

COMPOSITION OF THE SOIL MICROBIAL COMMUNITY UNDER SUGARCANE PRODUCTION AS INDICATED BY PHOSPHOLIPID FATTY ACID ANALYSIS

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Abstract

Phospholipid fatty acid (PLFA) analysis was used to characterise the microbial communities in the soil under long-term pre-harvest burning of sugarcane and several other land uses, and on a long-term experiment comparing sugarcane burnt before harvest and sugarcane harvested green with retention of a trash blanket. Trends in total PLFAs (total microbial biomass) followed the order: kikuyu pasture > native grassland > ryegrass pasture > maize > burnt sugarcane, and were higher under trashed than burnt sugarcane. Principal component analysis (PCA) of PLFA data revealed considerable differences with respect to PLFA composition in response to land use and trash management, suggesting substantial differences in microbial community structure. In both the comparison of land uses and the trash management trial, soil organic C content was significantly correlated with PC1, suggesting that changes in soil organic matter content can greatly affect soil microbial diversity. Among the land uses, burnt sugarcane had the lowest values for PLFA richness and Shannon's diversity index (indicators of microbial diversity) and conversion to green cane harvesting resulted in an increase in these values. The ratio of 18:2T6 fungal fatty acid: bacterial fatty acids was highest under improved pastures. The ratio of ester-linked monosaturated fatty acids : ester-linked saturated fatty acids was increased in land uses with a high organic matter content, suggesting an increase in the ratio of Gram-negative : Gram-positive bacteria in response to greater substrate availability. It was concluded that sugarcane production under pre-harvest burning is particularly detrimental to the structural diversity of soil microbial communities and that the conversion to green cane harvesting with trash retention increases soil microbial diversity.

Keywords: sugarcane, soil microbial content, phospholipid fatty acids

Introduction

A progressive loss of soil organic matter, microbial biomass and microbial activity is a major factor leading to soil degradation under pre-harvest burnt sugarcane (Dominy and Haynes, 2002; Dominy *et al.*, 2002). Conversion from pre-harvest burning to green cane harvesting with retention of a trash blanket has been shown to increase soil organic matter content and the size and activity of the soil microbial community (Graham *et al.*, 2002a,b).

Measuring the total microbial community size and activity focuses on the community as a whole, and questions remain as to how soil management and land use affect the structure and composition of the community. Indeed, it has been suggested that microbial community structure may be a more sensitive bioindicator of soil quality and function than general microbial processes or community size and activity (White and MacNaughton, 1997). The composition of the soil microbial community under sugarcane production, and the effects of

management practices (e.g. burning versus trash retention) on community structure are, as yet, unstudied.

Phospholipid fatty acid (PLFA) analysis is a relatively new biochemical procedure which is useful for evaluating microbial community structure. PLFAs are essential components of the cellular membranes of microflora, and the chemical composition of PLFAs differs in different organisms. PLFAs can be extracted from the soil and their composition then analysed by gas chromatography. Changes in PLFA profiles are indicative of changes in the overall structure of microbial communities, and signature PLFAs can provide information on specific groups of micro-organisms present in the community (Zelles, 1999). PLFA analysis offers an advantage over isolation-based techniques because it avoids the selectivity inherent in the isolation and culture of micro-organisms (White and MacNaughton, 1997).

The purpose of this study was to use PLFA analysis to assess the impacts of sugarcane production compared with other common agricultural land uses and also to compare the long-term effects of green cane harvesting versus pre-harvest burning on the structure of soil microbial communities.

Materials and methods

Experiment sites

For the comparison of land uses, sites previously described by Dominy and Haynes (2002) were used. They were on Baynesfield Estate (27° 22' S and 30° 45' E), and cropping histories of fields were: >50 years permanent kikuyu grass (*Pennisetum clandestinum*) pasture, >50 years annual ryegrass (*Lolium multiflorum*) pasture, >30 years continuous, pre-harvest burnt sugarcane (*Saccharum* spp.), >30 years continuous maize (*Zea mays*) under conventional tillage, and undisturbed native grassland. Annual rainfall at the site is approximately 844 mm. The soil is classified as the Hutton form (Farmingham series) (Soil Classification Working Group, 1991) or as a Rhodic Ferisol (FAO).

