

**THE EFFECT OF VARIOUS CROP RESIDUE MANAGEMENT PRACTICES  
UNDER SUGARCANE PRODUCTION ON SOIL QUALITY**

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## ABSTRACT

This study examined the influence of different management practices under sugarcane production on soil chemical, biological and physical properties on a long-term (59yr) field experiment in KwaZulu-Natal. These management practices included conventional post-harvest burning of crop residues, with the tops either left on the soil surface or with tops removed, and green cane harvesting with the retention of crop residues on the soil surface as a trash blanket. Each of these treatments were either fertilized or did not receive fertilizer. The data collected was used to evaluate the effect of crop residue management on soil quality under sugarcane production.

Soil organic matter content increased from 39 g C kg<sup>-1</sup> soil, under conventional burning to 55 g C kg<sup>-1</sup> soil in the surface 10 cm under green cane harvesting where crop residues are returned to the soil. It also resulted in greater recycling of nutrients and increases in exchangeable K and Ca and extractable P. Fertilizer application resulted in a build-up of soil nutrients in combination with trash retention. Fertilizer application induced increases in exchangeable P and also some accumulation in soil organic P. Exchangeable and non-exchangeable K concentrations were also higher in fertilized than non-fertilized treatments.

However, nitrogen fertilizer application and, to a lesser degree, organic matter mineralization, resulted in soil acidification to a depth of 30 cm. Acidification in the fertilized treatments resulted in a concomitant increase in exchangeable acidity and exchangeable Al, due to the increase in H<sup>+</sup> ions and solubilized Al species on exchange sites. Base cations moved into soil solution and were leaching to lower soil layers. The decrease in soil pH resulted in the surface charge conferred on the variable charge surfaces on soil colloids becoming less negative and as a result there was a decrease in ECEC. Acidification in fertilized treatment not only increased exchangeable Al but also the buffering reserve of non-exchangeable al; both that complexed with soil organic matter (CuCl<sub>2</sub>-extractible) and that present as hydroxy - Al associated with mineral colloids

(ammonium acetate - extractable). The increased organic matter content under trash retention resulted in an increase in ECEC. This enabled the soil to retain greater amounts of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  which were returned to the soil in the trash.

Both residue retention and fertilizer application had a positive effect on the microbial biomass C and N and the microbial quotient increased from 0.39% to 0.86% as organic C increased from  $39 \text{ g C kg}^{-1}$  soil under burnt treatments to  $55 \text{ g C kg}^{-1}$  soil under trashed, fertilized treatments. This increase was associated with increased concentrations of labile organic material ( $\text{K}_2\text{SO}_4$ -extractable) present as well as increased amounts of nutrients being cycled through the plant-soil system. The light fraction organic matter also increased with increasing returns of organic residues. However, the large active microbial biomass under the trashed, fertilized treatment resulted in an increased turnover rate of this fraction and consequently resulted in lower LF dry matter, C and N than in the unfertilized treatment.

Aggregate stability increased with increasing amounts of organic material returned due to trash retention. Nevertheless, fertilized treatments induced a lower aggregate stability than unfertilized ones, despite the tendency for the latter to have higher organic C and microbial biomass values. This was attributed to an increase in the proportion of exchangeable cations present in monovalent form (due to application of fertilizer K and leaching of Ca and Mg) favouring dispersion and a decline in aggregate stability.

Green cane harvesting resulted in an increase in microbial activity (basal respiration, FDA hydrolytic activity, arginine ammonification rate and dehydrogenase activity) and in the activity of specific soil enzymes involved in turnover of C, N, P and S to a depth of 30 cm. Increased activities of these enzymes reflect a higher rate of turnover of C, N, P and S. The metabolic quotient decreased with increasing residue return, indicating a more metabolically efficient microbial community. Fertilizer application resulted in a variable effect on enzyme activity. Long-term fertilizer application resulted in an increase in

invertase and acid phosphatase, a decrease in L-histidase and arylsulphatase and had little effect on protease and alkaline phosphatase. These variable effects were explained in terms of an interaction between fertilizer - induced increases in  $C_{org}$  and soil nutrient status and fertilizer - induced soil acidification.

