Microbial Ecology

Soil Microbial Community Response to Land Use Change in an Agricultural Landscape of Western Kenya

D.A. Bossio[1], M.S. Girvan[2], L. Verchot[1], J. Bullimore[2], T. Borelli[1], A. Albrecht[1,3], K.M. Scow[4] A.S. Ball[2], J.N. Pretty[2] and A.M. Osborn[2]

(1) World Agroforestry Center, ICRAF, P.O. Box 30677, Nairobi, Kenya
(2) Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester CO4 3SQ, UK
(3) Institut de Recherche pour le Développement (IRD), ICRAF, P.O. Box 30677, Nairobi, Kenya
(4) Department of Land, Air and Water Resources, University of California, Davis, Davis, CA 95616, USA

Received: 26 September 2003 / Accepted: 19 February 2004 / Online publication: 24 January 2005

Abstract

Tropical agroecosystems are subject to degradation processes such as losses in soil carbon, nutrient depletion, and reduced water holding capacity that occur rapidly resulting in a reduction in soil fertility that can be difficult to reverse. In this research, a polyphasic methodology has been used to investigate changes in microbial community structure and function in a series of tropical soils in western Kenya. These soils have different land usage with both wooded and agricultural soils at Kakamega and Ochinga, whereas at Ochinga, Leuro, Teso, and Ugunja a replicated field experiment compared traditional continuous maize cropping against an improved N-fixing fallow system. For all sites, principal component analysis of 16S rRNA gene denaturing gradient gel electrophoresis (DGGE) profiles revealed that soil type was the key determinant of total bacterial community structure, with secondary variation found between wooded and agricultural soils. Similarly, phospholipid fatty acid (PLFA) analysis also separated wooded from agricultural soils, primarily on the basis of higher abundance of monounsaturated fatty acids, anteiso- and iso-branched fatty acids, and methyl-branched fatty acids in the wooded soils. At Kakamega and Ochinga wooded soils had between five 5 and 10-fold higher levels of soil carbon and microbial biomass carbon than agricultural soils from the same location, whereas total enzyme activities were also lower in the agricultural sites. Soils with woody vegetation had a lower percentage of phosphatase activity and higher cellulase and chitinase activities than the agricultural soils. BIOLOG analysis showed woodland soils to have the greatest substrate diversity. Throughout the study the two functional indicators (enzyme activity and BIOLOG), however, showed lower specificity with respect to soil type and land usage than did the compositional indicators (DGGE and PLFA). In the field experiment comparing two types of maize cropping, both the maize yields and total microbial biomass were found to increase with the fallow system. Moreover, 16S rRNA gene and PLFA analyses revealed shifts in the total microbial community in response to the different management regimes, indicating that deliberate management of soils can have considerable impact on microbial community structure and function in tropical soils.

Introduction

Land use change alters the below-ground ecosystem, often leading to loss of biodiversity and depletion of soil carbon [14]. Some 1780 Mha of the world's soils are now known to be degraded in some way [39], with conversion of forests and grasslands to agriculture causing dramatic effects on physical and chemical properties [13, 36, 45]. Losses in soil carbon can be considerable, and indigenous microbial communities respond to such changes in carbon and other substrates [8, 11, 12, 22, 30, 46]. In tropical agroecosystems, where climate and edaphic factors may place less constraint on microbial activity [9], farmers commonly have limited access to inputs, and turnover of organic matter is rapid. Degradation processes such as losses in soil carbon, nutrient depletion, and reduced water holding capacity can occur quickly and be difficult to reverse [40, 42]. Thus land use and management factors may play a more important role in regulating microbial communities in tropical soils than

Present address of D.A. Bossio: International Water Management Institute, IWMI, P.O. Box 2075, Colombo, Sri Lanka
Correspondence to: D.A. Bossio; E-mail: d.bossio@cgiar.org

 DOI: 10.1007/s00248-003-0209-6 • Volume 49, 50–62 (2005)

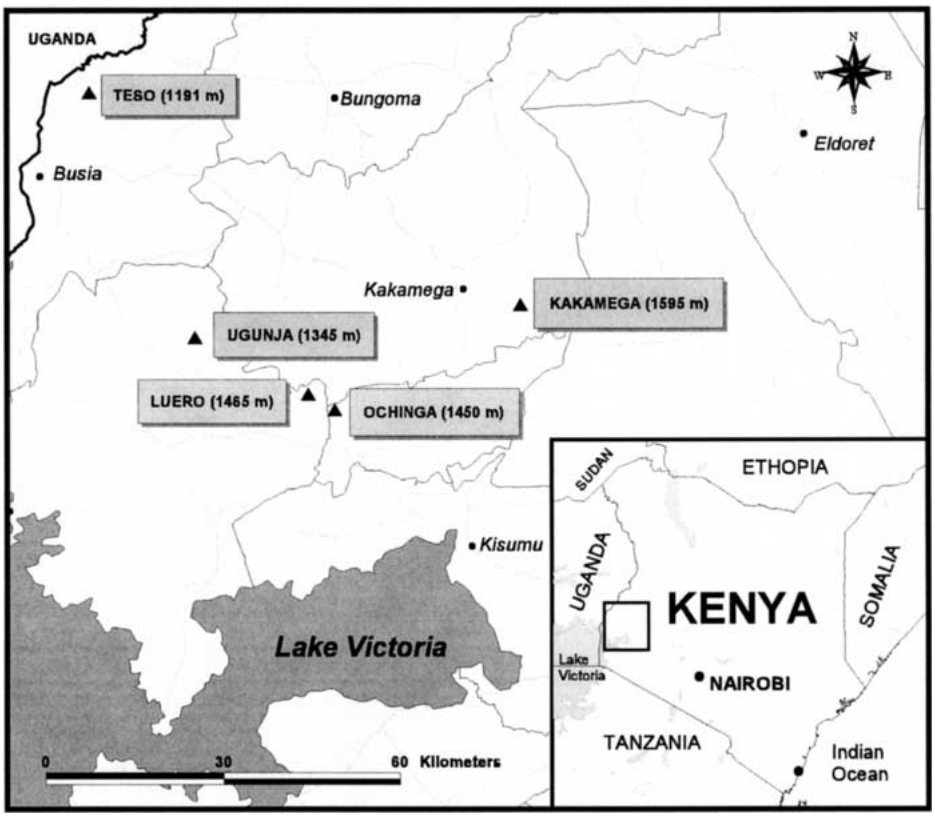

Figure 1. Map of study area in Western Kenya showing location of experiment sites.

in many temperate systems, where favorable climatic factors, combined with access to fertilizers and other soil amendments, help to buffer changes in the soil environment [20].

Research on tropical soil biota has primarily focused on macrofauna, including demonstration of the importance of earthworms and termites in regulating nutrient cycling in humid soils [5, 9, 17, 26], with less focus on microorganisms with the exception of several key microbially mediated processes such as nitrogen fixation [21, 27]. Analysis of changes in microbial communities in tropical soils has hitherto been limited to a few land use types using traditional methods to detect changes in microbial biomass and soil respiration [4, 31, 37, 41]. These analyses have shown increases in microbial and fungal biomass as a result of differing organic matter inputs [31], demonstrated the importance of microbial biomass as a source of nutrients to crops [44], quantified microbial biomass in relation to other functional groups in soils of tropical slash-and-burn systems [37], and found that decomposition pathways in tropical forests were more dominated by fungi than bacteria than in temperate forests [41]. Advanced molecular and biochemical ecological approaches have only been used to study microorganisms in a limited number of tropical systems [6, 23, 35, 51]. Dramatic changes in microbial community structure resulted from changes in vegetation in young Hawaiian soils [35], and conversion of forest to agriculture decreased microbial biomass and produced compositionally distinct microbial communities in Tahiti [51]. Borneman and Triplett [6] demonstrated significant differences between soil microbial populations in a mature forest and adjacent pasture in Eastern Amazonia. Many of these studies are anecdotal and are not based on replicated field trials. Therefore, little is known about how agricultural practices affect microbial communities in tropical soils.

The objectives of this research were to investigate the variation in tropical soil microbial communities associated with (i) differing soil types, (ii) differences in land use; comparing wooded (forest and woodlot) and agricultural soils, and (iii) differences in soil management practices within degraded soils.

