LONG-TERM EFFECTS OF GREEN CANE HARVESTING VERSUS BURNING ON THE SIZE AND DIVERSITY OF THE SOIL MICROBIAL COMMUNITY

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Abstract

The long-term effects of green cane harvesting with trash retention, compared with burning, on soil microbiological properties were evaluated using data from the long-term trash management trial at Mount Edgecombe (BT1). Total organic C content was greater under green cane harvesting than burning in the surface 0-10 cm layer of soil. Microbial biomass C was greater under green cane harvesting than burning to a depth of 30 cm. Both K_2SO_4-extractable C and light fraction C were also increased markedly by trash retention to a depth of 30 cm reflecting the downward leaching of soluble C and/or deposition of particulate C at depth below the trash blanket. This demonstrates the value of these labile C fractions as indicators of changes in soil C. Soil community structure was examined based on extraction and identification of phospholipid fatty acids (PLFA). Data from PLFA composition of soils were subjected to principal component analysis which accounted for over 78% of the total variance. Trash retention markedly changed the composition of the microbial community compared with burning. Fertiliser applications had very little effect on the community composition in the burnt treatments but changed the community greatly where trash was returned. The total quantity of PLFAs extracted was greater under trashed than burnt treatments reflecting the larger microbial biomass under trash retention. Principal component analysis was also used to separate PLFA profiles from soils under long-term burnt sugarcane, maize , annual ryegrass, kikuyu grass and undisturbed veld. Results clearly showed that the community composition under conventional sugarcane production where burning is practiced prior to harvest was very different from that under the other land management practices.

Keywords: light fraction; microbial biomass; organic matter; phospholipid fatty acids; soil management; sugarcane

Introduction

In South Africa the normal practice is to burn standing cane prior to harvest and to burn and/or remove post-harvest crop residues. During burning, large amounts of C, N and S are lost from the plant residues via volatilisation (Raison, 1979). It is, therefore, not surprising that many workers have measured an appreciable decrease in organic matter under long-term sugarcane production (Blair, *et al*., 1998; Blair, 2000). This decline is considered by many to be the most serious aspect of soil degradation that occurs under sugarcane monoculture (Wood, 1985; Haynes and Hamilton, 1999). The most obvious way to curtail such a decline is to cease burning and return all crop residues to the soil. This system is termed green cane harvesting and a blanket of cane trash is left at the soil surface after harvest. In Australia, burning prior to harvest is becoming less favoured and the vast majority of the crop is harvested green (Bramley *et al*., 1996). Short-term (< 5 yr) experiments on green cane harvesting have shown some beneficial effects on soil organic matter quality (i.e. increased labile organic matter content) (Wood, 1991; Blair, 2000).

Although total soil organic matter content is an important agronomic attribute, it is the size of the labile pools of organic matter that are important in relation to nutrient supply, soil structure and soil biological activity (Gregorich *et al*., 1994). In addition, short-term changes in organic C and total N content induced by changes in soil management are often not detectable (Haynes *et al*., 1991) since the large background levels of relatively stable organic matter (humic material) make it difficult to measure small changes in organic matter status. By contrast, due to their dynamic nature, more active (labile) fractions of organic matter can respond rapidly to changes in the rate of input or degradation of organic matter brought about by changes in soil management (Haynes and Beare, 1996). For example, microbial biomass C has been used as an indicator of early changes in soil organic matter status induced by management practices such as tillage methods, straw incorporation and use of grass leys in rotation (Carter, 1986; Powlson, *et al*., 1987; Haynes, 1999). In addition, the effects of long-term changes in soil management on labile organic matter fractions are important. Labile organic matter pools do not necessarily change in proportion to changes in total soil organic matter (Liang *et al*., 1998).

The importance of the biodiversity of soil biota to the integrity, function and long-term sustainability of natural and managed terrestrial ecosystems is becoming increasingly recognized (Pankhurst, 1997; Roper and Ophel-Keller, 1997). There are believed to be several million species of microorganisms present in soils and most cannot be characterised by conventional culture techniques and generally plate counts are not considered reliable measures of soil microbial diversity and activity (Roper and Ophel-Keller, 1997). This has led to approaches based on the quantitative description of communities rather than species. Community structure can be examined based on extraction and identification of phospholipid fatty acids (Zelles, 1999). Soil microbial communities are involved in key soil processes such

as biogeochemical cycling of nutrients and maintenance of soil structure.