The size and activity of the soil microbial biomass was studied in the plant row and in the inter-row of a sugarcane field under burning or green cane harvesting. Soils were sampled to 30 cm depth in (i) the centre of the plant row, (ii) 30 cm out from the row centre and (iii) 60 cm out from the row centre (i.e. the middle of the inter-row area). Under burning, the only substantial input of organic matter to the soil was from root turnover in the row area where the root biomass was concentrated. As a consequence, the size (microbial biomass C) and activity (basal respiration of the soil microbial community) were concentrated in the row. However, under green cane harvesting there was a large input of organic matter in the inter-row area in the form of the trash blanket itself and through turnover of crop roots that were concentrated in the surface 10 cm of the soil below the blanket. As a result, soil microbial activity was considerably higher in the inter-row area under green cane harvesting than under burning.

Phospholipids are essential membrane components of microorganisms and a good correlation was found between the total PLFA's extracted from soils and the microbial biomass C, indicating that phospholipids are an accurate measurement of living biomass. Multivariate statistical analysis (PCA) was used to separate different PLFA profiles under burning versus trash retention and under different land uses (sugarcane, maize, annual and perennial pasture and undisturbed veld). Soil organic matter content contributed the greatest variance in the data along the first axis. That is increasing soil organic matter return not only increased the size of the microbial biomass, but also affected the composition of the microbial community. There was a shift in the different sub-fractions under different management practices. MUFA's are general biomarkers of Gram negative bacteria and were found to be a sensitive indicator of higher substrate availability (i.e they increased under green cane harvesting). Fungal biomarkers

indicated an increased fungal biomass associated with surface application of residues. Soil physical conditions were considered to be a contributing factor to the shift in microbial community structure. Increased organic matter content improved soil physical conditions and preferentially stimulated the growth of aerobic microorganisms. In addition to this, the proportion of SATFA (gram positive bacteria) was found to increase in response to burning. This increase was attributed to the survival mechanisms of these microorganisms (i.e. endospore formation). It was found that the conversion from burning to trash management changes the composition of the soil microbial community.

The effect of management practices on soil functional diversity was also evaluated using two methods (i.e. Biolog plates and substrate induced respiration (SIR)). Biolog plates are a selective technique that stimulate growth of a small proportion of the soil microbial community whereas the SIR technique measures the activity of the metabolically active microbial community *in situ*. As a result the SIR method separated treatments more effectively than Biolog plates (i.e. annually tilled treatments, permanent grassland sites and fertilized and unfertilized treatments). The quantity and the quality of organic C supply influenced the catabolic diversity. Conversion from burning to green cane harvesting greatly increased catabolic evenness and richness and therefore presumably also tended to increase the resilience of the soil to stress and disturbance particularly in relation to decomposition functions.

It was concluded that conversion from preharvest burning to green cane harvesting results in an increase in soil organic matter content, an improvement in soil structure and soil nutrient status, an increase in the size, activity, taxonomic and functional diversity of the soil microbial community. The practice should therefore be promoted to the South African sugar industry.

### Pre-Publication of Parts of this Thesis

A major portion of chapter 3 has been submitted for publication, entitled: Changes in soil chemistry and aggregate stability induced by fertilizer applications, burning and trash retention on a long-term sugarcane experiment in South Africa. By: M.H. Graham, R.J. Haynes and J.H. Meyer. *European Journal of Soil Science*. 53: 589 - 598.

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## DECLARATION

I hereby certify that the research reported in this thesis is my own work, except where otherwise indicated in the text, and that the work has not been submitted for a higher degree at any other instruction.

Signed:

A handwritten signature in blue ink that reads "M.H. Graham". The signature is written over a horizontal dotted line.

Martha Helena Graham

Signed:

A handwritten signature in blue ink that reads "R.J. Haynes". The signature is written over a horizontal dotted line.

Prof R.J. Haynes

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## CHAPTER ONE

### 1. General introduction

Research into the evaluation of soil quality has increased over the past few years due to concern regarding soil degradation and agricultural sustainability (Doran and Parkin, 1994; Nannipieri, 1994). An assessment of soil quality that includes soil biological, chemical and physical properties can provide valuable information for evaluation of the sustainability of land management practices (Doran and Parkin, 1994). Soil chemical properties that define soil fertility (e.g. pH, organic C, extractable P, exchangeable cations) are often well documented. Soil physical properties are normally less well documented since they often need to be measured under field conditions and they may change markedly during the season (e.g. after conventional cultivation). Normally least is known about the biological properties of soils.