Methods

Study Sites. Study sites were selected in Western Kenya (Fig. 1) to represent three comparisons: (i) variation in soil type (with soils ranging in texture from sandy to clay); (ii) comparison of land use (forest, woodlot, or agriculture) at two locations (Ochinga and Kakamega); and (iii) comparison of agricultural management practices, focusing on improved fallow (increased organic nitrogen and carbon inputs through a fallow season

Table 1. Soil properties, environment, and productivity characteristics at field sites

Site	Precipitation[a] (mm)	Soil texture[a] (% clay)	pH[a]	Fallow biomass[a,b] (tha^{-1})	Maize yield traditional[a,c] (tha^{-1})	Maize, yield, improved[a,d] (tha^{-1})
Teso	1500	Sandy (5%)	5.0	13	1.02	2.19
Ugunja	1600	Sandy loam (15%)	5.1	5	1.77	1.86
Ochinga	1700	Clay (40%)	5.1	10	3.07	5.06
Luero	1700	Clay (60%)	5.8	27	1.93	4.47
Kakamega	1900	Clay (40%)	4.1	NA	0	NA

[a]Soil properties and other data from Albrecht [1].
[b]Fallow biomass yields are the total biomass of *Tephrosia candida* produced during an 18-month fallow period in Teso, Ugunja, and Luero and 9-month fallow period in Ochinga, and incorporated into soil in improved system plots in February 2001.
[c]Maize yields from experimental plots in which continuous maize cropping was practiced with no nitrogen-fixing fallow.
[d]Maize yields from crop following growth and incorporation of *Tephrosia candida* fallow crop.

growth of the N-fixing *Tephrosia candida*), and traditional agricultural management (double cropping of maize with no fallow) across four soil types (Teso, Ugunja, Ochinga, and Luero) and perennial tea cultivation (Kakamega).

Four soils were sampled. They represented the major classes of soils in the region and, though separated by up to 80 km (Fig. 1), were spread over a narrow precipitation gradient (Table 1). Agricultural productivity was generally very low at all sites (Table 1). For land use comparison a primary forest, a perennial tea plantation, and unimproved maize fields were sampled at the Kakamega site, and maize fields and a woodlot established 20 years earlier on previously tilled fields were sampled at the Ochinga site. Three of the agricultural sites (Teso, Ugunja, and Luero) were managed as part of the IMPALA project [1]. These sites were located on farmers' fields representing the main soil types of the region. All IMPALA experiments were on degraded soils that were no longer productive following years of agricultural management that did not maintain soil fertility levels. At each site, a randomized complete block experiment included continuous maize treatments, consisting of the traditional practice of growing two maize crops per year, as well as improved fallow treatments, in which maize is followed in the rotation by the nitrogen-fixing tree *Tephrosia candida*. Both management treatments received 75 kg ha^{-1} phosphorus as triple super phosphate and 75 kg ha^{-1} potassium as potassium chloride. *Tephrosia* is undersown in maize during the long rainy season, grows during the short rains, and is cut and incorporated into the soil before the next year's maize crop. The loss of the short-rain maize crop is more than offset by the increase in subsequent long-rain maize yields.

At all but one site (Ugunja: two replicates), three replicates from continuous maize and maize-fallow plots (with *T. candida*) were sampled. These experiments were initiated in 1999 and, at the time of this study, improved system plots were in the first maize rotation following an 18-month fallow. The Ochinga site had the same treatments as IMPALA sites, also in a randomized complete block experiment, but the improved system had been in place for three seasons prior to sampling.

Soil Sampling. At the wooded sites at Kakamega (forest) and Ochinga (woodlot) three separate sample locations were established at each site. Within each of these locations, 20 separate sub-samples of litter and mineral soil were taken that were pooled into a composite sample per location. Tea field soils were sampled by randomly choosing three rows at least 25 m apart. For each row 20 soil cores were taken and pooled to form a single composite sample per row.

Agricultural soils, except tea, were sampled under the maize crop in late May 2001. Green biomass from fallows had been incorporated 3 months earlier in the fallow treatment plots. The long rains (May to June) began 2 weeks prior to sampling, and soils were moist. Soils from each of the three replicate plots (Ugunja; two replicate plots) were sampled by taking twenty 5-cm cores per plot with sterile core samplers (diameter 2.5 cm). These samples were then pooled into one composite sample per plot. Enzyme assays, microbial biomass, and total C and N quantification were performed on soil stored at 4°C. Subsamples from each plot were frozen and transported to UC Davis for phospholipid fatty acid (PLFA) analysis. The remainder of the soil was transported immediately to the UK where subsamples were frozen for subsequent denaturing gradient gel electrophoresis (DGGE) analysis, whereas BIOLOG assays were performed within 4 days on fresh soil.

Microbial Biomass and Total Soil C and N Analysis. Microbial biomass C and N were determined using the fumigation extraction method [50]. Briefly, 25 g of soil was fumigated with chloroform for 24 h. Following fumigation, soil microbial C and N were extracted with 100 ml 0.5 M K_2SO_4. Microbial biomass C was determined using a heated sulfuric acid–dichromate digestion, and microbial biomass N was measured in a persulfate digestion of the extracts and analyzed for total N. Total soil C and N were determined by the dry combustion method on a CHN analyzer [34].

Enzyme Assays. Five soil enzymes that are components of important nutrient cycles were chosen for

this study. They were β-glucosidase and cellobiohydrolase acid in the carbon cycle, the chitinase enzyme, D-acetylglucosaminidase, involved in both the N and C cycles, and acid and alkaline phosphatases; and orthophosphoric monoester phosphohydrolase and orthophosphoric monoester phosphohydrolase in the phosphorus cycle. Enzyme assays, modified from Tabatabai and Bremner's [47] original method, used *p*-nitrophenol (*p*NP) linked substrates and were based on the colorimetric determination of *p*NP released by each enzyme when soil is incubated with a buffered substrate solution. For each enzyme, 15 g of soil per sample was mixed with 100 mL of 0.05 M acetate buffer (pH 9.5 for alkaline phosphate; pH 5.0 for all other enzymes) in 25 mL Nalgene bottles and placed on a magnetic stirrer. Aliquots (2 mL) of slurry were pipetted from each bottle and transferred to polypropylene test tubes, which were kept chilled pending incubation. At the beginning of each incubation, 2 mL of substrate (5 mM for all substrates except chitobiose, which were prepared at 2 mM concentration) solution were added to each sample test tube. The tubes were then capped and placed on a rotary shaker for 2 h at 25°C. Following incubation, tubes were centrifuged at 3900 *g* for 5 min and 1-mL aliquots of clear supernatant were taken from each tube and transferred to 15-mL glass test tubes containing 0.2 mL of 1 N NaOH, to stop the reaction and cause color change. The solution was brought to a volume of 10 mL using deionized water. The assay mixtures were then vortexed and absorbance measured with a spectrophotometer at 410 nm. Parallel sample and substrate controls were run by adding 2 mL of acetate buffer with 2 mL of soil slurry, and 2 mL of substrate solution with 2 mL of acetate buffer, respectively. The concentration of p-NP detected in samples after incubation was corrected by subtracting the combined absorption results for the sample and substrate controls from the analytical samples.

BIOLOG and Phospholipid Fatty Acid Analysis. BIOLOG analysis (31 substrates) was carried out as described by Girvan et al. [22] using BIOLOG Eco-Plates (BIOLOG Inc.). PLFA was carried out as described by Bossio and Scow [7]. Out of a possible total of 85 fatty acids identified in any sample, analyses were based on 49 fatty acids that appeared in at least 25% of the samples.

16S rRNA Gene DGGE Analysis. Extraction, quantification, and purification of DNA were performed as described by Girvan et al. [22]. PCR and subsequent denaturing gradient gel electrophoresis (DGGE) analysis were performed on DNA using 16S rRNA gene universal bacterial DGGE primers 2 and 3 (synthesized by Invitrogen Custom Primers, Paisley, UK) from Muyzer et al. [33] as detailed in Girvan et al. [22].