The purpose of this study was to evaluate the long-term effect of burning versus green-cane harvesting on the quantity and quality of organic matter. Preliminary results on the use of phospholipid fatty acid analysis to describe soil microbial community structures are also reported and discussed. Diversity is also compared with that under other common agricultural land uses.

Materials and Methods

The trial (designated BT1) is situated at Mount Edgecombe on a vertisol (Arcadia form, Lonehill family; Soil Classification Working Group, 1991) with an 'A' horizon of about 500 mm. Mean annual precipitation at the site (longitude 31° 04'29" and latitude 29° 43'20") is 950 mm.

The main experimental treatments are: (i) green cane harvested with retention of a trash blanket (100% cover) (T), (ii) burning prior to harvest with tops left scattered on plots (67% cover) (Bt) and (iii) burning prior to harvest with all tops raked off plots (Bto). The treatments are either (a) unfertilised (Fo) or (b) fertilised annually with 140 kg N/ha, 20 kg P/ha and 140 kg K/ha (F). The experiment is replicated four times in a randomized split-plot design.

Three replications of the experiment were sampled in March 1998, 59 years after the experiment was initiated. Plots were sampled randomly over the whole area using a 50 mm diameter soil sampler (10 samples per plot, 0-30 cm) and sectioned into the 0-2.5, 2.5-5, 5-10, 10-20 and 20-30 cm layers. 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 under undisturbed grassy vegetation as it is possible to find in the vicinity of the experiment.

The field-moist soil was sieved (< 2 mm) and stored at 1°C for not more than 72 h prior to subsequent microbiological and biochemical analysis. Part of this sample was subsequently air-dried and finely-ground (< 150 µm) and used for organic C analysis. Organic C (C_{org}) was determined by a dichromate wet oxidation procedure (Yeomans and Bremner, 1988).

Microbial biomass C (C_{mic}) was estimated by the method of Vance *et al*. (1987) based on the difference between C extracted with 0.5 M K_2SO_4 from chloroform-fumigated and unfumigated soil samples using a K_c factor of 0.38. The microbial quotient was calculated by expressing microbial biomass C as a percentage of total soil organic C.

Basal respiration was determined by placing 30 g oven-dry equivalent of field moist soil in 50 ml beakers and incubating the sample in the dark at 25° C in a 1 litre air-tight jar along with 20 mL of 0.5 M NaOH. The CO_2 evolved was determined after seven days by titration (Anderson, 1982). The metabolic quotient (q CO_2) was calculated as basal respiration (µg CO_2 - C h^{-1}) per mg of microbial biomass C.

To study the effect of different crop residue management practices on the soil community structure additional samples from the 0-5 cm layer were taken from the grass, BtoFo, BtoF, TFo and TF treatments. For comparison, samples were also taken from the 0-5 cm layer from fields under various land uses at a site in the KwaZulu-Natal midlands. The fields were on "Baynesfield" estate and cropping histories were > 50 yr kikuyu grass pasture, > 50 yr annual ryegrass pasture, > 30 yr continuous burnt sugarcane, > 30 yr continuous maize and undisturbed grassveld. The soil at the site was a Hutton form (Farmingham series) (Rhodic Ferralsol, FAO) with a clay content of about 62%. Its mineralogy is dominated by kaolinite plus halloysite and there are also appreciable amounts of crystalline sesquioxides, gibbsite and interlayered chlorite.

The phospholipid fatty acids (PLFAs) were extracted from these field-moist soil samples using a mixture of buffer solution, methanol and chloroform and this was followed by a mild alkaline hydrolysis step (Zelles, 1999). The fatty acid methyl esters (FAMES) were then fractionated into various chemically relevant groups and determined by gas chromatography. Statis-