Because of its large influence on the fertility, biological activity of soil and organic matter content are considered key attributes of soil quality (Gregorich *et al.*, 1994).

The effects of short-term changes in soil management practice on total soil organic matter content are often difficult to quantify because of the large background level of stable humus that is present in soils. For this reason, attention has turned to defining labile organic matter fractions (e.g. microbial biomass C, light fraction C, water soluble C) that are early indicators of a change in soil organic matter status (Gregorich *et al.*, 1994).

A decrease in organic matter when virgin land is put under sugarcane has been observed in many sugar producing areas of the world including South Africa (Masilaca *et al.*, 1985; Wood, 1985; Alaban *et al.*, 1990; Henry and Ellis, 1995; Hartemink and Kuniata, 1996; van Antwerpen and

Meyer, 1996; Hartemink, 1998a). In Queensland, some workers have suggested that the main contributing factor to soil degradation is incorrect management practice (particularly burning of large amounts of crop residue) that results in organic matter

decline (Bramley *et al.*, 1996; Skjemstad *et al.*, 1999). An obvious way to improve the soil organic matter status of sugarcane soils is to change from the usual practice of burning to green cane harvesting with the retention of a trash blanket over the soil surface. This practice is not used to any great extent in the South African sugar industry and its benefits need to be quantified and demonstrated before it will be taken up on a large scale.

This study concentrates on the effect of crop residue management practices (burning versus green cane harvesting with retention of a trash blanket) and fertilizer additions on soil fertility and on the quantity and quality of soil organic matter present. The research site is a long-term (59 yr) trash management trial situated at the Mount Edgecombe at the South African Sugar Association Experiment Station. The main objectives are as follows:

1. Determine how trash management and annual fertilizer additions have affected soil chemical properties that define soil fertility and what the various constraints associated with soil fertility are at the site.
2. Quantify the organic C and total N content of soil that has been under various crop management practices for 59 years.
3. Quantify the various labile organic matter fractions that have been identified as indicators of soil quality by Gregorich *et al.* (1994), to evaluate how these labile pools respond to various management practices in the long-term.
4. Quantify how treatments have affected soil physical condition as defined by aggregate stability.
5. Determine how residue management practices and annual fertilizer additions have affected microbial and enzyme activity.

6. Determine how residue management practices in combination with ratoon row cropping have affected the distribution of labile soil organic matter pools across the field.
7. Determine how residue management practices and annual fertilizer additions have affected microbial community structure and functional diversity.

This thesis is divided into eight chapters. Following this introductory chapter an overview of the current literature on the concept of soil quality and the effect that different agricultural management practices have is presented. Included in this chapter is a review of the current knowledge on sugarcane management practices and their effect on soil quality.

Chapter 3 describes and discusses the effect of three contrasting post-harvest residue management practices on the fertility status of the soil. Chapter 4 focuses on how labile organic matter pools, that are used to define soil quality, respond to these management practices. For comparison, the values obtained from the experimental treatments were compared to values obtained from grass rows in between the treatments that have not been cultivated for the duration of the experiment (i.e. control). Chapter 5 focuses on how microbial and enzyme activity are affected by crop residue management and annual fertilizer application.

Since sugarcane is a ratoon row crop, it was thought that the root biomass in the row would increase the organic C level in the row, resulting in an uneven distribution of organic matter return to the soil across the field. As a result, chapter 6 describes and discusses the distribution of labile organic matter pools and microbial activity from samples taken with increasing distance from the row centre into the inter-row. Chapter 7 focuses on soil microbial diversity measured by phospholipid fatty acids and chapter 8 discusses microbial functional diversity with regards to changes in crop residue management changes. The thesis ends with a short conclusions chapter.

## CHAPTER TWO

### 2. Literature review

#### 2.1 Introduction

According to the latest population estimates released by the United Nations (1999) there are approximately 6 billion human beings on earth. On average, there is a net gain of three people per second. These are alarming values when considering the impact that this population growth has on land use and the intensity of agricultural production.