Statistical Analysis. Multivariate analysis of BIOLOG, enzyme, PLFA, and DGGE profiles was done using CANOCO software [49] from Microcomputer Power (Ithaca, NY). Each data set was standardized to total activity or biomass to emphasize changes in community characteristics of that were independent of changes in biomass. Therefore, BIOLOG substrate absorbance was standardized to whole-plate color development; individual enzyme activities were represented as a percentage of the total measured activity ("specific activity") as defined by [51]; mole percent of individual fatty acids was used in the analyses of PLFA profiles; and for DGGE analysis, each ribotype (band) was identified and its intensity measured after image capture and analysis using the Phoretix ID Advanced software (Non Linear Dynamics, Newcastle UK). This band intensity was then expressed as a proportion of the total intensity of all of the bands comprising a particular community profile. The software eliminates background and automatically detects peaks when noise levels and minimum peak thresholds are set and was used as described in detail in Girvan et al. [22]. Principal component (PCA) and redundancy (RDA) analyses were used to analyze the data. RDA is an ordination technique based on PCA, in which ordination axes are constrained to be linear combinations of environmental variables [48], thus allowing direct assessment of the relationship between environmental variables and variation in the multivariate data. Environmental variables tested in this study included soil type, land use (wooded or agriculture), and management system (continuous maize or improved fallow system). The Monte Carlo permutation test [49] was used to test the statistical significance of the relationships between environmental variables and variation in BIOLOG substrate utilization, enzyme activities, and PLFA or DGGE profiles.

Shannon indices [43]

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where H′ is the value of the Shannon index, p_i is the number of individuals of species (ribotype) i, and s is the number of species (ribotype) found in the community profile, and UPGMA dendrograms were calculated from the 16S rRNA gene DGGE community profiles as described by Girvan et al. [22]. Relative comparison of diversity indices for DGGE data and also BIOLOG data were made as these indices do not represent absolute measures of diversity that are the norm in classical macroecology studies [22]. Simple linear regression and ANOVA were used to test for significant relationships between diversity indices and soil factors, and to test for significance of treatment effects on univariate measures.

Results

Soil and Microbial Community Characteristics in Relation to Soil Type and Land Use. Soil organic carbon varied according to land use, with the highest content in the forest soils and then the woodlot soils. For both forest and woodlot soils organic carbon was greater in the litter than in mineral soil (Table 2). In annually cropped fields, the organic carbon content varied from ~1% at the Teso site with the lightest texture soil, to just over 2% in heavier textured soils at Luero (Table 2). Microbial biomass was highly correlated with soil carbon ($r = 0.98$). Total enzyme activity also correlated highly with both soil organic carbon and microbial biomass carbon ($r = 0.99$ and $r = 0.95$ respectively).

DGGE analysis of 16S rRNA gene fragments (Fig. 2A) separated microbial communities by soil type, with the exception that Ochinga woodlot samples clustered with Kakamega soils, and separated wooded soils from agricultural soils along a first axis that explained 16% of variability in the profiles. Agricultural locations were more different from each other than from wooded sites. Analysis of PLFAs (Fig. 2B) showed separation of wooded from agricultural soils, based on relatively higher abundance in wooded soils of monounsaturated fatty acids (15:lω8c, 16:1ω7t, 16:lω7c, 16:lω5c, il7:1ω5c, 17:1ω9c, 18:lω6c, 18:lω9c, and 18:lω7c), a few anteiso-, iso- and hydroxy-branched fatty acids (i13:0, a13:0, i14:0, 12:02OH, 14:03OH, 16:02OH), and 18:2ω6c. Soils from forest and woodlot had relatively more anteiso- and iso-branched fatty acids (i15:0, i16:0, i17:0, a17:0, i18:0, i19:0, i20:0), and methyl-branched fatty acids (16:010Me, 17:010Me, 18:010Me) than were found in agricultural soils. Agricultural soils separated into two groups; the first included Luero, Ochinga and Kakamega tea, with higher relative abundances of branched fatty acids, and the second comprised Ugunja, Teso, and Kakamega continuous maize, with higher relative abundances of monounsaturated fatty acids.

Enzyme analysis (Fig. 2c) contrasted with DGGE and PLFA analyses in that the main separation of samples along the first axis was Kakamega maize, and also Kakamega tea and forest mineral soil from other sites. This separation was driven by the relatively higher activity of acid phosphatase, in Kakamega samples (an acidic clay soil), and relatively higher activity of β-glucosidase, chitinase, and alkaline phosphatase at the other sites (Table 3). Relationships of soil microbial communities based on BIOLOG profiles (Fig. 2D) were broadly similar to what was observed with the enzyme analyses with separation of the acidic clay agricultural samples, Kakamega maize and tea, from other samples. This was based primarily on the very high utilization of between 40 and 80 and very low utilization of β-methyl-δ-glucoside that dominated the first axis (22%) (Fig. 2D), and separation of wooded sites from agricultural fields, due to high utilization of cellulose, lactose and glycogen, along the second axis (eigenvalue 17%).

There were some differences among sites in Shannon's index based on DGGE analysis of 16S rRNA gene fragments (Table 2), most notably a significantly lower relative diversity at the Ugunja site than at the other sites. However, there were no significant differences in community diversity between wooded and agricultural sites, nor any consistent trend with respect to agricultural management treatment. Relative diversity indices generated from BIOLOG analysis showed wooded sites to have the greatest substrate diversity (Table 2). These values for the wooded sites were, however, only significantly greater than those determined for the Kakamega and Leuro continuous maize plots.

Variation between Wooded and Agricultural Soils. The land use conversion represented by forest and woodlot soils and the same soils under agricultural management at two locations (Kakamega and Ochinga) showed dramatically lower soil carbon, microbial biomass, and total enzyme activities under annual cropping (Table 2). At Kakamega and Ochinga, land covers characterized by perennial woody vegetation (forest, woodlot, tea plantation) had a higher percentage of total enzyme activity of cellulases and chitinase (Table 3). Redundancy analysis (RDA) and Monte Carlo testing were used to provide a statistical analysis of the microbial community response to changes in land usage using data from the two sites (Fig. 3). In general, community composition and activity in forest and woodlot soils were significantly different ($p < 0.01$) from agricultural soils at both sites, as determined by Monte Carlo permutation testing (data not shown). The single exception to this was that the enzyme activities of the Ochinga woodlot soil were not significantly different from those of Ochinga agricultural soil. Forest (Kakamega) and woodlot (Ochinga) soil communities were also significantly different ($p < 0.01$) from each other based on all methods, although inspection of ordination plots (Fig. 3) revealed less discrimination of forest from woodlot in PLFA and BIOLOG analysis as compared with DGGE analysis of 16S rRNA gene fragments, and greater variability among replicates in enzyme profiles.

Effects of Agricultural Management Practices. Identical replicated experiments comparing the traditional continuous maize cropping (two crops per year) with a cereal/improved fallow rotation were established on farmers' fields, on four soil types (Ochinga, Leuro, Ugunja and Teso; Table 1). Soil carbon content was increased significantly ($p < 0.001$) with improved fallow management. Similarly, microbial biomass carbon, although variable, increased significantly ($p < 0.05$) under

Table 2. Soil organic carbon, microbial biomass carbon, total enzyme activities, and Shannon diversity indices at the study sites

Site	*Soil carbon (%)*	*Microbial biomass carbon (mg kg^{-1})*	*Total ezyme activity (μmol pNP g-soil^{-1} h^{-1})*	*Shannon's diversity index (based on 16S rRNA gene DGGE)*	*Shannon's diversity index (based on BIOLOG carbon source utilization)*
Kakamega					
Primary forest litter	26.75 (3.02)a	3151 (326)a	72.05 (7.65)a	3.66 (0.10)	3.22 (0.03)a
Primary forest mineral soil	9.05 (1.39)b	1177 (74)b	18.09 (0.62)b	3.41 (0.06)	3.26 (0.01)a
Tea	2.92 (0.36)c	293 (16)c	12.43 (0.80)b	3.58 (0.02)	3.15 (0.02)a
Continuous maize	2.26 (0.05)c	373 (18)c	5.22 (0.75)b	3.64 (0.02)	2.97 (0.07)b
Ochinga					
Woodlot litter	10.49 (0.70)a	1751 (472)a	28.81 (2.57)a	3.46 (0.02)	3.29 (0.01)
Woodlot mineral soil	4.32 (0.32)b	963 (96)b	15.07 (0.35)b	3.43 (0.07)	3.25 (0.01)
Fallow	1.60 (0.05)c	200 (26)b	4.78 (0.13)c	3.65 (0.04)	3.10 (0.03)
Continuous maize	1.42 (0.06)c	112 (17)b	2.77 (0.17)c	3.53 (0.08)	3.11 (0.04)
Luero					
Improved Fallow Maize	2.25 (0.06)a	315 (53)	7.63 (0.76)a	3.35 (0.00)	3.13 (0.07)
Continuous maize	2.01 (0.06)b	228 (8)	4.21 (0.88)b	3.22 (0.05)	3.04 (0.02)
Ugunja					
Improved Fallow maize	1.49 (0.13)	207 (7)	2.16 (0.49)	2.49 (0.06)	Nd
Continuous maize	1.21 (0.04)	204 (12)	2.88 (0.24)	2.59 (0.01)	Nd
Teso					
Improved Fallow maize	1.14 (0.06)	171 (21)	3.74 (0.32)a	3.22 (0.07)	3.18 (0.08)
Continuous maize	0.91 (0.09)	141 (25)	1.89 (0.23)b	3.33 (0.01)	3.24 (0.05)

Diversity indices are based on DGGE analysis of 16S rRNA gene fragments and carbon substrate utilization profiles using BIOLOG plates. Standard errors are in parentheses, and different letters following the SE indicate significant differences within sites ($p < 0.05$, ANOVA). The absence of a letter indicates that there was no significant variation between different land uses at a particular site for the measurement considered. $N = 3$ except for the Ugunja site at which $N = 2$. ND: Not determined.