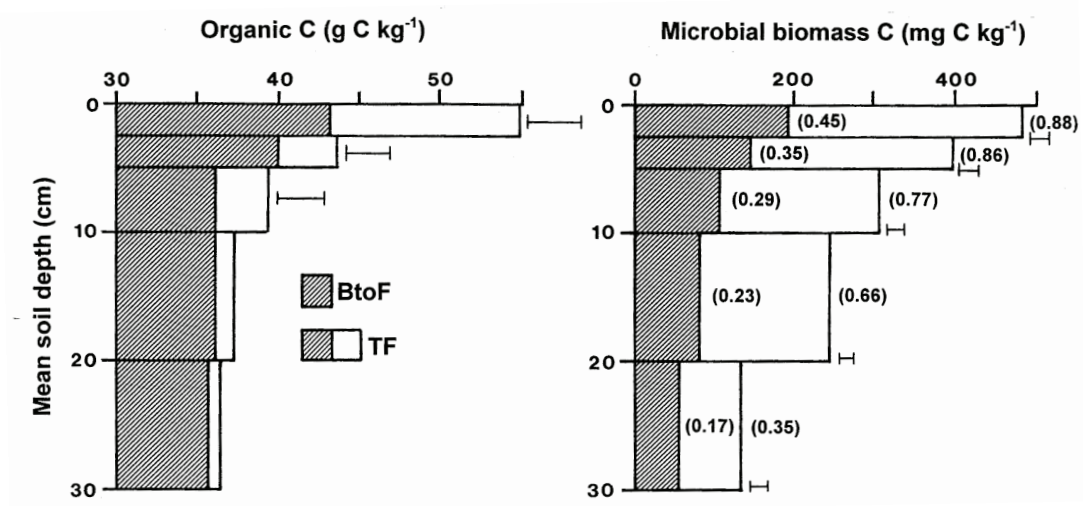

Figure 1. Distribution of organic C and microbial biomass C in the soil profile in the BtoF and TF treatments. Values for the microbial quotient at each depth are shown in brackets. Bto = burnt with harvest residues removed, T = green cane harvested with retention of a trash blanket, F = fertilised annually with N, P and K. LSD (P ≤ 0.05) shown for treatment differences for each soil layer.

tical analysis was carried out with Statistical Package for Social Sciences (SPSS) for Windows. The changes in PLFA composition were analysed by principal component analysis (PCA) to elucidate variations in patterns using $\log_{10}$ mol% values for individual PLFAs.

Results

Significant increases in C_{org} due to trash retention were observed only in the surface 10 cm of soil. Data for C_{org} in the fertilised treatments (BtoF and TF) are shown in Figure 1. By contrast, significant increases in C_{mic} (Figure 1) were observed to a depth of 30 cm in response to trash retention. The microbial quotient decreased with increasing soil depth but was markedly increased by trash retention to a depth of 30 cm (Figure1).

Concentrations of K_2SO_4-extractable C and light fraction carbon (LFC) (Figure 2) were higher under trash retention than burning to a depth of 30 cm. Significant increases in basal respiration (Figure 3) were also observed to a depth of 30 cm in response to trash retention. The qCO_2 increased with increasing soil depth but was markedly decreased by trash retention (Figure 3).

The total amount of PLFAs generally represents the total microbial biomass (Balkwill *et al*., 1988; Petersen *et al*., 1991; Bääth *et al*., 1992; Zelles *et al*, 1994; 1997). For comparison the results of both C_{mic} and total PLFAs in the 0-5 cm layer are shown in Figure 4. The concentration of C_{mic} and PLFAs generally followed the order BtoFo ≤ BtFo ≤ BtoF ≤ BtF ≤ TFo < TF. The results of the C_{mic} and total PLFA's under other common agricultural land uses are shown in Figure5. The concentration of both C_{mic} and PLFA's generally followed the order: long-term burnt sugarcane ≤ maize ≤ annual rye grass ≤ undisturbed veld ≤ kikuyu grass.

Data from PLFA composition of soil were subjected to principal component analysis (PCA). Principal component analysis can be regarded as a qualitative distribution of PLFA data in a two dimensional space, based on the similarity of the PLFA profiles of the different treatments.

The PCA was applied to the $\log_{10}$ mol % values for all individual PLFAs of soil samples derived from the long-term management trial (Figure 6) and the other land uses (Figure7). The component weights were plotted for the first two variables. The first principal component (PC1) accounted for 78 % and 77% of the total variance, respectively.

Trash retention markedly changed the composition of the microbial community compared with burning. Fertiliser application had very little effect on the community composition in the burnt treatments but changed the community greatly where trash was returned. Results in Figure 7 clearly show that community composition under sugarcane that was burnt prior to harvest was very different from that under the other land management practices (i.e. maize, kikuyu grass, undisturbed veld and annual ryegrass).

Discussion

As expected, quantities of total and labile soil organic matter present in the surface 10 cm of soil were greater under green cane harvesting than burning. This is not surprising since annual returns of trash for the TF treatment are estimated to be about 16 Mg/ha.