A growing demand for food results in the need for continuous crop production with, an increased use of crop monocultures and greater reliance on chemical fertilizers and pesticides. A combination of these factors has resulted in an increased crop yield and on-farm labor efficiency (Power and Papendick, 1985). However, these management practices also contribute to the degradation of an important medium for plant growth, namely soil. Soil degradation can result in an eventual decline in crop yield (Doran and Parkin, 1994). Findings from a project of the United Nations Environment Program on "Global Assessment of Soil Degradation" indicate that almost 40% of agricultural land has been adversely affected by human-induced soil degradation and that more than 6 % is degraded to such a degree that restoration of its original productive capacity is only possible through major capital investments (Oldeman, 1994). This awareness of the degradation of our soil resources under agricultural production kindled a concern for soil quality evaluation (Larson and Pierce, 1991). Soil quality is concerned with chemical, physical and biological properties of the soil.

Many of the soils biological, physical, and chemical properties are a function of soil organic matter content (Larson and Pierce, 1991; Doran and Parkin, 1994). The soil organic matter content is, thus, a vital component in the maintenance and sustainability of soil quality (Gregorich *et al.*, 1994) and is therefore considered a key

attribute of soil quality. The large amounts of crop residues produced annually are a valuable resource of organic matter and should not be considered as a waste product. Management of these crop residues is necessary to prevent soil organic matter decline and a consequent decrease in soil quality.

This review chapter is written in three main sections. Firstly, the concept of soil quality is reviewed with particular reference to soil organic matter content and soil microbial activity. Secondly, the effects of sugarcane monoculture on soil quality and soil degradation are discussed. Thirdly, the effects of crop residue management on soil quality, with particular reference to sugarcane production, are reviewed.

## 2.2 Soil quality

Concern regarding the effect of anthropogenic actions on the environment has focussed attention on the evaluation of air and water and recently on soil quality. Various definitions have been formulated to define soil quality (Anderson and Gregorich, 1983; Power and Meyer, 1989; Larson and Pierce, 1991; Parr *et al.*, 1992; Pierce and Larson, 1993; Doran and Parkin, 1994). All these definitions have common themes. For the purpose of this review the following definition is most appropriate: **The capacity of a soil to support crop growth in a sustained manner over the long-term, to maintain soil organic matter levels, thereby supporting biological productivity and biodiversity, maintaining soil nutrient capacity and physical conditions, and to act positively with the surrounding environment.** This definition coincides with the statement of Larson and Pierce (1991), that the multiplicity of physical, chemical, and biological factors and their variations in time and space should be considered when practically assessing soil quality. The complex interactions between soil chemical, biological and physical properties give a soil its capacity to function and define its quality. Soil quality is defined by means of soil quality indicators which quantify the chemical, biological and physical properties of the soil in relation to the soil's capacity to function (Doran and Parkin, 1994; Lal, 1998).

Soil quality indicators should be sensitive to variations in management practices and climatic conditions (Gregorich *et al.*, 1994; Doran *et al.*, 1996). Indeed, if indicators of soil quality are insensitive to changes in management practices, they will be of little use in monitoring changes in soil quality and for proposing management changes to enhance soil quality. Larson and Pierce (1991) proposed that a minimum data set (MDS) of soil parameters be adopted for assessing the quality of soils and that standardized methodologies and procedures be established to assess changes in the quality of those factors (Table 2.1).

A central concept of soil quality is considering the soil as a living system (Doran *et al.*, 1996). For that reason, measurement of the size and activity of the microbial biomass and soil enzyme activity are of particular importance. In addition, in recent years, there has been an increasing awareness that the biodiversity and functional diversity of soil organisms should be considered as soil quality indicators (Pankhurst, 1997) since they are responsible for decomposition transformation of organic compounds and other nutrient transformations e.g. asymbiotic nitrogen fixation, protein and amino acid decomposition, mineralization and immobilization of nitrogen and mineral transformations (Alexander, 1977; Roper, 1983; Sikora and McCoy, 1990). Some recently developed approaches to measure biodiversity include analysis of community structure based on extraction and chromatography of phospholipid fatty acids (Zelles, 1999) and analysis of community functioning based on utilization of selected substrates (Garland and Mills, 1991; Degens and Harris, 1997).