For the comparison of trash management, an experiment site situated at the South African Sugar Association Experiment Station at Mount Edgecombe (31° 04' S and 29° 43' E) was used. The site, BT1, previously described by Graham *et al.* (2002a), was established in 1939. Experiment treatments are (i) green cane harvested with retention of a trash blanket (100% cover), (ii) burnt with harvest residues (i.e. unburnt tops) left scattered on plots (67% cover) and (iii) burnt with tops raked off. The treatments are either (a) unfertilised or (b) fertilised annually with 140 kg N/ha, 20 kg P/ha and 140 kg K/ha. The experiment is replicated four times in a randomised split plot design with trash management treatments as main plots. Annual rainfall at the site is approximately 950 mm. The soil is classified as the Arcadia form, Lonehill family (Soil Classification Working Group, 1991) or as a Chromic Vertisol (FAO).

Site sampling

For the different land uses, soil samples were taken from three separate randomly chosen plots (approximately 60 m²) within each field. Twenty-five samples (0-5 cm) were sampled randomly from within each plot and bulked. Soil samples were taken randomly from within the interrow areas of maize and sugarcane plots, and randomly over the entire area of the pasture plots.

Three replicates of the long-term trash management trial were sampled in April 1999, 60 years after the experiment was initiated. Only the green cane harvested and burnt with tops raked off treatments were sampled. Plots were sampled randomly over the whole area

(0-5 cm) and 25 samples from each plot were bulked. Sites between the experimental blocks that have been under grass for the duration of the experiment (no fertiliser applied) were also sampled. These sites were considered to be as close to the condition of undisturbed grassy vegetation as it is possible to find in the vicinity of the experiment.

Within 48 hours of collection, bulk field-moist samples were thoroughly mixed and sieved (<2 mm). One subsample was stored at 2°C prior to microbial analysis, while the other was air-dried.

Soil analysis

Organic C was analysed on ground soil by the Walkley and Black dichromate oxidation method (Blakemore *et al.*, 1972). Soil pH was measured in a 1:2.5 soil : water slurry using a glass electrode. Exchangeable cations were extracted with 1M ammonium acetate (1:4 soil : extractant ratio) for two hours and Ca, Mg, K and Na were measured by atomic absorption spectrophotometry. Available P was extracted with Truog reagent. Bulk density was measured in quadruplet in the 0-5 cm layer for each plot using the core method.

Microbial biomass C was estimated by the fumigation-extraction method based on the difference between C extracted with 0.5M K₂SO₄ from chloroform-fumigated and unfumigated soil samples using a K_c factor of 0.38 (Vance *et al.*, 1987). Soluble C in the K₂SO₄ extracts was analysed using a Shimadzu 5000A soluble C analyser.

Phospholipid fatty acid analysis

Analysis of PLFAs was performed as described by Zelles (1999) and Steinberger *et al.* (1999). In short, a sample of approximately 100 g of field-moist soil was extracted in a one-phase mixture of chloroform, methanol and phosphate buffer solution. The total lipids contained in the chloroform phase were fractionated into neutral lipids, glycolipids and phospholipids on a silica-bonded phase column (SPE-SI Analytichem International, California) by eluting them sequentially with chloroform, acetone and methanol respectively. The phospholipid fraction was subjected to mild alkaline hydrolysis and the products were separated on an aminopropyl solid-phase column (SPE-NH₂). Unsubstituted fatty acids were further separated by silver-ion chromatography while unsaponifiable lipids were hydrolysed and separated on a SPE-NH₂ column. The fatty acids were subjected to methanolysis to synthesize fatty acid methyl esters (FAMES). Analysis of FAMES was performed by gas chromatography using a Hewlett-Packard 5971A MSD, combined with a 5890 series II GC system. GC operation was conducted as described by Steinberger *et al.* (1999).

Fatty acid and biomarkers

Fatty acid nomenclature and PLFA signatures followed those of Zelles (1999). Total PLFAs were taken to represent total microbial biomass. Total PLFAs are composed of the ester-linked (EL-PLFA) and non-ester-linked PLFAs (NEL-PLFA). The main EL-PLFAs are subdivided into saturated (EL-SATFA), monosaturated (EL-MUFA) and polyunsaturated (EL-PUFA) fatty acids. Common PLFA biomarkers used were for Gram-negative bacteria (EL-MUFAs), Gram-positive bacteria (branched chain (BRANC) EL-SATFA), actinomycetes (methyl branching on the 10th C atom (10ME) of EL-SATFA) and fungi (linoleic acid (18:2T6)). The fungal : bacterial ratio was calculated as 18:2T6 : bacterial PLFAs as outlined by Bardgett and McAlister (1999).