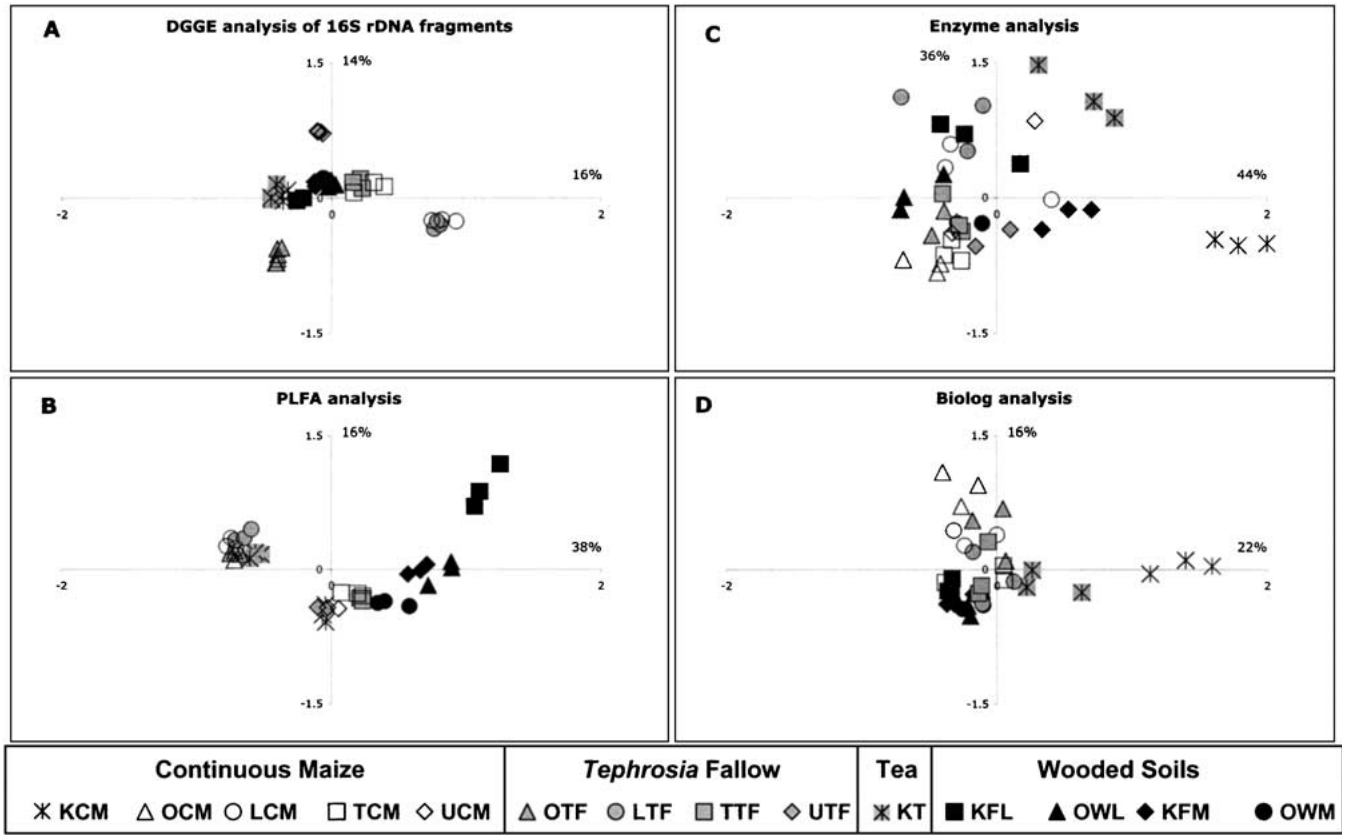

Figure 2. Principal component analysis (PCA) of data from all sample sites based on (A) DGGE analysis of 16S rRNA gene fragments; (B) PLFA profiles; (C) enzyme analysis; and (D) BIOLOG analysis. Symbols in the key correspond to specific treatments at the sampling sites with letters as follows, K: Kakamega; L: Luero; O: Ochinga; T: Teso; U: Ugunja; CM: continuous maize; TF: *Tephrosia* fallow; T: tea; FL: forest litter; WL: woodlot litter; FM: forest mineral; WM: woodlot mineral.

Table 3. Standardized enzyme activities at the study sites

Site	β-*Glucosidase*	*Cellobiohydrolase*	*Chitinase* (μmol *pN g-soil*$^{-1}$ *h*$^{-1}$)	*Acid phosphatase*	*Alkaline phosphatase*
Kakamega					
Primary forest litter	19.96 (2.15)a	4.42 (0.15)b	7.61 (1.46)a	51.01 (3.45)c	16.99 (0.91)a
Primary forest mineral soil	12.18 (1.04)a	1.12 (0.47)c	3.42 (0.31)bc	64.36 (2.74)bc	18.92 (2.75)a
Tea	13.47 (3.01)a	9.41 (0.5 l)a	5.75 (0.46)ab	69.82 (3.83)b	1.55 (1.02) b
Continuous maize	2.82 (0.74) b	−0.24 (0.43)d	1.72 (0.18)c	97.88 (4.46)a	−2.18 (3.53) b
Ochinga					
Woodlot litter	12.77 (1.58)	2.46 (0.34)a	18.31 (2.62)	45.57 (1.27)ab	20.90 (0.72)c
Woodlot mineral soil	9.57 (0.92)	1.93 (0.12)ab	12.24 (1.41)	50.06 (1.36)a	26.21 (1.03)c
Fallow	11.61 (0.28)	2.67 (0.39)a	9.42 (0.89)	43.29 (1.05)b	33.01 (1.66)b
Continuous maize	7.84 (0.15)	0.95 (0.14)b	10.14 (2.05)	38.04 (1.36)c	43.03 (2.54)a
Luero					
Fallow	17.59 (0.86)	7.64 (1.11)	12.52 (2.08)	47.95 (4.17)	14.29 (3.67)
Continuous maize	11.54 (2.05)	6.17 (0.72)	10.69 (1.15)	51.24 (5.43)	20.36 (2.09)
Ugunja					
Fallow	13.50 (3.31)	3.44 (0.82)	19.58 (1.21)	58.53 (3.31)	4.95 (12.43)
Continuous maize	8.79 (0.95)	1.71 (0.13)	9.64 (1.14)	53.26 (6.89)	26.61 (7.20)
Teso					
Fallow	11.13 (1.19)	2.50 (0.31)	11.39 (1.72)	47.31 (0.15)a	27.67 (2.88)
Continuous maize	7.96 (1.01)	2.14 (0.10)	8.62 (0.49)	43.79 (0.67)b	37.49 (0.87)

Standard errors are in parentheses, and values followed by different letters within the same site are significantly different at $p < 0.05$ (ANOVA). The absence of a letter indicates that there was no significant variation for different land uses at a particular site for the particular enzyme activity. $N = 3$ for all sites, except Ugunja at which $N = 2$.