Soil quality can be viewed as the net effect of the difference between resilience and degradation. Resilience is soils inherent properties that provide it with the natural ability to recover following perturbation (Lal, 1998). Degradation is the loss of actual or potential productivity and utility (Lal, 1998). Both soil resilience and degradation are affected by management practices; such practices can influence soil quality in three different ways, by degrading, sustaining, or aggrading the quality (Figure 2.1).

Soil organic matter is thought to be a key attribute of soil quality (Gregorich *et al.*, 1994) since it influences physical, chemical and biological properties of soils. For example, it serves as both a source and sink for available nutrients, it profoundly affects the activities of microfloral and faunal soil organisms and it promotes good structure thereby improving soil tilth, aeration and retention of moisture (Stevenson, 1994). That is, in addition to being one of the chemical indicators of soil quality listed in Table 2.1, it also greatly influences many of the physical and biological indicators listed. For this reason, minimum data sets to assess soil organic matter content and quality in agricultural soils have been suggested as an integral part of soil quality evaluation (Larson and Pierce, 1991; Gregorich *et al.*, 1994; Doran *et al.*, 1996).

### **2.2.1 Soil organic matter and its importance**

Soil degradation is a major global issue because of its adverse impact on agricultural productivity and sustainability. The effects of cultivation on soil degradation, with the emphasis on soil organic matter content have been reported in Australia (Dalal and Meyer, 1986), Brazil (Lepsch *et al.*, 1994), Denmark (Schjøning *et al.*, 1994), Germany (Capriel *et al.*, 1992; Zhang, 1994), Italy (Saviozzi *et al.*, 1994), Nigeria (Hulugalle, 1994), UK (Jenkinson and Colemand, 1994), USA (Karlen *et al.*, 1994), New Zealand (Hart *et al.*, 1988; Haynes and Tregurtha, 1998), and South Africa (du Toit and du Preez, 1995). All these studies concluded that soil organic matter loss is generally the most important factor contributing to soil degradation under arable agriculture.

Soil organic matter comprises a range of humified and biologically active compounds, including readily decomposable material, plant litter and roots, and dead and living organisms (Stevenson, 1994). Litter originating from above and below ground plant parts such as coarse plant debris and colloidal and soluble organic compounds from root exudates are responsible for the principal input of organic carbon into soils, and soil microorganisms are largely responsible for degradation of organic matter.

The dynamic nature and complex chemistry of soil organic matter makes it a major source of plant nutrients. Approximately 95 % of soil nitrogen (N), 40 % of soil phosphorus (P) and 90 % of soil sulfur (S) are associated with the soil organic matter fraction (Stevenson, 1994). During decomposition microorganisms assimilate complex organic substances for energy, cellular carbon and nutrients and plant available mineral forms of nutrients ( $\text{NH}_4^+$ ,  $\text{H}_2\text{PO}_4^-$  and  $\text{SO}_4^{2-}$ ) are released (during mineralization).

The cation exchange capacity (CEC) improves with increased levels of soil organic matter (Russell, 1971). Humic substances contain carboxylic and phenolic groups which play a major role in retaining exchangeable inorganic ions. Upon ionization of the hydrogen ions from these groups, the negatively charged sites hold the cations ( $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ) against leaching, providing a temporary sink for plant nutrients.