Statistical analysis

Soil analysis data and concentrations of total and signature PLFAs were analysed by Analysis of Variance using the Genstat 5 package. Least significant differences were calculated at the 5% level.

To demonstrate the similarities and differences in the PLFA profiles due to land use and management, concentrations of all the fatty acids were subjected to principal component analysis (PCA) using CANOCO software (Microcomputer Power, Ithica, NY) Using this software, redundancy analysis (RDA) was employed to correlate variation in PLFAs with selected soil chemical properties (organic C, pH, exchangeable Ca, Mg, K and Na and extractable P) and the significance of these relationships was tested using the Monte Carlo permutation test.

The PLFA data was analysed according to Zak *et al.* (1994) to give PLFA richness (the number of PLFAs identified), PLFA evenness (a measure of the equitability of the distribution of PLFAs) and Shannon's diversity index (a composite measure of richness and evenness).

Results and discussion

Soil properties

Some chemical properties and bulk density of the experiment site soils are shown in Table 1. For the different land uses, organic C content was greatest under kikuyu pasture and least under sugarcane and maize. High bulk density values suggested there was some compaction of the topsoil under maize, sugarcane and annual ryegrass.

For the trash management trial, organic C was greater under trashing than burning. Soil pH and exchangeable Ca and Mg were less in fertilised than unfertilised plots, while the reverse was the case for exchangeable K and extractable P. Bulk density was less under trashed than burnt sugarcane.

Total PLFAs

Total concentrations of PLFAs extracted from the study soils are shown in Figure 1. Concentrations were closely correlated with those for microbial biomass C ($r=0.87$, $P\leq 0.001$) and the two parameters showed similar trends with land use and trash management. Thus, total PLFA content is a good measure of microbial biomass (Zelles *et al.*, 1997). A supply of organic substrate is frequently a limiting factor for growth of heterotrophic soil micro-organisms, and the size of the microbial biomass generally increases as soil organic matter content increases. Thus, the large PLFA content under kikuyu pasture and native grassland is the result of the large organic matter content arising mainly from turnover of the large, ramified root system of grasses (Haynes and Beare, 1996). For ryegrass pasture, the large organic matter returns are balanced by the degrading effect of annual tillage and, as a result, organic matter and total PLFA content was lower than that under native grassland.

Organic C, microbial biomass C and total PLFA content were lowest under sugarcane and maize. In addition to tillage-inducing organic matter breakdown, both these crops are reasonably widely spaced, so below-ground organic matter inputs are low, while much of the above-ground dry matter is harvested and removed. Although sugarcane fields are not tilled each year, organic matter inputs to the interrow area under pre-harvest burning are minimal. The lower PLFA content under pre-harvest burnt sugarcane than maize may be because the interrow under sugarcane is effectively fallow, while annual organic matter inputs from the maize crop are distributed throughout the field because it is resown in a different position each year.

When sugarcane is harvested green, the interrow area is covered with decaying trash. The increased organic matter inputs result in an increased soil organic matter content and a large microbial biomass content (and total PLFA content) (Figure 1). The moister, more fertile

conditions below the trash mulch and the greater crop root growth in the surface soil below the mulch also help stimulate microbial growth and activity.