Because of the dynamic nature of C_{mic}, this small, but labile, component of soil organic matter responds rapidly to changes in C supply and therefore can be a good indicator of early changes in soil organic matter status (Gregorich *et al*., 1994; Haynes and Beare, 1996). Effects are often evident long before changes in C_{org} can be detected. Similarly, in this study, changes in C_{mic} in the surface 5 cm in response to long-term trash retention were much more pronounced than for C_{org}.

The importance of organic matter in enhancing soil microbial activity is reflected in the vertical distribution of C_{mic} in the soil profile. That is, C_{mic} declined rapidly with soil depth as did C_{org}, K_2SO_4-extractable C and LFC. Such a rapid decline in C_{mic} with depth is typical in soils under zero tillage or pasture (Haynes and Beare, 1996) since major organic matter inputs occur principally at, or near, the soil surface. Due to the decline in the proportion of readily metabolisable C with increasing depth, there was also a decline in the microbial quotient.

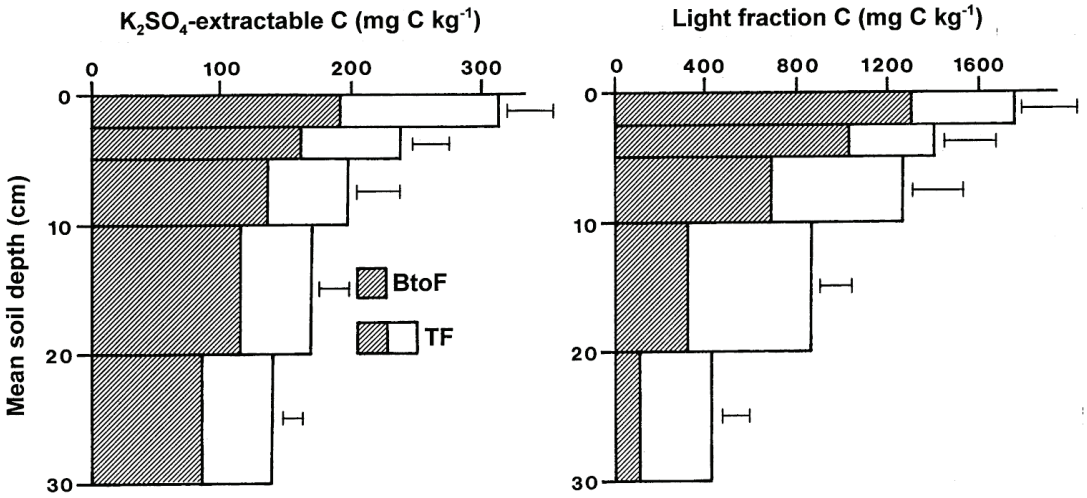

Figure 2. Distribution of K_2SO_4-extractable and light fraction C in the soil profile in the BtoF and TF treatments. Bto = burnt with harvest residues removed, T = green cane harvested with retention of a trash blanket, F = fertilised annually with N, P and K. LSD (P ≤ 0.05) shown for treatment differences for each soil layer.

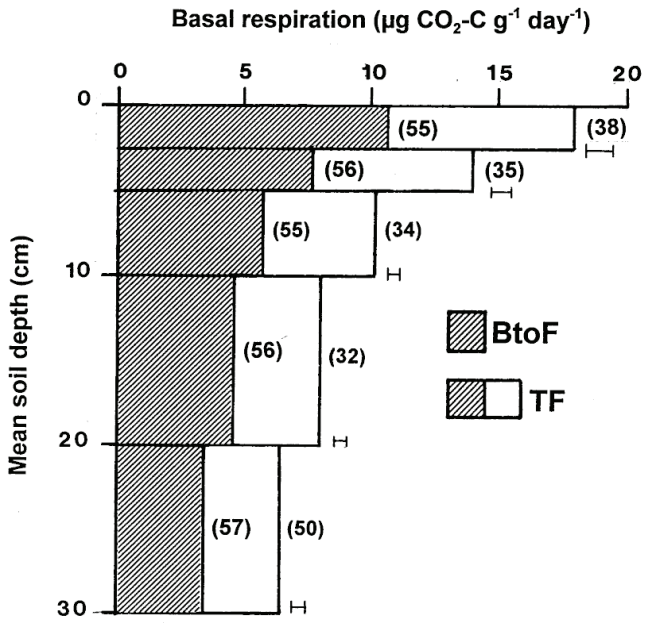

Figure 3. Vertical distribution of the basal respiration rate in the BtoF and TF treatments to a depth of 30 cm. Values for the metabolic quotient at each depth are shown in brackets. Bto = burnt with harvest residues removed, T = green cane harvested with retention of a trash blanket, F = fertilised annually with N, P and K. LSD ($P \leq 0.05$) shown for treatment differences for each soil layer.