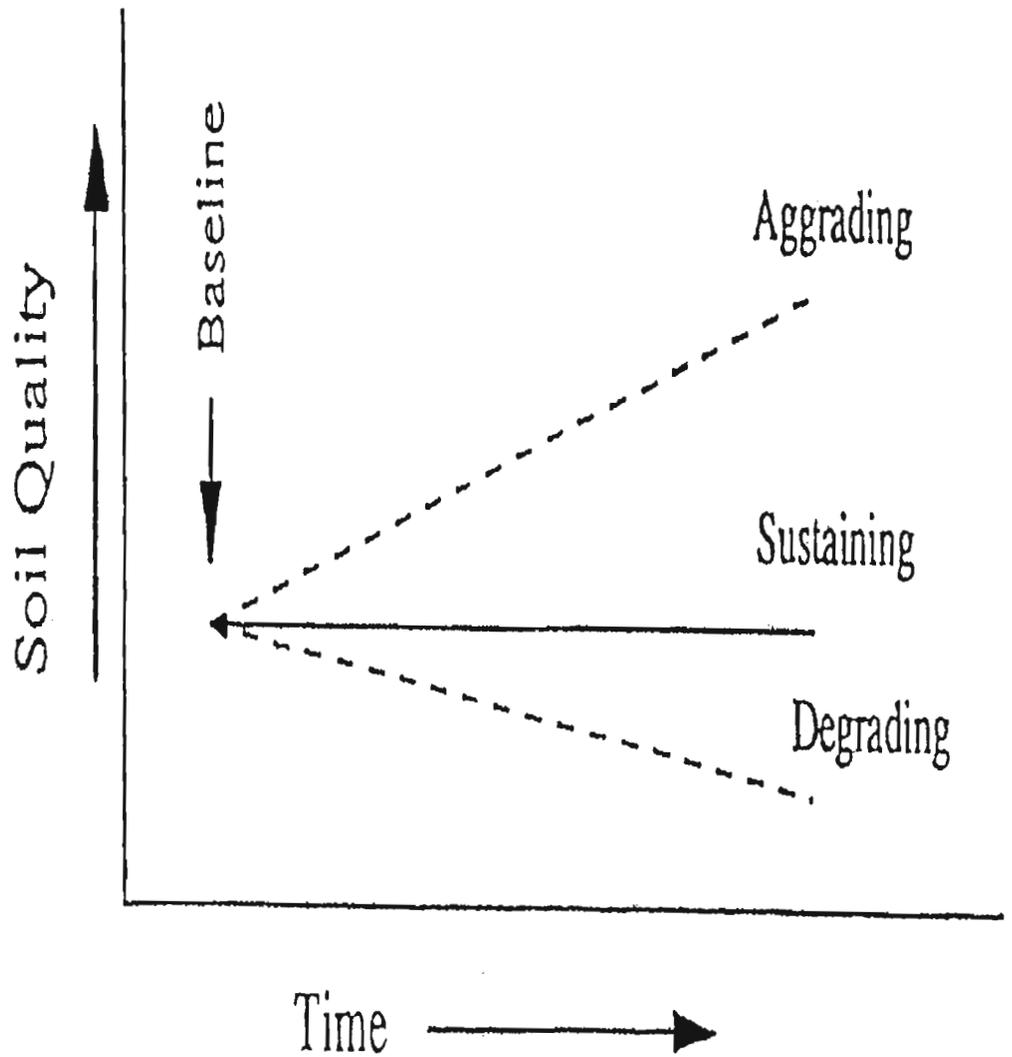
Soil organic matter has a profound effect on the structure of many soils. The organic matter acts as a binding agent for aggregate formation (Tisdall and Oades, 1982). These aggregates create a loose porous structure and result in better water infiltration, promoting water retention. Large pores also permit better gaseous exchange between the soil and the atmosphere improving aeration for microbial and plant root activity.

Adequate organic matter levels reduce the risk of compaction and simplify tillage operations and seedbed preparation (Soane and Kershaw, 1987). Other important functions of organic matter include its ability to buffer the soil against large changes in pH, its ability to form stable complexes with metal ions and its ability to complex xenobiotics such as herbicides and pesticides, affecting their bioactivity (Stevenson, 1994). It is clear that the loss of soil organic matter can have an adverse effect on soil properties and that the management of organic matter is important in maintaining soil quality and productivity.

**TABLE 2.1:** Proposed minimum data set of physical, chemical, and biological indicators for screening the quality of soils