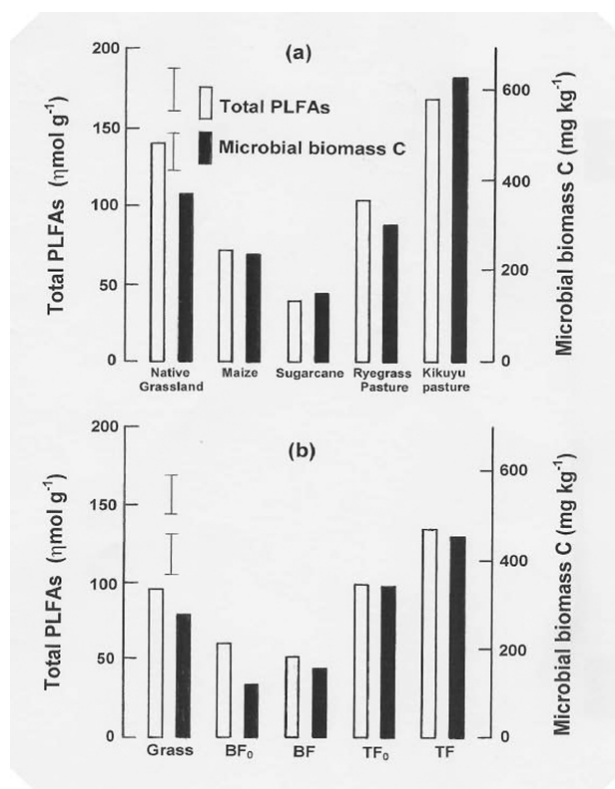


Figure 1. Total phospholipid fatty acid (PLFA) concentrations and microbial biomass C concentrations in soils under (a) five long-term land uses and (b) a long-term sugarcane trash management experiment. Grass = unfertilised grass, B = pre-harvest burnt, T = green cane harvested with retention of a trash mulch, F₀ = unfertilised, F = fertilised annually with N, P and K. LSD (P≤0.05) shown.

Table 1. Some key chemical properties and bulk densities in soils (0-5 cm) at the study sites.

Treatment	Organic C (g/kg)	pH (water)	Exchangeable Ca (mmol _e /kg)	Exchangeable Mg (mmol _e /kg)	Exchangeable K (mmol _e /kg)	Truog P (mg/kg)	Bulk density (g/cm ³)
Land use							
Native grassland	44	5.0	30	7.2	2.3	8	1.11
Maize	28	5.3	34	6.4	3.8	49	1.28
Sugarcane	27	5.3	33	6.6	4.1	26	1.37
Annual ryegrass	40	5.4	36	9.9	4.6	35	1.25
Kikuyu pasture	67	5.6	42	10.5	4.7	29	1.05
Trash management							
Burnt (unfertilised)	38	5.8	95	61	2.4	8	1.20
Burnt (fertilised)	41	5.1	66	33	4.3	31	1.18
Trashed (unfertilised)	47	5.7	121	62	4.0	12	1.12
Trashed (fertilised)	50	4.8	80	50	7.1	35	1.18
Grass	42	5.7	97	56	4.4	8	1.21

Principal component analysis

Ordination biplots for PCA analysis of PLFA data for different land uses and trash managements are shown in Figure 2. The different positions due to land use, trash management practice and fertiliser application in the plane of the first two principal components indicates that there are clear differences in PLFA components (and microbial communities). For the land management experiment, the first variable (PC1) accounted for 74% and the second variable (PC2) for 23% of the total variance in the PLFA data. For the trash management trial, PC1 accounted for 85% and PC2 for only 8% of the total variance in the data.

Results of RDA are displayed on the biplots (Figure 2). The closer the vector for an individual variable aligns with a principal component axis, the more that particular variable can be used to explain variation in the data along that axis. For the land use experiment, organic C was significantly correlated ($P \leq 0.005$) with PC1 and accounted for 66.4% of explained variation. The vertical distribution of land uses was significantly correlated with soil pH and accounted for 24% of the explained variance. RDA on the trash management data showed that organic C and exchangeable K were significantly correlated with PC1 and accounted for 67.1 and 10.2% respectively, of the explained variance.