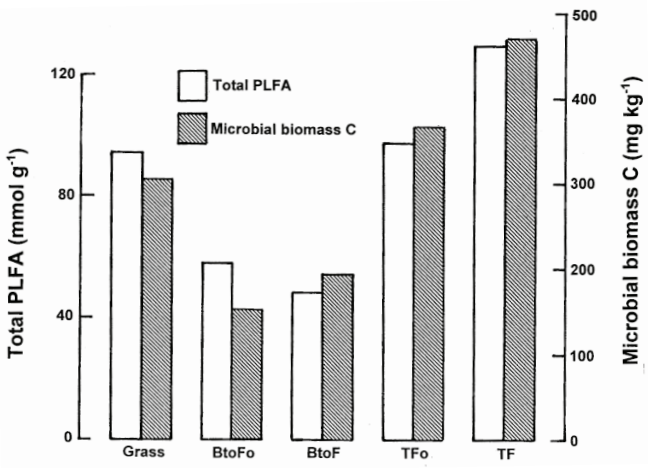

Figure 4. Effect of long-term trash management practices and fertiliser applications on the total amount of PLFAs and microbial biomass C in the 0-5 cm soil layer. Grass = undisturbed grassed area, Bto = burnt with harvest residues removed, T = green cane harvested with retention of a trash blanket, Fo = unfertilised and F = fertilised annually with N, P and K.

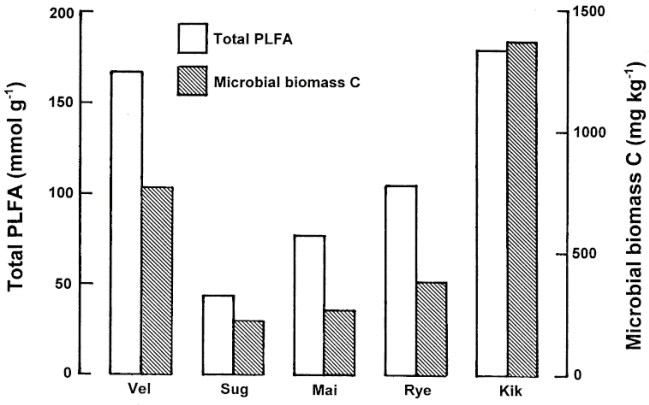

Figure 5. Effect of various different land management practices in comparison with burnt sugarcane on the total amount of PLFAs and microbial biomass C in the 0-5 cm soil layer. Sug = sugarcane was burnt with harvest residues removed, Mai = maize, Vel = undisturbed veld, Kik = kikuyu, Rye = annual ryegrass.

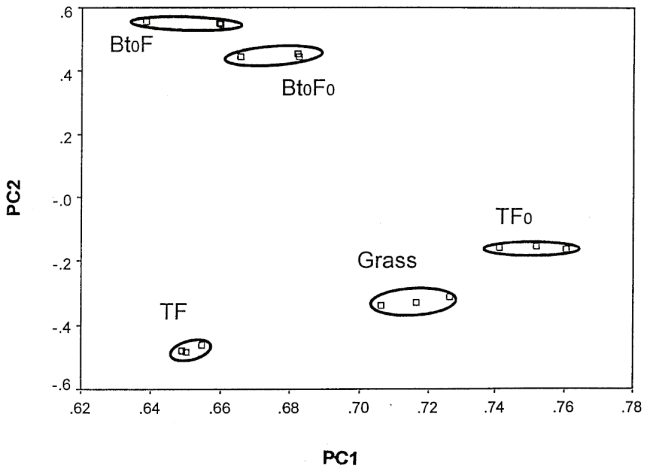

Figure 6. Principal component analysis of individual PLFA profiles, expressed as $\log_{10}$ mol % in soils under different crop residue management practices, either fertilised or unfertilised. Grass = undisturbed grassed area, Bto = burnt with harvest residues removed, T = green cane harvested with retention of a trash blanket, Fo = unfertilised and F = fertilised annually with N, P and K. PC1 accounted for 78% of the total variance in the data.