<u>Indicators</u>	<u>Relationship to soil condition and function: rationale as a priority measurement</u>
	<b><u>Physical</u></b>
Texture	Retention and transport of water and chemicals; modeling use, soil erosion and variability estimate
Depth of soil rooting	Estimate of productivity potential and erosion; normalizes landscape & geographic variability
Infiltration and bulk density	Potential for leaching, productivity, and erosivity; bulk density: soil bulk density needed to adjust analyses to volumetric basis
Water holding	Related to water retention, transport, and erosivity; capacity available H <sub>2</sub> O; calculate from soil bulk density, texture, and organic matter
	<b><u>Chemical</u></b>
Soil organic matter	Defines soil fertility, stability, and erosion extent; organic matter; use in process models and for site normalization
pH	Defines biological and chemical activity thresholds; essential to process modeling
Electrical conductivity	Defines plant and microbial activity thresholds; presently lacking in most process models
Extractable N, P, and K	Plant available nutrients and potential for N loss; productivity and environmental quality indicators
	<b><u>Biological</u></b>
Microbial biomass C and N	Microbial catalytic potential and repository for C and N; modeling: warning of management effects on organic matter
Potentially	Soil productivity and N supplying potential; mineralizable N; process mineralizable N modeling (surrogate indicator of biomass)
Soil respiration	Microbial activity measure (in some cases plants); process modeling; Estimate of biomass activity

(Larson and Pierce, 1991).



**FIGURE 2.1:** Monitoring trends in soil quality over time can result in aggrading, sustaining, or degrading soil conditions (Lal, 1998).

### 2.2.2 Soil organic matter pools.

Organic matter can be partitioned into two major pools: “stable” and “labile” (Stevenson, 1994). The concept of pools of organic matter is based on the susceptibility of the organic matter fractions to microbial decomposition (Stevenson, 1994). The distribution of soil organic matter within functional pools is an important consideration in developing a better understanding of soil organic matter dynamics and the diverse roles of soil organic matter in ecosystems and on soil quality.

The stable organic matter pool consists mainly of relatively recalcitrant humus and makes up the bulk of the soil organic matter (70 - 80%) (Stevenson, 1994). It consists of complex polymeric organic compounds with a high molecular weight and are intimately associated with soil inorganic constituents. It is made up of a core of phenolic polymers produced from the products of biological degradation of plant and animal residues and the synthetic activity of microorganisms (Stevenson, 1994). The complex chemical structure of humic substances makes them more resistant to decomposition than non-humic, labile materials.

The labile pool comprises a small fraction of the total soil organic matter and can be sub-divided into a number of fractions including: light fraction organic matter, microbial biomass C and N, readily mineralizable C and N, easily extractable carbohydrate-C, and soil enzymes (Gregorich *et al.*, 1994; Stevenson, 1994).

Although total soil organic matter content is an important agronomic attribute, it is the size of the labile pools of organic matter that are particularly important in relation to nutrient supply (nutrient rich material), soil structure (as binding agents) and soil biological activity (as substrate) (Gregorich *et al.*, 1994). Such pools of organic matter are of particular interest in relation to changes in soil organic matter status induced by short-term changes in soil management practice. Thus, short-term changes in labile soil organic matter may be useful for predicting long-term changes in total soil organic matter (Dalal and Meyer, 1987; Janzen *et al.*, 1992; Bremer *et al.*, 1993). Due to their dynamic nature, pools of labile organic matter can respond

rapidly to changes in the rates of input or degradation of organic matter brought about by changes in crop residue management.

In the following sections a short description and discussion of the individual labile organic matter pools is provided since they are considered as key attributes of soil quality.

#### **2.2.2.1 Light fraction**

The light fraction consists mainly of plant residues in various stages of decomposition (Gregorich *et al.*, 1994). Residues derived from animal feces, macrofauna and microorganisms in various stages of decomposition may also be present. Chemical characterization of the light fraction has indicated that it is in an intermediate state of decomposition between the fresh organic material and soil humic material (Gregorich *et al.*, 1994). The light fraction usually represents about 0.1 to 4 % of the total mass of top soil and can constitute as much as 30 % of the total organic matter in some soils (Stevenson, 1994).