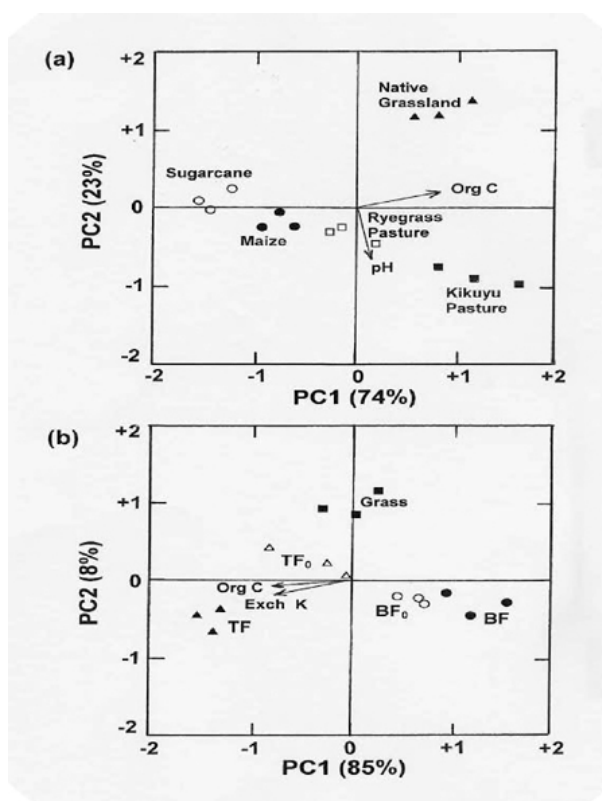


Figure 2. Ordination biplots of principal component analysis (PCA) of phospholipid fatty acid profiles from (a) five long-term land uses and (b) a long-term sugarcane trash management experiment. For explanation of abbreviations, see Figure 1. Redundancy analysis (RDA) results are shown by vectors.

The fact that organic C was closely correlated with PC1 at both sites demonstrates its importance in influencing the structural diversity of soil microbial communities. When organic matter is lost from soils, readily decomposable fractions are lost preferentially (Haynes and Beare, 1996). These fractions support a substantial portion of the heterotrophic microbial community and their loss could therefore reduce structural and functional diversity.

PLFA richness, evenness and diversity under the different land uses are shown in Figure 3. Richness was least under burnt sugarcane. Evenness followed the order sugarcane = maize > ryegrass > kikuyu > native grassland. Shannon's diversity index was least under sugarcane, intermediate under kikuyu and native grassland, and greatest under ryegrass and maize. For the trash management experiment, PLFA richness was greater, and evenness lower, under trashing than burning (Figure 3). Shannon's diversity index was greater under trashing than burning, and greatest under grass.

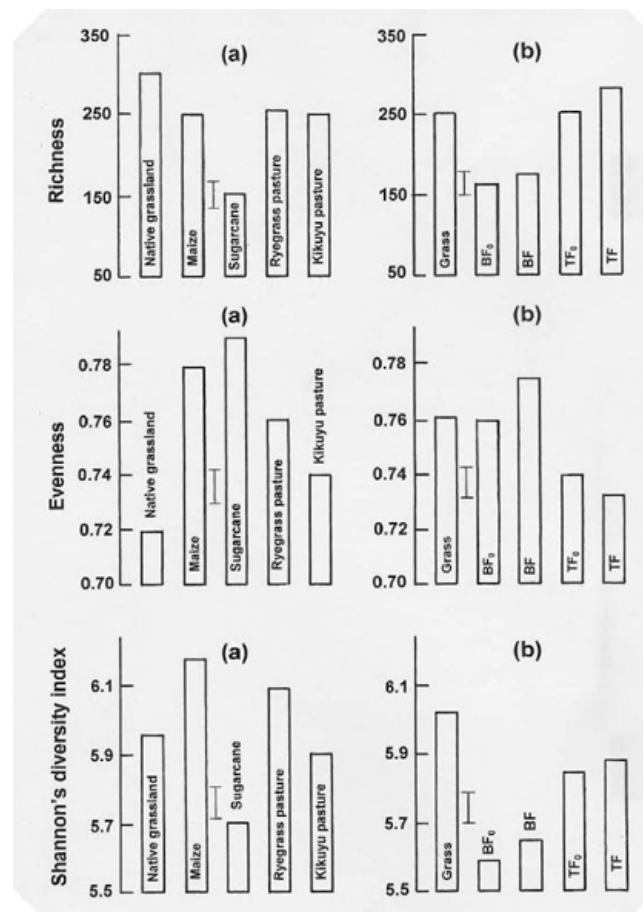


Figure 3. Richness, evenness and Shannon's diversity index for PLFA profiles under (a) five long-term land uses and (b) a long-term sugarcane trash management experiment. For explanation of abbreviations, see Figure 1. LSD ($P \leq 0.05$) shown.