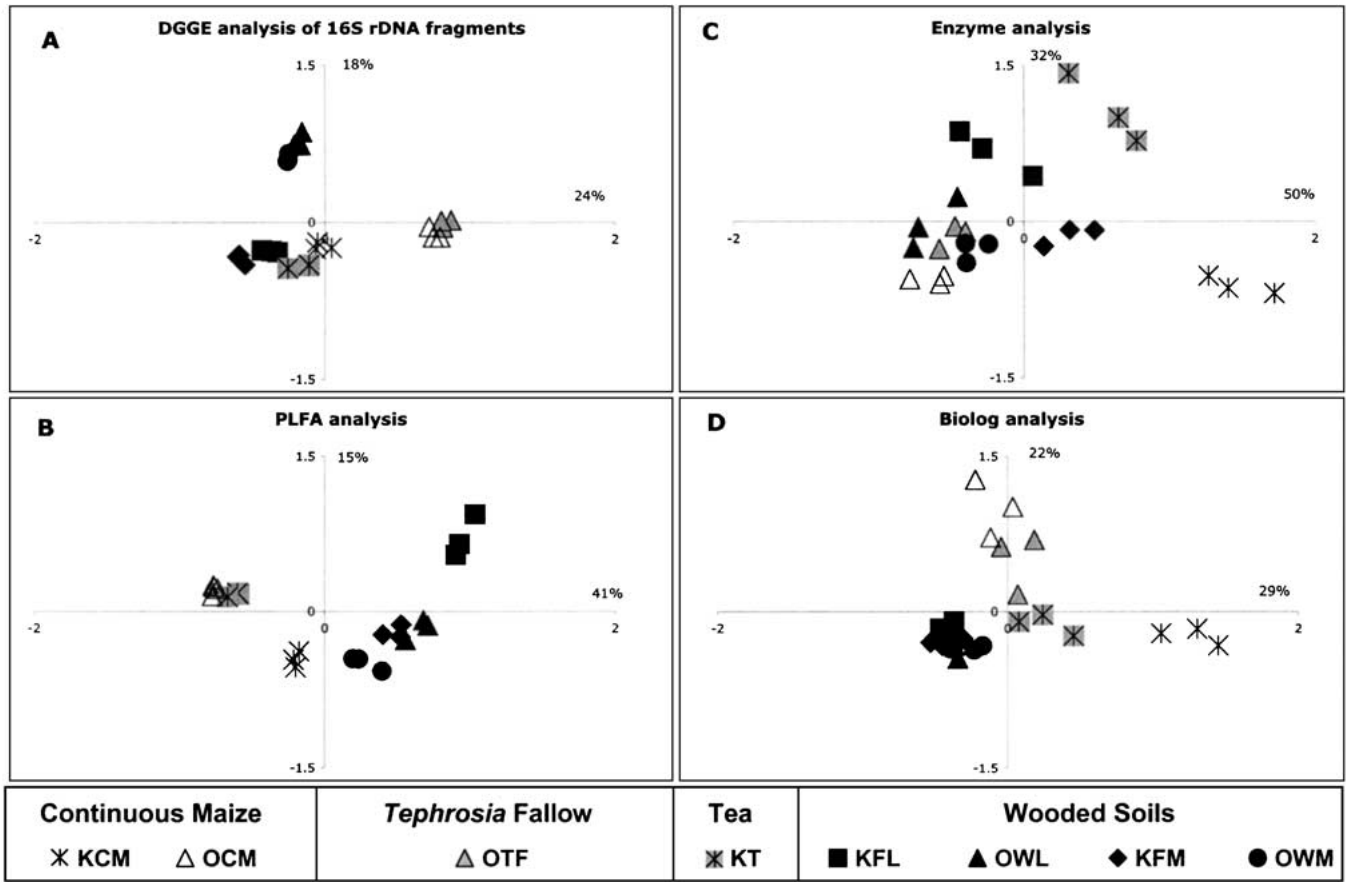

Figure 3. Principal-component analysis (PCA) of data from sites with a land use comparison between wooded areas and agricultural fields based on (A) DGGE analysis of 16S rRNA gene fragments; (B) PLFA profiles; (C) enzyme analysis; and (D) BIOLOG analysis. Symbols in the key correspond to specific treatments at the sampling sites with letters as follows, K: Kakamega; O: Ochinga; CM: continuous maize; TF: *Tephrosia* fallow; T: tea; FL: forest litter; WL: woodlot litter; FM: forest mineral; WM: woodlot mineral.

improved fallow. Total enzyme activity was significantly greater at the Luero site compared to the other three sites (Ochinga, Ugnuja, and Teso; $p = 0.0003$, Table 2). Total activity was also significantly greater in the cereal-fallow rotations compared to the continuous maize cropping system ($p = 0.0010$), except for Ugunja at which there was no significant difference (Table 2). Comparisons of the standardized activities of individual enzymes between the four sites showed a trend where Luero had significantly higher activities for the two cellulases, β-glucosidase and cellobiohydrolase (Table 3). Across all four sites, standardized activities of β-glucosidase, cellobiohydrolase, and chitinase were greater in the fallow–cereal rotations than in the continuous maize plots ($p = 0.0004$, 0.0057, and 0.0212, respectively). There was no difference in the four sites between cropping systems for the standardized activity of the acid phosphatase ($p = 0.3029$), but significant differences were found for the alkaline phosphatase ($p = 0.0012$), where standardized activities were higher under continuous maize cropping.

The importance of soil type and management as determinants of soil microbial communities was examined using RDA and Monte Carlo testing. DGGE analysis of 16S rRNA gene fragments showed significant separation of the communities by soil type ($p < 0.001$) that dominated the ordination (Fig. 4); a significant effect of management systems ($p < 0.01$) was also measured. These two variables explained 47% of the variation in the 16S rRNA gene DGGE data on the first two ordination axes. Soil type and management explained 69% of the variation in PLFA profile (Fig. 4B) with strong separation of the soils into two groups, namely Ochinga and Leuro, versus Ugunja and Teso, mainly along the first axis. The different management systems within soil type were separated along the second axis. Both soil type and management were highly significant ($p < 0.001$). Separation along the first axis was based on higher relative abundance of anteiso-, iso-, methyl- and hydroxy-branched and saturated fatty acids (i15:0, a15:0, 15:0, i16:0, 16:0, i17:0, a17:0, 17:0, 18:0, 12:02OH, 14:03OH, 16:02OH, 16:010Me, 17:010Me, 18:010Me) to the left, and higher monounsaturated fatty acids (16:1ω7c, 16:lω5c, i17:lω5c, 17:lω9c, 18:lω9c, and 18:lω7c) on the right. In contrast to the compositional measures (DGGE