Even so, changes in C_{mic} and other labile organic fractions did not necessarily mirror those of C_{org}. For example, there were no significant treatment differences in C_{org} below 10 cm yet C_{mic}, K_2SO_4-extractable C and LFC were greater under trash retention than burning in the 10-20 and 20-30 cm layers. The downward leaching of labile organic matter originating from the trash blanket would have favoured this. Downward redistribution of particulate organic material originating from the trash blanket via faunal activity (e.g. earthworms) probably also occurred. It is also possible that the presence of the trash blanket favoured a crop root distribution more concentrated in the interrow spaces and nearer the soil surface. This would have resulted in a greater turnover of root material in these layers and greater deposition of LF material.

The most common method of measuring microbial activity is to measure the respiratory activity of the soil. As soil organic matter content and microbial biomass C increased due to trash retention, so too did basal respiration. This increase was evident to a depth of 30 cm, reflecting the metabolic response of the C_{mic} to the readily mineralisable C fractions.

It has been proposed that the metabolic quotient can be used as a measure of changes in microbial activity in response to adverse environmental conditions (either environmental stress or disturbance) (Anderson and Domsch, 1985; 1993). The higher value of qCO_2 for the burnt treatment compared with the trashed

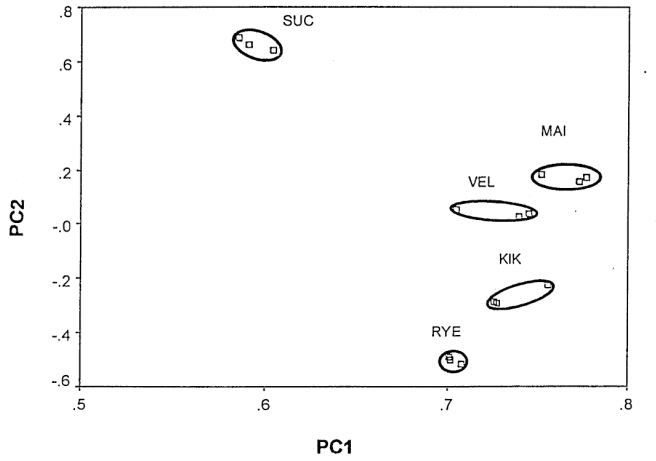

Figure 7. Principal component analysis of individual PLFA profiles, expressed as log_{10}mol % in soil under different land management practices. Sug = sugarcane that was burnt with harvest residues removed, Mai = Maize, Vel = undisturbed veld, Kik = kikuyu, Rye = annual rye grass. PC1 accounted for 77% of the total variance in the data.

treatment suggests that burning induced a decrease in the efficiency of use of C substrates by the soil microbial community. Since this treatment has a low organic C content (i.e. low supply of C substrate for microbial activity) and is also nutrient deficient (van Antwerpen and Meyer, 1997) a stressed microbial population is not unexpected. On the other hand, the trashed fertilised treatment had the lowest metabolic quotient. This treatment had the highest organic C and microbial biomass C content and was also fertilised. Other factors could also contribute to the decreased qCO_2. For example, fungal communities, that are preferentially stimulated by surface applied residues, are more efficient at converting substrate C into cellular C than bacterial communities (Kazunori and Oba, 1994).

Because phospholipids are present in the membranes of living cells and break down rapidly in soils, it is argued that analysis of this fraction in soils can be related directly to the viable soil biomass (Frostegård *et al*., 1991; Frostegård and Bååth, 1996; Petersen *et al*., 1991; Zelles, 1999). In this study good correlation (r = 0.91) was found between the total amount of phospholipids and the microbial biomass determined by fumigation extraction. Other workers have recorded similar results. For example, the r value for the correlation of the total amount of phospholipids to biomass, measured by the substrate-induced respiration (SIR) technique, varied between 0.9 and 0.984 (Zelles *et al*., 1992, 1994,1995) and the correlation between PLFA and total adenine nucleotides was also high (r > 0.959, Zelles *et al*., 1994). The large amount of PLFAs under kikuyu and undisturbed veld was not surprising, since organic matter inputs are large and arise from senescing plant tops and particularly roots and exudation of organic compounds from roots (Haynes and Beare, 1996). By comparison, the total amount of PLFAs under the burnt sugarcane reflect the negative effect that the removal of residues and intensive cultivation have on soil quality. Nevertheless, the increase in the total amount of PLFAs extracted from soil under the green cane harvested treatment compared with the burnt treatments clearly indicate a larger microbial biomass under trash retention.