The fact that soil under burnt sugarcane had the lowest PLFA richness and diversity index values of all the land uses studied suggests that it not only had the smallest microbial community, but also the least diverse one. This negative effect of sugarcane production on soil microbial biodiversity was somewhat nullified by converting to green cane harvesting. That is, in the trash management trial, trashing increased richness and diversity values, indicating that there was an increase in the structural diversity of the microbial community as well as an increase in organic C and microbial biomass content.

The generally negative relationship between PLFA richness and evenness observed (Figure 3) indicates that, where there are a small number of PLFAs present, they accumulate in relatively similar amounts, whereas where there are a relatively large number of PLFAs, a small number predominate. A possible explanation for this is that the lack of microbial diversity leads to a small community consisting of a relatively small number of resilient groups and populations. A diverse community, however, tends to be dominated by a

relatively small group of populations and associations.

Signature PLFAs

Values for EL-PLFA, NEL-PLFA, EL-MUFA, BRANC, 10ME and 18:2T6 with land use (Figures 4, 5 and 6) showed broadly similar trends to total PLFAs (Figure 1), except that NEL-PLFA and 18:2T6 values were greater under native grassland than kikuyu pasture. Similarly, trends in values for EL-PLFA, NEL-PLFA, EL-MUFA, BRANC, 10ME and 18:2T6 (Figures 4, 5 and 6) for the trash management experiment were similar to those for total PLFAs (Figure 1). Nonetheless, some interesting trends were observed, particularly when comparing the ratios of various PLFAs present under different land uses and/or trash managements. Such changes can be related to changes in the structural composition of the soil microbial community.

The distribution of PLFAs between EL-PLFA and NEL-PLFA has been suggested to be indicative of the ratio of aerobic : anaerobic bacteria present (Zelles, 1999). A low soil organic matter content generally results in a decrease in aggregate stability and greater susceptibility to compaction, waterlogging and anaerobic conditions (Gabriels *et al.*, 1997). Thus, soil conditions under burnt sugarcane, which had the lowest organic C and greatest bulk density of any of the land uses studied (Table 1), favoured an anaerobic environment. Consequently, it had the highest proportion of NEL-PLFAs (Figure 4.)

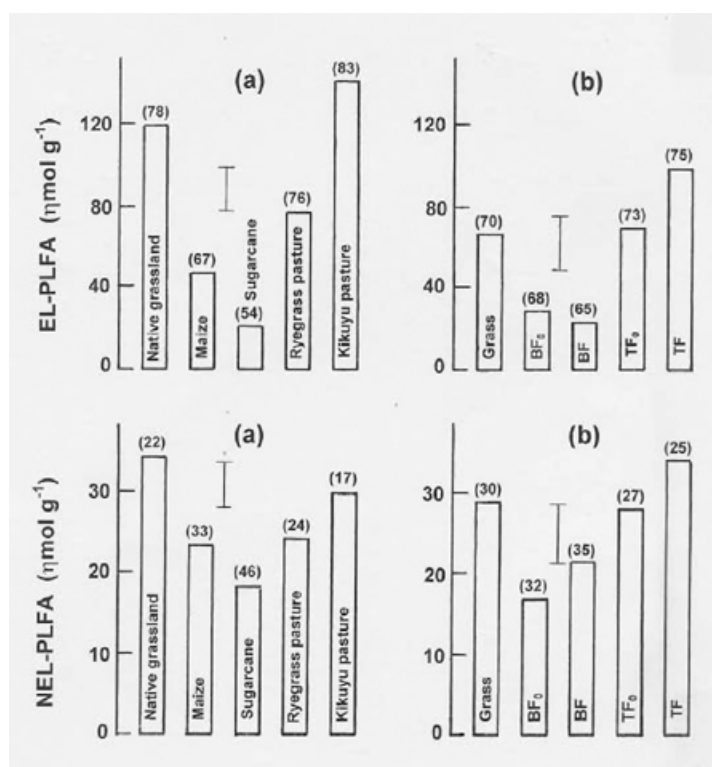


Figure 4. Concentrations of ester-linked (EL-PLFA) and non-ester-linked (NEL-PLFA) phospholipid fatty acids in soils under (a) five long-term land uses and (b) a long-term sugarcane trash management experiment. For explanation of abbreviations, see Figure 1. LSD ($P \leq 0.05$) shown.

