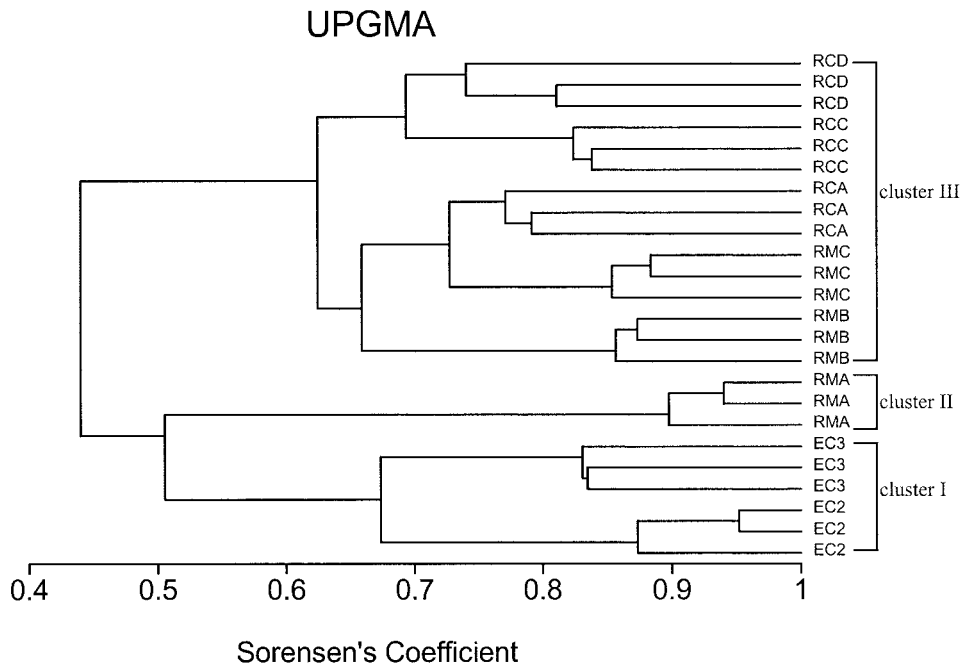

FIG. 7. UPGMA dendrogram constructed from similarity matching data (Dice-Sorensen index) for all T-RFLP fragments (5′ and 3′ T-RF frequencies generated after *Alu*I and *Cfo*I digestion) of 16S rDNA amplified from soil and generated by using MVSP version 3.12h. The mean and standard deviation number of T-RFs analyzed was as stated in the legend to Fig. 6. The scale bar represents percent similarity.

In the present study, however, the organically managed fields did not show significantly greater levels of organic matter than the fields under GAP management. Similarly, elevated bacterial numbers were not found in organically managed fields. Fields with high levels of organic matter did, however, show elevated nitrate concentrations.

The substrate utilization data separated the EC fields from all other fields as the most dissimilar, as did profiles generated from the total (rDNA) and active (rRNA) bacterial communities by DGGE and by T-RFLP analysis of products amplified from rDNA. The EC soils were distinguished from the other soils on the basis of soil type and physicochemical properties, in particular, by a significant increase in the inorganic carbon content due to the chalky nature of the soils and by a water-holding capacity significantly lower than those of all other soils in the study.

Despite variations in many chemical and biological soil characteristics, five fields of the typical stagnogley soil type on two geographically distinct farms (RMB, RMC, RCA, RCC, and RCD) exhibited almost identical rDNA-derived DGGE profiles. Likewise, these soil communities formed a similar cluster after analysis of rDNA T-RFLP profiles. Therefore, it is likely that the total bacterial community compositions have been determined primarily by the underlying soil chemistry and structure rather than by the different management practices or cropping regimens at these sites.