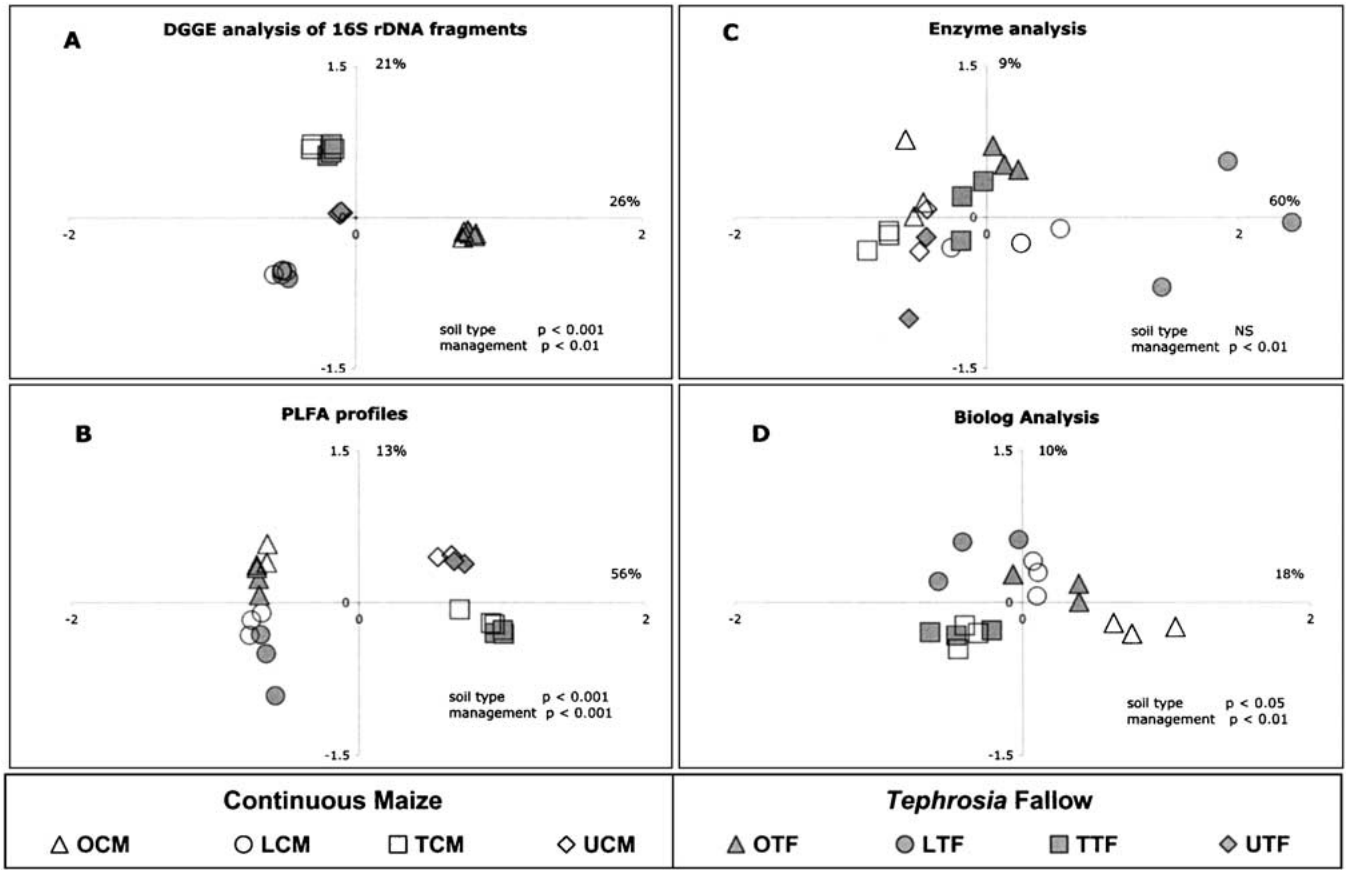

Figure 4. Redundancy analysis (RDA) of data from the four soil types with an agricultural management comparison based on (A) DGGE analysis of 16S rRNA gene fragments; (B) PLFA profiles; (C) enzyme analysis; and (D) BIOLOG analysis. Soil type and management system were included as constraining variables in the RDA and significance testing (p values on figure) of those variables was done using Monte Carlo permutation analysis. Symbols in the key correspond to specific treatments at the sampling sites with letters as follows, L: Luero; O: Ochinga; T: Teso; U: Ugunja; CM: continuous maize; TF: *Tephrosia* fallow.

and PLFA), soil management had a highly significant effect on some enzyme activities ($p < 0.001$), but soil type, with the exception of Luero, was not significant (Fig. 4C). Separation based on management was due to higher relative activity of the carbon cycling enzymes, β-glucosidase and cellobiohydrolase, and lower relative activity of alkaline phosphatase in improved fallow plots. The first two ordination axes captured 69% of variability. For BIOLOG profiles, both soil type and soil management variables (Fig. 4D) were significant ($p < 0.01$), and with consistent separation of fallow and continuous maize fields for most locations. The first two ordination axes constrained by these variables accounted for 28% of total variability.

The data from DGGE analysis of 16S rRNA gene fragments were also analyzed by direct similarity comparison using the Dice/Sorensen index (Fig. 5). Discrete reproducible differences in the community composition due to land management were evident. As with RDA, agricultural sites clustered separately from their wooded counterpoints, and further separation within the agricultural sites due to management was also observed. Sites cropping continuous maize clustered separately from the maize intercropped with *T. Candida* at all sites.

Discussion

All four multivariate methods were highly sensitive to differences in the microbial community. DGGE analyses of 16S rRNA gene fragments provided a measure of the composition and structure of the dominant species within the bacterial community and produced highly reproducible, site-specific profiles. These profiles primarily varied with regard to soil site, for which each of the five sites had different soil types and/or pH (Table 1). These results were consistent with the findings of several other studies on temperate agricultural soils that found soil type to be the primary determinant of bacterial community structure [10, 19, 22]. Smaller secondary influences were also found due to differing management practices. In this study, PLFA profiles were found to be primarily sensitive to land use conversion, but they also

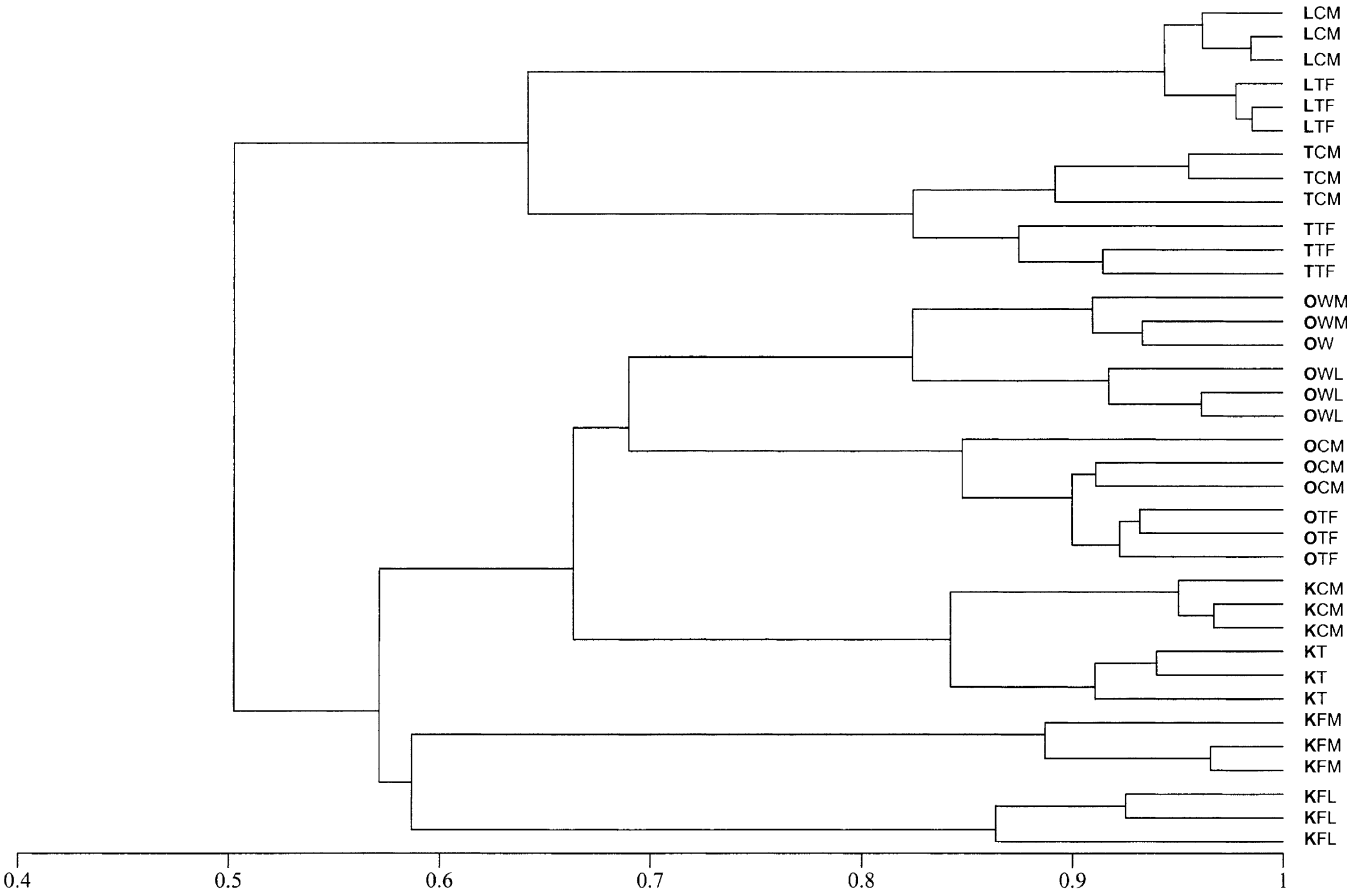

Figure 5. UPGMA dendrogram constructed from the similarity matching data (Dice-Sorensen index) produced from the DGGE profiles of 16S rRNA genes amplified from soil and generated by using MVSP version 3.12h. The scale bar represents percent similarity. The letters represent the sites and treatments as follows, K: Kakamega; L: Luero; O: Ochinga; T: Teso; CM: continuous maize; TF: *Tephrosia* fallow; T: tea; FL: forest litter; WL: woodlot litter; FM: forest mineral; WM: woodlot mineral.

significantly separated soil types and soil management effects on communities. Forest and woodlot sample profiles were indicative of a higher relative abundance of Gram-negative bacteria, associated with monounsaturated fatty acids [53], that grow rapidly and make use of a variety of different carbon sources, together with greater fungal abundance (18:2ω6c) [15]. Conversely, agricultural soil profiles indicated higher levels of actinomycetes (associated with methyl-branched fatty acids) [28] and Gram-positive bacteria (associated with branched fatty acids) [24]. Separation of agricultural soils into two groups based on greater relative abundance of anaerobic organisms (branched fatty acids) [24] indicated higher soil moisture in the heavier textured soils at Ochinga and Luero. These results are consistent with other studies in which PLFA analysis has been demonstrated to be highly sensitive to both environmental and management effects on soil microbial communities [3, 8, 30, 38, 52], and carbon and water availability were found to explain large portions of the variability in PLFA profiles [7].