Conversion from pre-harvest burning to green cane harvesting means larger amounts of organic matter are returned, there is more crop root growth in the surface soil, and greater soil faunal activity (Graham *et al.*, 2002c). These factors result in increased porosity, decreased bulk density and an increase in the proportion of aerobic microflora as indicated by the

greater proportion of EL-PLFAs present.

At the land use site, the fungi : bacterial ratio (18:2T6 : bacterial PLFAs) was greatest under sugarcane and followed the order: sugarcane > maize > native grassland > ryegrass pasture ≥ kikuyu pasture (Figure 5). The fungi : bacterial ratio was, however, unaffected by trash management practice. The greater fungi : bacterial ratio under sugarcane (and maize) than other land uses does not support the findings of Stahl *et al.* (1999), who suggested that fungi are proportionately more repressed than other components of the microbial community by repeated tillage under arable crop production. As already noted, labile organic matter components are lost first when soil organic matter degradation occurs under arable agriculture. A loss of soluble organic matter is likely to inhibit bacteria more than fungi, thus increasing the fungi : bacterial ratio.

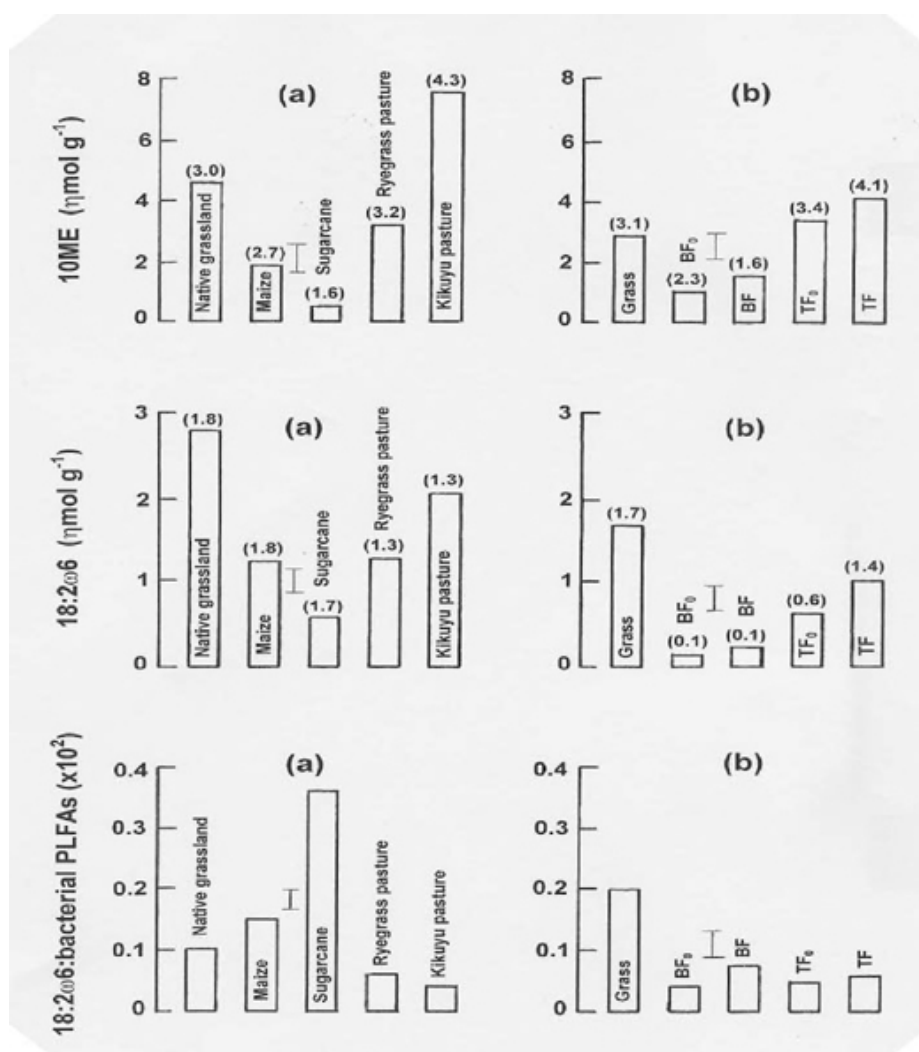


Figure 5. Concentrations of ester-linked saturated PLFAs with branching on the 10th C atom (10ME), linoleic acid (18:2w6) and the ratio of 18:2w6 : bacterial PLFAs in soils under (a) five long-term land uses and (b) on a long-term trash management experiment. For explanation of abbreviations, see Figure 1. LSD (P ≤ 0.05) shown.