Spatial homogeneity in soil over distances of several hundred meters was also revealed in Dutch meadows by 16S rRNA and rDNA fingerprinting (temperature gradient gel electrophoresis [TGGE]) by Felske and Akkermans (17). Almost identical bacterial profiles were generated between samples, suggesting that despite heterogeneity in soil properties, the dominant members of the microbial community appeared to be ubiquitous in this area (as determined with universal bacterial primers). Buckley and Schmidt (5) also highlighted the fact that in the published literature on the average abundance of 16S rDNA clones from the alpha, beta, and gamma *Proteobacteria* and *Actinobacteria*, originating from diverse soils from three different continents, samples showed remarkable similarities (3, 4, 33, 35, 36, 39). This finding suggests that certain characteristics of soil environments can lead to overall similarities. Spatial and temporal variations in soil chemical, physical, and biological properties, such as water content, nutrient and mineral availability, pH, and soil structure, contribute to immense heterogeneity and complexity of niches available within the soil environment (41, 64). This variability results in the formation of many discrete microhabitats, thereby conferring suitable habitats on a much smaller scale; habitat specificity is therefore not prohibitive. In addition, microorganisms have the ability to persist despite unfavorable conditions for growth, thereby permitting almost a universal presence of species, provided that initial dispersal was permitted (21). These factors may explain the lack of differentiation in the total bacterial community structures on two geographically distinct farms (>65 km apart), observed in the present study.

The T-RFLP data derived from DNA were similar to the data obtained from DGGE, forming three discrete clusters (Fig. 3 and 7). T-RFLP analysis provided slightly greater discrimination than DGGE, probably due to the inclusion of the V1, V2, and V3 hypervariable regions (65), whereas primers 2 and 3 covered only the V3 region. This factor, in addition to the combination of results from two restriction enzymes and both forward and reverse primers, may also explain the greater variability between replicates seen with T-RFLP analysis than with DGGE. However, the PCA ordination of the T-RFs produced slightly different groupings than the cluster dendrograms constructed from similarity matching data (Fig. 6 and 7). Samples RMA, RCC, and RCD were grouped together by PCA due to the more even distribution of phylotypes in their communities than in those of other samples, as determined by the equitability index (Table 3). These groupings were similar to those produced by the plotting of total carbon content against loss-on-ignition data (Fig. 1), perhaps suggesting a relationship between organic and inorganic carbon ratios and distribution within bacterial communities. The fact that ordination takes into account not only the presence and absence of species but also the distribution in the community highlights the need for comprehensive and combinational analysis methods. When Buckley and Schmidt (5) examined five fields, on the same site, that had been cultivated long term but with five different historically cultivated treatments, they found that the microbial community structures (T-RFLP analysis of 16S rDNA) were remarkably similar among these fields. Similarly, comparing improved and unimproved grassland soils, McCaig et al. (40) did not observe differences between the diversities of individual unimproved and improved grassland communities on the basis of 16S rDNA-derived DGGE profiles. However, DGGE banding profiles obtained from triplicate samples, pooled prior to analysis, indicated that there was less evenness in improved soils, suggesting that selection for specific bacterial groups had occurred.

As it has been well established that most of the bacterial soil community is inactive, a description of community structure on the basis of rDNA diversity provides a historical perspective rather than an immediate description of the present soil bacterial community (18, 64). Many bacterial species are known to vary their ribosome numbers in accordance with their cellular activities (66); thus, studying a bacterial community by rRNA-based approaches permits a description of the active community. The rRNA-derived DGGE profiles showed greater discrimination than either of the rDNA-based methods. Treatment differences were highlighted in the different relative abundances of sequences between EC2 (organic) and EC3,and between RMB (manure treated) and RMC. The five fields (RMB, RMC, RCA, RCC, and RCD) previously found to form one cluster on the basis of rDNA profiles (Fig. 3 and 7) were further separated by farm location into two clusters (Fig. 5). Fields RCC and RCD (typical stagnogley soil type with leguminous cropping) were also separated from field RCA (typical stagnogley soil type with nonleguminous cropping). Therefore, the rRNA diversity analysis would seem to be more indicative of short-term changes in land-use management.