By applying multivariate statistical methods such as PCA to the PLFA data, qualitative changes in the soil microbial community structure can be measured. These shifts in community structure are a function of the changes in the environment in which the organisms live. Preliminary analysis of the data suggest that two main factors contributed most variance to the data; these were pH (PC1) and organic matter levels (PC2).

Research into the effects of fertiliser applications on C_{mic} has yielded rather contradictory results with both increases and decreases being reported (Wardle, 1992). A decline in C_{mic} is frequently related to soil acidification induced by the application of nitrogenous fertilisers (Grace *et al*., 1994). In this study, both C_{mic} and the total amount of PLFAs were increased by fertiliser applications. This suggests that the increase in C_{org} and labile organic matter (e.g. K_2SO_4-extractable and LFC) induced by fertiliser applications (data not shown) effectively nullified any negative effects that acidification may have had. Nevertheless, pH can have a significant effect on the microbial community structure related to the fact that each microbial strain has an intrinsic range of pH within which it can function. Generally, fungi are more tolerant of acid conditions than bacteria, including actinomycetes (Doran, 1980), leading to a changing balance in the microbial composition of soil as the pH varies. This shift in the microbial community was evident in both burnt and more particularly trashed treatments and is thought to be the result of fertiliser induced acidification that occurred [mean pH (0-5 cm) was 5.8 for unfertilised plots and 5.1 for fertilised plots] (Graham *et al*., 2000). This change in microbial composition could have an impact on key soil processes which influence crop productivity. For example, nitrification is thought to occur primarily in soils with a pH greater than 5.5, since it is catalysed mainly by a small group of autotrophic bacterial species which are pH sensitive (Dommergues *et al*., 1978), and can greatly affect N availability to the crop.

Organic matter is frequently the growth limiting factor for heterotrophic microorganisms which are generally stimulated by organic amendments(Knapp *et al*., 1983 ; Schnürer *et al*., 1985). Thus the shift in the microbial community under green cane harvesting, is a result of increasing resource heterogeneity and a consequent increase in microbial diversity (Wander *et al*., 1995). However, specific organic substrates often favour the growth of particular populations, that would further accentuate the shift in community structure. As a result, fungal biomass is generally larger in the proximity of surface-applied residues (Grace *et al*., 1994). Thus, management practices with large organic matter returns (i.e. unfertilised trashed treatments, undisturbed veld and kikuyu grass) showed clear similarities in their PLFA profiles. Other factors could also contribute to the similarities in PLFA profiles. For example, similar physical conditions under these land uses may result in the composition of factors determining growth and structure of the microbial population being similar.

Remarkably the position of the BtoF treatment within the ordinates of Figure 6 was almost identical to that of the fertilised, burnt sugarcane in Figure 7. This similarity is particularly significant since the soils at the two sites are very different as is the climate. The same principal factors do, however, seem to be

strongly influencing the soil microbial community under conventional sugarcane production at both sites.

It is evident that green cane harvesting with trash retention is an effective way of arresting the loss of soil organic matter that characteristically occurs under conventional sugarcane production where burning is practiced prior to harvest. Not only did the practice increase total soil organic matter content in the surface 10 cm, but it also increased concentrations of various labile organic matter fractions as well as soil microbial activity to a depth of 30 cm. These changes in organic matter content and quality may greatly affect other soil properties and processes such as aggregation, soil structural condition, and N supply to the crop via mineralisation.

Crop residue management also resulted in a shift in microbial composition. Not only did green cane harvesting increase the microbial biomass C and total PLFAs, but it also significantly altered the soil microbial community structure. As a result of increased biodiveristy the PLFA profile of unfertilised trashed sugarcane was very similar to that under undisturbed veld and kikuyu grass. Thus, PLFA analysis proves to be a promising technique to determine effects of land management on microbial community structure. Phospholipid fatty acid analysis can also be used to characterise specific groups of microorganisms, providing essential information with regard to a shift in specific functional subsets of the community. These aspects will be investigated in future studies.

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