Gram-negative bacteria are nutritionally a very diverse group of micro-organisms that use a diverse range of C sources, are fast growing and adapt quickly to a variety of environments (Zelles *et al.*, 1992). Thus, in this study, there was a selective stimulation of Gram-negative bacteria (i.e. an increase in the EL-MUFA : BRANC ratio) in soils under land uses with higher C inputs (e.g. native grassland and improved pastures) and to a lesser extent under

trashed compared with burnt sugarcane (Figure 6).

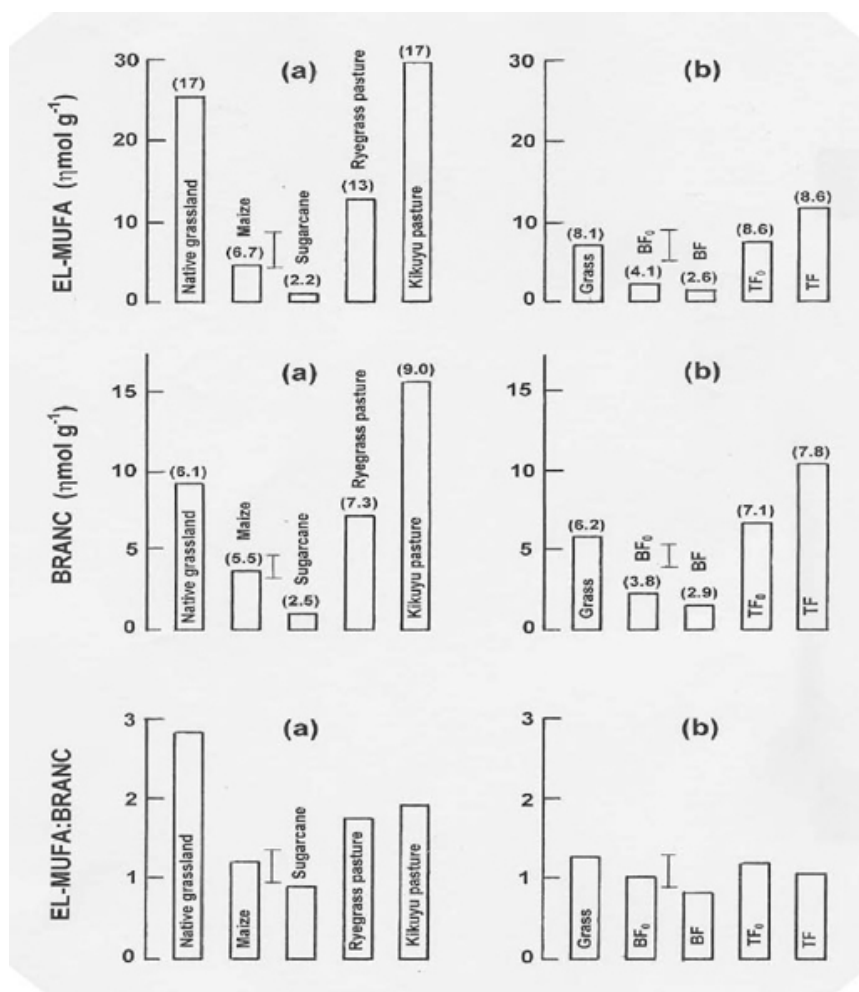


Figure 6. Concentrations of ester-linked, monosaturated (EL-MUFA) PLFAs, branched chain, ester-linked, saturated (BRANC) PLFAs and the ratio of EL-MUFA : BRANC in soils under (a) five long-term uses and (b) a long-term sugarcane trash management experiment. For explanation of abbreviations, see Figure 1. LSD ($P \leq 0.05$) shown.

Conclusions

It is concluded that land use and management can greatly influence the structural diversity of soil microbial communities. A lack of structural diversity could lead to a reduction in the effectiveness with which microflora perform key soil functions and a community less resilient to environmental stress and disturbance. Sugarcane production under pre-harvest burning is particularly detrimental to structural diversity, and conversion to trash retention has the advantage of not only increasing organic matter status, but also the diversity of the soil microbial community.

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