In a study of soil bacterial community profiles in five Dutch grassland meadows, Felske et al. (19), using multiple competitive RT-PCR and DGGE analyses, discovered an increase in the total number of rRNAs after these fields were taken out of agricultural production. However, although the multiple competitive RT-PCR analysis indicated activity shifts for the predominant soil bacteria during the subsequent vegetational suc-

cession, quantitative dot blot hybridization failed to detect differences at a higher taxonomic level; although the vegetation clearly changed, the general composition of the bacterial community remained remarkably stable. Similarly, Buckley and Schmidt (5) failed to detect significant differences in the microbial group rRNA abundances in five fields despite differences in chemical inputs, tillage, plant community composition, and productivity. Therefore, it would appear that agricultural soil habitats are immensely stable environments where competition among microbial organisms is not severe, allowing species to persist throughout fluctuations in above-ground vegetation and in soil chemical properties.

Despite exhibiting the highest substrate utilization diversities, the EC fields showed the lowest relative diversities on the basis of DGGE analysis of rDNA (Table 3). Smalla et al. (58) discovered, by comparing the TGGE profiles of the original inoculum with those in the BIOLOG wells following incubation, that fast-growing bacteria that adapted to high substrate concentrations were numerically dominant in the BIOLOG wells. Therefore, the comparatively high diversities of sequences generated from rRNA-derived DGGE profiles of the EC soils may more accurately reflect the functional community. The high substrate utilization diversities in these soils may suggest an overlap between the metabolically active members of the soil community, as assessed from rRNA, and those active in the BIOLOG wells.

There seemed to be a strong link between the elevated nitrate concentrations within these soils and the significant increases in organic matter in these soils compared to their nonleguminous counterparts. This finding may indicate the presence of nitrogen-fixing bacteria and, in conjunction with the higher organic matter concentrations, may advocate the use of leguminous crop planting for improving soil health. The benefit to agriculture of nitrogen fixation from nodulated legumes has long been established (31, 57, 62, 68). In long-term field experiments on loamy sand and sandy loam, leguminous cropping stimulated microbial activity in the rhizosphere more than cereals, maize, or oil flax. Moreover, normal GAP farming may also decrease nitrogen fixation activity. Hoflich et al. (28) demonstrated that the leghemoglobin content of pea nodules (an indicator of nitrogen fixation activity) was reduced by the application of high levels of nitrogen in crop rotation. Herbicides widely applied for the suppression of weed growth in white clover seed crops have also been shown to be toxic to the growth of the nodule-forming bacterium *Rhizobium trifolii* and to the nitrogen-fixing symbiosis of these organisms (8).

In conclusion, the polyphasic approaches used to analyze bacterial community structure were generally in broad agreement, with samples being separated primarily by soil type. The community within the EC fields was discriminated by all methods, including BIOLOG. The community within field RMA was likewise discriminated by rDNA and rRNA analyses. Cluster analysis of the T-RFLP data and the rDNA-derived DGGE data grouped the remaining fields on the basis of soil type. Thus, we hypothesize that soil type is the overriding factor in community determinations at these sites, followed by the planting of, specifically, leguminous crops rather than other management practices. The bacterial profiles generated by RT-PCR of rRNA further separated the soil by geographic location and also revealed small treatment differences, providing the greatest discrimination between sites and emphasizing the importance of studying both total and active communities. Soil has been shown to have an immense capacity for diversity and therefore a large buffering capacity before the results of management practices will affect the dominant members of the community. However, the longer-term impacts of management practices may be much more significant, and their effects upon the fungal community were not examined in this study; therefore further attention is warranted on this subject.

ACKNOWLEDGMENTS

We thank Ed Cross, Robert Carey, and Robert Middleditch for cooperation during this research and for permitting sampling on their farms. We also thank A. Prieto-Fernandez and B. Nogales-Fernandez for advice and assistance with T-RFLP analysis and Steve Parry and Mike Lane for continuing advice and help.

We are grateful for the financial support of this research by Unilever and Syngenta.