Enzyme activity and BIOLOG substrate utilization analyses that provide indications of biological function produced profiles that were less specific with regard to soil type than resulted from 16S rRNA gene DGGE and PLFA approaches. Different soil types have specific microhabitats due to widely ranging abiotic factors. This encourages differences revealed by DGGE analysis, which identifies the most dominant ribotypes (i.e., on the basis of 16S rRNA gene sequences) within a community. However, although bacteria may be separated on a 16S RNA gene DGGE profile because of small genetic variations, it is still possible for these bacteria to maintain a similar function within the community. Conversely, similar substrate utilization profiles may be generated by BIOLOG analysis of soil samples but may include genetically distinct organisms. It is this functional redundancy among soil bacterial communities that promotes soil stability and protects soil processes from species loss [18, 25]. Therefore, overall functional capacity need not be determined by soil type [2, 29]. The soils from wooded sites showed the greatest substrate

diversity, significantly greater than those from the Kakamega and Luero continuous maize sites. Overall, however, functional diversity was generally high in all soils, despite large differences in community structure, a result that supported the functional redundancy theory. Relative functional parameters, including specific enzyme activity and standardized substrate utilization, appeared to be predominantly affected by soil chemical properties, rather than carbon and water availability, as suggested by separation of acid soil samples from other soils along the first axis. Although results indicate overlaps in functional capacity between bacteria, it should also be considered that greater variability with respect to site-specific clustering the basis of functional indicators might also be a consequence of analyzing a smaller number of variables, i.e., five enzymes and 31 BIOLOG substrates (functional variables), as opposed to 49 PLFAs and 102 DGGE bands (community structure indicators).

Dramatic difference is the below-ground environment in terms of carbon content were accompanied by decreased microbial biomass and changes in microbial community structure and function in the wooded soils, in agreement with earlier research [51]. One anomalous result, however, was that although overall enzyme activity was much lower in tilled fields than the 20-year-old woodlot at the Ochinga site (Table 3), the specific activity of each enzyme was similar, regardless of land use (Fig. 3C), suggesting that these functions were conserved in both the woodlot and agricultural soil at the Ochinga site. Woodlot communities were significantly different from primary forest according to all methods (Fig. 3), although measures of the metabolically active component of the communities (PLFA, enzymes, and BIOLOG) displayed lower specificity with respect to site than was found by 16S rRNA gene DGGE analysis indicative of the total communities. Mineral soil samples from both the Kakamega forest and Ochinga woodlot had similar PLFA, enzyme and BIOLOG 22 profiles.

Analysis of 16S rRNA genes has been proposed to represent a more historical than immediate description of the microbial community [16]. However, a notable finding of this current research was that DGGE analysis of amplified 16S rRNA gene fragments revealed differences in the microbial community structure in relation to differing soil management practices (Fig. 4 and 5). Nusslein and Tiedje [35] had previously identified large vegetation-induced changes in the soil microbial structure in a tropical Hawaiian soil, as inferred from differing G+C compositions, whereas deforestation in the Eastern Amazon was accompanied by significant change in microbial populations based on rRNA intergenic spacer analysis [6]. However, in general, the similarity of bulk community composition, measured by 16S rRNA gene analysis, across landscapes with similar soil types, despite differences in management, is more commonly observed [22, 32]. This suggests that changes in the composition of microbial communities that were attributable to agricultural management in this present study were more pronounced than found in many previous studies. Nusslein and Tiedje [35] suggested that the differences in the Hawaiian soil were due to the young age of the soil, and that young soils are more susceptible to change than well-established soils. The consistency of results across the four soil types in our study (Fig. 4) could be interpreted to indicate that management more rapidly affects the composition of microbial communities in tropical environments than those from temperate ecosystems. Moreover, PLFA analysis was found to strongly discriminate between differing management practices (Fig. 4), providing additional evidence that deliberate management of soils will have considerable impact on microbial community structure in tropical soils.

Conclusions

The polyphasic methodology used in this research provides considerable advantages over conventional measures for analysis of the soil microbial community, offering two key opportunities. First, the approach enhances our ability to determine rates of change in the total and specific functional components of microbial communities. Second, it facilitates an improved understanding of the environmental conditions that result in changes to soil properties and their indigenous microbiota. These data indicate that the functional and compositional measures used in this study are sufficiently sensitive to permit investigation of management-induced impacts upon microbial community structure and function.

In this study, 16S rRNA gene analysis showed soil type to be the primary determinant of the total bacterial community in these tropical soils. 16S rRNA gene and PLFA analyses revealed additional specific differences between wooded and agricultural soils. Functional indicators (BIOLOG and enzyme activities) showed less specificity with respect to soil type, and greater variability overall than DNA- and PLFA-based measures. In replicated field experiments comparing traditional continuous maize cropping with an improved N-fixing fallow system in which maize yields are increased, 16S rRNA gene and PLFA analyses revealed distinct separation of microbial communities between treatments, although this separation was not necessarily associated with increases in microbial diversity. Microbial biomass and enzyme activities were generally found to increase in soils using the cereal fallow rotation. Thus, management practices were found to affect both microbial community composition and function. Future research will focus on determining whether such changes are correlated with

improvements in soil health, and on identifying key functional microbial components that respond to differing management practices.

Acknowledgments

Drs. Bossio and Girvan contributed equally to this work. The authors thank Margaret Edwards, James Rowe, Moses Mathuva, and Edith Anyango for their contributions to this research. We are grateful for the financial support of this research by the Rockefeller Foundation (Grant Number 2001-FS-089), the IMPALA project funded by the European Union (Grant Number ICAS4-CT-2000-30011), the Government of Italy, Syngenta, and Unilever.