REFERENCES

1. **Ball, D. F.** 1964. Loss-on-ignition as an estimate of organic matter and organic carbon in non-calcareous soils. J. Soil Sci. **15:**84–92.
2. **Boddington, C. L., and J. C. Dodd.** 2000. The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian Ultisol. Plant Soil **218:**137–144.
3. **Borneman, J., P. W. Skroch, K. M. O'Sullivan, J. A. Palus, N. G. Rumjanek, J. L. Jansen, J. Neinhuis, and E. W. Triplett.** 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. Appl. Environ. Microbiol. **62:**1935–1943.
4. **Borneman, J., and E. W. Triplett.** 1997. Molecular microbial diversity in soils from eastern Amazonia: evidence for unusual microorganisms and microbial population shifts associated with deforestation. Appl. Environ. Microbiol. **63:**2647–2653.
5. **Buckley, D. H., and T. M. Schmidt.** 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microb. Ecol. **42:**11–21.
6. **Buresh, R. J., P. A. Sanchez, and F. Calhoun (ed.).** 1997. Replenishing soil fertility in Africa. Soil Science Society of America publication 51. Soil Science Society of America, Madison, Wis.
7. **Cassel, D. K., and D. R. Nielsen.** 1986. Field capacity and available water capacity. *In* A. Klute (ed.), Methods of soil analysis. Part 1. Physical and mineralogical methods, 2nd ed. Soil Science Society of America, Madison, Wis.
8. **Clark, S. A., and H. K. Mahanty.** 1991. Influence of herbicides on growth and nodulation of white clover, *Trifolium repens*. Soil Biol. Biochem. **23:**725–730.
9. **Clegg, C. D., K. Ritz, and B. S. Griffiths.** 1998. Broad-scale analysis of soil microbial community DNA from upland grasslands. Antonie Leeuwenhoek Int. J. Gen. Mol. Microbiol. **73:**9–14.
10. **Clegg, C. D., K. Ritz, and B. S. Griffiths.** 2000. %G+C profiling and cross hybridisation of microbial DNA reveals great variation in below-ground community structure in UK upland grasslands. Appl. Soil Ecol. **14:**125–134.
11. **Curci, M., M. D. R. Pizzigallo, C. Crecchio, R. Mininni, and P. Ruggiero.** 1997. Effects of conventional tillage on biochemical properties of soils. Biol. Fertil. Soil **25:**1–6.
12. **Daily, G. C. (ed.).** Nature's services. Island Press, Washington, D.C.
13. **Doran, J. W., and M. R. Zeiss.** 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. **15:**3–11.
14. **Drinkwater, L. E., P. Wagoner, and M. Sarrantonio.** 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature **396:**262–265.
15. **Dumanski, J., and C. Pieri.** 2000. Land quality indicators: research plan. Agric. Ecosyst. Environ. **82:**93–102.
16. **Farrelly, V., F. A. Rainey, and E. Stackebrandt.** 1995. Effect of genome size and rrn copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. Appl. Environ. Microbiol. **61:**2798–2801.
17. **Felske, A., and A. D. L. Akkermans.** 1998. Spatial homogeneity of abundant bacterial 16S rRNA molecules in grassland soils. Microb. Ecol. **36:**31–36.
18. **Felske, A., A. Wolterink, R. van Lis, and A. D. L. Akkermans.** 1998. Phylogeny of the main bacterial 16S rRNA sequences in Drentse A grassland soils (The Netherlands). Appl. Environ. Microbiol. **64:**871–879.
19. **Felske, A., A. Wolterink, R. van Lis, W. M. de Vos, and A. D. L. Akkermans.** 2000. Response of a soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. Appl. Environ. Microbiol. **66:**3998–4003.
20. **Ferris, M. J., and D. M. Ward.** 1997. Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. **63:**1375–1381.

21. **Finlay, B. J., G. F. Esteban, J. L. Olmo, and P. A. Tyler.** 1999. Global distribution of free-living microbial species. Ecography **22:**138–144.
22. **Garland, J. L.** 1996. Analytical approaches to the characterisation of samples of microbial communities using patterns of potential C source utilisation tests. Soil Biol. Biochem. **28:**213–221.
23. **Giller, K. E.** 2001. Nitrogen fixation in tropical cropping systems, 2nd ed. CAB International, Wallingford, Oxon, United Kingdom.
24. **Grayston, S. J., and C. D. Campbell.** 1996. Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). Tree Physiol. **16:**11–12.
25. **Hansen, B., H. F. Alroe, and E. S. Kristensen.** 2001. Approaches to assess the environmental impact of organic farming with particular regard to Denmark. Agric. Ecosyst. Environ. **63:**11–26.
26. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33:**1225–1228.
27. **Hodge, C. A. H., R. G. O. Burton, W. M. Corbett, R. Evans, and R. S. Seale.** 1984. Soils and their use in eastern England. Bulletin 13. Soil Survey of England and Wales, Harpenden, United Kingdom.
28. **Hoflich, G., M. Tauschke, G. Kuhn, and J. Rogasik.** 2000. Influence of agricultural crops and fertilization on microbial activity and microorganisms in the rhizosphere. J. Agron. Crop Sci. **184:**49–54.
29. **Hurni, H.** 2000. Assessing sustainable land management. Agric. Ecosyst. Environ. **82:**83–92.
30. **Ibekwe, A. M., S. K. Papiernik, J. Gan, S. R. Yates, C. H. Yang, and D. E. Crowley.** 2001. Impact of fumigants on soil microbial communities. Appl. Environ. Microbiol. **67:**3245–3257.
31. **Kennedy, I. R., and N. Islam.** 2001. The current potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: a review. Aust. J. Exp. Agric. **41:**447–457.
32. **Killham, K.** 1994. Soil ecology. Cambridge University Press, Cambridge, United Kingdom.
33. **Kuske, C. R., S. M. Barns, and J. D. Busch.** 1997. Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. Appl. Environ. Microbiol. **63:**3614–3621.
34. **Lane, D. J.** 1991. 16S/23S sequencing, p. 115–175. *In* E. Stackebrandt and M. Goodfellow (ed.), Nucleic acid techniques in bacterial systematics. John Wiley & Sons, Chichester, United Kingdom.
35. **Lee, S.-Y., J. Bollinger, D. Bezdicek, and A. Ogram.** 1996. Estimation of the abundance of an uncultured soil bacterial strain by a competitive quantitative PCR method. Appl. Environ. Microbiol. **62:**3787–3793.
36. **Liesack, W., and E. Stackebrandt.** 1992. Occurence of novel groups of the domain *Bacteria* as revealed by analysis of genetic material isolated from an Australian terrestrial environment. J. Bacteriol. **174:**5072–5078.
37. **Lockeretz, W., G. Shearer, and D. H. Kohl.** 1981. Organic farming in the corn belt. Science **211:**540–547.
38. **Marchesi, J. R., T. Sato, A. J. Weightman, T. A. Martin, J. C. Fry, S. J. Hiom, D. Dymock, and W. G. Wade.** 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. **64:**795–799.
39. **McCaig, A. E., L. A. Glover, and J. I. Prosser.** 1999. Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl. Environ. Microbiol. **65:**1721–1730.
40. **McCaig, A. E., L. A. Glover, and J. I. Prosser.** 2001. Numerical analysis of grassland bacterial community structure under different land management regimens by using 16S ribosomal DNA sequence data and denaturing gradient gel electrophoresis banding patterns. Appl. Environ. Microbiol. **67:** 4554–4559.
41. **McDougald, D., S. A. Rice, D. Weichart, and S. Kjelleberg.** 1998. Nonculturability: adaption or debilitation. FEMS Microbiol. Ecol. **25:**1–9.
42. **Ministry of Agriculture, Fisheries, and Food.** 1993. Code of good agricultural practice for the protection of soil. MAFF Publications, London, United Kingdom.
43. **Muyzer, G., E. C. de Waal, and A. G. Uitterlinden.** 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. **59:**695–700.
44. **Nusslein, K., and J. M. Tiedje.** 1998. Characterisation of the dominant and rare members of a young Hawaiian soil bacterial community with small-subunit ribosomal DNA amplified from DNA fractionated on the basis of its guanine and cytosine composition. Appl. Environ. Microbiol. **64:**1283–1289.
45. **Nusslein, K., and J. M. Tiedje.** 1999. Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Appl. Environ. Microbiol. **65:**3622–3626.
46. **O'Donnell, A. G., M. Goodfellow, and D. L. Hawksworth.** 1994. Theoretical practical aspects of the quantification of biodiversity among microorganism. Philos. Trans. R. Soc. Lond. B **345:**65–73.
47. **Oldeman, L. R.** 1994. The global extent of soil degradation, p. 99–118. *In* D. J. Greenland and I. Szabolcs (ed.), Soil resilience and sustainable land use. CAB International, Wallingford, Oxon, United Kingdom.
48. **Osborn, A. M., E. R. B. Moore, and K. N. Timmis.** 2000. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. Environ. Microbiol. **2:**39–50.
49. **Palm, C. A., C. N. Gachengo, R. J. Delve, G. Cadisch, and K. E. Giller.** 2001. Organic inputs for soil fertility management in tropical agroecosystems: application of an organic resource database. Agric. Ecosyst. Environ. **83:**27–42.
50. **Pielou, E. C.** 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. **13:**131–144.