References

1. Albrecht, A (2002) Improved fallow by legume plants (trees, shrubs and grasses) in Eastern and Southern Africa. Impact of soil biota (SOM, roots, BNF microbes, mycorrhiza, and soil fauna), improvement on bean and maize yields, soil organic matter dynamics and soil conservation. First Annual Report. IMPALA project, INCO-DEV (1998–2002)
2. Andrén, O, Bengtsson, J, Clarholm, M (1995) "Biodiversity and species redundancy among litter decomposers." In: Collins, HP, Robertson, GP, Klug, MJ (Eds.) The Significance and Relation of Soil Biodiversity, Kluwer, Dordrecht, pp 141–151
3. Bååth, E, Frostegård, A, Pennanen, T, Fritze, H (1995) Microbial community structure and pH response in relation to soil organic matter quality in wood-ash fertilized, clear-cut or burned coniferous forest soils. Soil Biol Biochem 2: 229–240
4. Beare, MH, Tian, G, Reddy, UM, Srivastava, SC (1997) Agricultural intensification, soil biodiversity and agroecosystems function: the role of decomposer biota. Appl Soil Ecol 6: 87–108
5. Black, H, Okwakol, M (1997) Agricultural intensification, soil biodiversity and agroecosystems function: the role of termites. Appl Soil Ecol 6: 37–53
6. Borneman, J, Triplett, EW (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
7. Bossio, DA, Scow, KM (1998) Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microb Ecol 35: 265–278
8. Bossio, DA, Scow, KM, Gunapala, N, Graham, KJ (1998) Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microb Ecol 36: 1–12
9. Brussaard, L, Hauser, S, Tian, G (1993) "Soil faunal activity in relation to the sustainability of agricultural systems in the humid tropics." In: Soil Organic Matter Dynamics and the Sustainability of Tropical Agriculture. Proceedings of an International Symposium, Leuven, Belgium, 4–6 November 1991, pp 241–256
10. Buckley, DH, Schmidt, TM (2001) The structure of microbial communities in soil and the lasting impact of cultivation. Microb Ecol 42: 11–21
11. Clegg, CD, Ritz, K, Griffiths, BS (1998) Broad-scale analysis of soil microbial community DNA from upland grasslands. Anton Leeuwen Int J Gen Mol Microbiol 73: 9–14
12. Clegg, CD, Ritz, K, Griffiths, BS (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
13. Davidson, EA, Ackerman, IL (1993) Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochem 20: 161–193
14. Doran, JW, Zeiss, MR (2000) Soil health and sustainability: managing the biotic component of soil quality. Appl Soil Ecol 15: 3–11
15. Federle, TW (1986) "Microbial distribution in soil—new techniques." In: Megusar, T, Ganthar, M (Eds.) Perspectives in Microbial Ecology, Ljubljana, pp 493–498
16. Felske, A, Wolterink, A, van Lis, R, Akkermans, ADL (1998) Phylogeny of the main bacterial 16S rRNA sequences in Drentse A grassland soils (The Netherlands). Appl Environ Microbiol 64: 871–879
17. Fragoso, C, Brown, G, Parton, C, Blanchartg, E, Lavelle, P, Pashanasi, B, Senapati, B (1997) Agricultural intensification, soil biodiversity and agroecosystems function: the role of earthworms. Appl Soil Ecol 6: 17–35
18. Fredrickson, JK, Balkwill, DL, Zachara, JM, Li, SMW, Brockman, FJ, Simmons, MA (1991) Physiological diversity and distributions of heterotrophic bacteria in deep Cretaceous sediments of the Atlantic coastal plain. Appl Environ Microbiol 57: 402–411
19. Gelsomino, A, Keijzer-Wolters, AC, Cacco, G, van Elsas, JD (1999) Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis electrophoresis. J Microbiol Methods 38: l–15
20. Giller, KE, Beare, MH, Lavelle, P, Izac, AMN, Swift, MJ (1997) Agricultural intensification, soil biodiversity and agroecosystem function. Appl Soil Ecol 6: 3–16
21. Giller, KE (2001) Nitrogen Fixation in Tropical Cropping Systems, 2nd ed. CAB International, Wallingford, UK
22. Girvan, MS, Bullimore, J, Pretty, JN, Osborn, AM, Ball, AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol 69: 1800–1809
23. Gomes, NCM, Fagbola, O, Costa, R, Rumjanek, NG, Buchner, A, Mendona-Hagler, L, Smalla, K (2003) Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Appl Environ Microbiol 69: 3758–3766
24. Haack, SK, Garchow, H, Odelson, DA, Forney, LJ, Klug, MJ (1994) Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Appl Environ Microbio 60: 2483–2493
25. Heneghan, L, Coleman, DC, Crossley, DA, Zou, X (1999) Nitrogen dynamics in decomposing chestnut oak (*Quercus pinus L.*) in mesic temperate and tropical forest. Appl Soil Ecol 13: 169–175
26. Jimenez, JJ, Moreno, AG, Lavelle, P, Decaens, T, Angel, AF, Thomas, RJ (1996) "Ecology and biology of a new tropical earthworm *Martiodrilus carimaguensis* (Oligochaeta, Glossoscolecidae)." In: Swift, MJ (Ed.) The Biology and Fertility of Tropical Soils, Report of the Tropical Soil Biology and Fertility Programme, TSBF, pp 15
27. Kahindi, JHP, Woomer, P, George, T, Moreira, FMD, Karanja, NK, Giller, KE (1997) Agricultural intensification, soil biodiversity and ecosystem function in the tropics: the role of nitrogen-fixing bacteria. Appl Soil Ecol 6: 55–76
28. Kroppenstedt, Rm (1985) "Fatty acid and menaquinone analysis of actinomycetes and related organisms." In: Goodfellow, M, Minnikin, DE (Eds.) Chemical Methods in Bacterial Systematics, Academic Press, London, pp 173–199
29. Lawton, JH, Brown, VK (1993) "Redundancy in ecosystem." In: Schultz, ED, Moonet, HA (Eds.) Biodiversity and Ecosystem Function, Springer, Berlin
30. Lundquist, E, Scow, K, Jackson, L, Uesugi, S, Johnson, C (1999) Rapid response of soil microbial communities from conventional,

low input, and organic farming systems to a wet/dry cycle. Soil Biol Biochem 31: 1661–1675
31. Mafongoya, PL, Mpepereki, S, Dzowela, E, Mangwayana, E, Makonese, F (1996) ''Soil biota: effect of pruning quality and the method of pruning placement on soil microbial composition.'' In: Swift, MJ (Ed.) The Biology and Fertility of Tropical Soils, Report of the Tropical Soil Biology and Fertility Programme, TSBF, pp 31–32
32. McCaig, AE, Glover, LA, Prosser, JI (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
33. Muyzer, G, de Waal, EC, Uitterlinden, AG (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
34. Nelson, DW, Sommers, LE (1994) ''Total carbon, organic carbon, and organic matter.'' In: Weaver, RW, Angle, S, Bottomley, P, Bezdicek, D, Smith, S, Tbatai, A, Wollum, A (Eds.) Methods of Soil Analysis: Part 2. Microbial and Biochemical Properties, Soil Science Society of America, Madison, WI, pp 539–579
35. Nusslein, K, Tiedje, JM (1999) Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. Appl Environ Microbiol 65: 3622–3626
36. Oldeman, LR (1994) ''The global extent of soil degradation.'' In: Greenland, DJ, Szabolcs, I (Eds.) Soil Resilience and Sustainable Land Use, CAB International, Wallingford, UK, pp 99–118
37. Palm, CA, Swift, MJ, Woomer, PL (1996) Soil biological dynamics in slash-and-burn agriculture. Agr Ecosys Environ 58: 61–74
38. Ponder, F, Tadros, M (2002) Phospholipid fatty acids in forest soil four years after organic matter removal and soil compaction. Appl Soil Ecol 19: 173–182
39. Pretty, J (2002) Agri-Culture. Earthscan, London
40. Qafoku, NP, Van Ranst, E, Noble, A, Baert, G (2003) ''Mineralogy and chemistry of variable charge soils.'' In: Lal, R (Ed.) Encyclopedia of Soil Science, Marcel Dekker, New York
41. Salamanca, EF, Raubuch, M, Joergensen, RG (2002) Relationships between soil microbial indices in secondary tropical forest soils. Appl Soil Ecol 21: 211–219
42. Sanchez, PA, Palm, CA, Szott, LT, Cuevas, E, Lal, R (1989) ''Organic input management in tropical agroecosystems.'' In: Coleman, DC, Oades, JM, Uehara, G (Eds.) Dynamics of Soil Organic Matter in Tropical Ecosystems, NifTAL Project, University of Hawaii, Honolulu, HI, pp 125–152
43. Shannon, CE, Weaver, W (1949) The Mathematical Theory of Communication. University of Dlinois Press, Champaign, IL
44. Srivastava, SC (1996) ''Microbial biomass and activity in dryland agriculture.'' In: Swift, MJ (Ed.) The Biology and Fertility of Tropical Soils, Report of the Tropical Soil Biology and Fertility Programme, TSBF, pp 35
45. Swift, MJ, Andren, O, Brussaard, L, Briones, M, Couteaux, M-M, Ekschmitt, K, Kjoller, A, Loiseau, P, Smith, P (1998) Global change, soil biodiversity, and nitrogen cycling in terrestrial ecosystems: three case studies. Global Change Biol 4: 729–743
46. Swift, MJ, Mafongoya, P, Ramakrishnan, PS (1998) ''Soil biodiversity: an essential foundation for sustainable soil fertility.'' In: Chopra, VL, Single, RB, Varma, A (Eds.) Crop Productivity and Sustainability: Shaping the Future. Proceedings of the 2nd International Crop Science Congress, New Delhi, IBH Publishers, New Delhi, pp 321–333
47. Tabatabai, MA, Bremner, JM (1969) Use of *p*-nitrophenol phosphate in assay of soil phosphatase activity. Soil Biol Biochem 1: 301–307
48. ter Braak, CJF (1987) Data Analysis in Community and Landscape. Ecology Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands
49. ter Braak, CJF (1990) CANOCO: A Fortranrogram for Canonical Community Ordination; Update Notes. Agricultural Mathematics Group, Wageningen, The Netherlands
50. Voroney, RP, Winter, JP, Bayaert, RP (1993) ''Soil microbial biomass C and N.'' In: Carter, MP (Ed.) Soil Sampling and Methods of Analysis, Lewis Publishers, London, pp 277–286
51. Waldrop, MP, Balser, TC, Firestone, MK (2000) Linking microbial community composition to function in a tropical soil. Soil Biol Biochem 32: 1837–1846
52. Zelles, L, Bai, QY, Beck, T, Beese, F (1992) Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biol Biochem 24: 317–323
53. Zelles, L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol Fertil Soils 29: 111–129