50a. **Pielou, E. C.** 1972. Niche width and niche overlap: a method for measuring them. Ecology **53:**687–692.

51. **Pretty, J. N.** 1995. Regenerating agriculture, p. 320. Earthscan Publications, London, United Kingdom.
52. **Pretty, J. N., and P. Shah.** 1997. Making soil and water conservation sustainable: from coercion and control to partnerships and participation. Land Degrad. Dev. **8:**39–58.
53. **Reganold, J. P., A. S. Palmer, J. C. Lockhart, and A. N. Macgregor.** 1993. Soil quality and financial performance of biodynamic and conventional farms in New Zealand. Science **260:**344–349.
54. **Royal Commission on Environmental Pollution.** 1996. Sustainable use of soil. 19th Report of the Royal Commission on Environmental Pollution. Command 3165. Her Majesty's Stationery Office, London, United Kingdom.
55. **Sarathchandra, S. U., G. Burch, and N. R. Cox.** 1997. Growth patterns of bacterial communities in the rhizoplane and rhizosphere of white clover (Trifolium repens L.) and perennial ryegrass (Lolium perenne L.) in long-term pasture. Appl. Soil Ecol. **6:**293–299.
56. **Shannon, C. E., and W. Weaver.** 1949. The mathematical theory of communication. University of Illinois Press, Champaign.
57. **Singh, N. B., P. P. Singh, and K. P. P. Nair.** 1986. Effect of legume intercropping on enrichment of soil nitrogen, bacterial activity and productivity of associated maize crops. Exp. Agr. **22:**339–344.
58. **Smalla, K., U. Wachtendorf, H. Heuer, W.-T. Liu, and L. Forney.** 1998. Analysis of BIOLOG GN substrate utilization patterns by microbial communities. Appl. Environ. Microbiol. **64:**1220–1225.
59. **Steffan, R. J., J. Goksoyr, A. K. Bej, and R. M. Atlas.** 1988. Recovery of DNA from soils and sediments. Appl. Environ. Microbiol. **54:**2908–2915.
60. **Suzuki, M., M. S. Rappé, and S. J. Giovannoni.** 1998. Kinetic bias in estimates of coastal picoplankton community structure obtained by measurements of small-subunit rRNA gene PCR amplicon length heterogeneity. Appl. Environ. Microbiol. **64:**4522–4529.
61. **Suzuki, M. T., and S. J. Giovannoni.** 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. Environ. Microbiol. **62:**625–630.
62. **Tate, R. L.** 1995. Soil microbiology (symbiotic nitrogen fixation), p. 307–333. John Wiley & Sons, Inc., New York, N.Y.
63. **Tilman, D.** 1998. The greening of the green revolution. Nature **396:**211–212.
64. **Torsvik, V., R. Sørheim, and J. Goksøyr.** 1996. Total bacterial diversity in soil and sediment communities—a review. J. Ind. Microbiol. **17:**170–178.
65. **Van der Peer, Y., S. Chappelle, and R. DeWachter.** 1996. A quantitative map of nucleotide substitution rates in bacterial rRNA. Nucleic Acids Res. **24:** 3381–3391.
66. **Wagner, R.** 1994. The regulation of ribosomal-RNA synthesis and bacterial-cell growth. Arch. Microbiol. **161:**100–109.
67. **Weller, R., and D. M. Ward.** 1989. Selective recovery of 16s ribosomal-rna sequences from natural microbial communities in the form of cDNA. Appl. Environ. Microbiol. **55:**1818–1822.
68. **Zahran, H. H.** 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev. **63:**968–989.