The light fraction pool is significant to soil organic matter turnover because it is generally free of mineral particles and therefore lacks protection from decomposers and serves as a readily decomposable substrate for microorganisms and as a major reservoir of mineralizable nutrients (Janzen *et al.*, 1992; Gregorich and Ellert, 1993). Its measurement thus provides information on the extent to which plant residues have been processed by the decomposer community in soil (Gregorich *et al.*, 1994). Studies with isotopic tracers indicate that despite a wide C/N ratio, light fraction organic matter has a faster turnover rate (5 - 25 years) than organic matter associated with sand, silt and clay-sized particles (Gregorich and Ellert, 1993). For example, Gregorich *et al.* (1995) reported, after a period of 25 years of maize cropping, that more than 70 % of the light fraction C had turned over whereas the corresponding value was only 16 % for C associated with the coarse silt fraction. The initial cultivation of virgin land typically results in a large loss of light fraction

organic matter (Cambardella and Elliott, 1992). Tiessen and Stewart (1983) reported a 40 % decrease in light fraction after only 4 years of cultivation when compared to a soil under native vegetation. Thus, the light fraction, may be used as an early predictor of organic matter changes (Cambardella and Elliott, 1992). This pool is responsive to management practices and may provide an earlier indication of the effects of crop residue management (Gregorich and Janzen, 1996) on soil quality.

#### **2.2.2.2 Readily mineralizable C and N**

More than 70% of soil organic matter exists as compounds that are slowly decomposable; the remainder is present as readily decomposable; or mineralizable compounds. The readily mineralizable C levels estimated in laboratory incubations (i.e. CO<sub>2</sub> evolution rate) reflect the total metabolic activity of the heterotrophic microbial community. Several incubation studies have shown that mineralizable C is directly related to the soluble C content which acts as a substrate for the microbial biomass. The mineralizable C potential fluctuates with seasonal changes (e.g. pattern of rhizodeposition) (Franzluebbers *et al.*, 1995), tillage methods and residue placement (Carter and Rennie, 1982), fertilizer application (Janzen, 1987) and crop rotations (Biederbeck *et al.*, 1984; Campbell *et al.*, 1991; Campbell *et al.*, 1992) indicating its sensitivity to different management practices.

Mineralizable N reflects a balance between gross mineralization and immobilization by the soil microbial biomass. These two processes occur simultaneously and the C:N ratio of the mineralizable fraction indicates the composition of this active fraction, reflecting net mineralization (narrow C:N ratio) or net immobilization (wide C:N ratio) (Gregorich *et al.*, 1994). The magnitude of net mineralization has important agronomic implications and is often measured to assess the capacity of soil organic matter to supply plant-available inorganic N, (NH<sub>4</sub><sup>+</sup> plus NO<sub>3</sub><sup>-</sup> - N).

### 2.2.2.3 Soil carbohydrates

Carbohydrates represent a significant pool of soil organic matter (5 - 20 % of total soil organic matter). Soil carbohydrates originate from plant, macrofauna and soil microorganisms. Most of the carbohydrate fractions are present as a mixture of complex polysaccharides.

Carbohydrates contribute to the formation and stabilization of soil macroaggregates (Haynes and Swift, 1990; Angers *et al.*, 1993). Their chemical structures indicate that they are also likely to be a readily-available source of energy for microorganisms (Gregorich *et al.*, 1994). Angers *et al.* (1993) found that the ratio of carbohydrate C to total organic C was greater under no-till than under moldboard-plowed soil, after only three cropping seasons, suggesting an enrichment of labile carbohydrates in the organic matter under reduced tillage. This illustrates the sensitivity of this pool to management practice.

### 2.2.2.4 Microbial biomass

The microbial biomass is defined as the living component of soil organic matter (Jenkinson and Ladd, 1981; Dalal, 1998) but excludes macrofauna and plant roots. The microbial component accounts for 1-3 % (Stevenson, 1994) and 2.6 % of soil organic C and N, respectively (Jenkinson, 1987). Microbial biomass functions both as an agent for the transformations and cycling of organic matter and plant nutrients within the soil, and as a sink (during net immobilization) or source (during net mineralization) of labile nutrients. The soil microbial biomass is also very important in maintaining soil structure (Haynes and Beare, 1996). Microorganisms exude mucilaginous carbohydrate material which acts as a glue and helps cement soil aggregates together (Tisdall and Oades, 1982). In addition, fungal hyphae can help bind aggregates by their enmeshing effects (Clarke *et al.*, 1967; Coughlan *et al.*, 1973; Forster, 1979).

The rate of microbial turnover in soil is typically 0.2 - 6 years compared to > 20 years for the bulk of the organic matter (Jenkinson, 1990). Due to its dynamic nature the soil microbial biomass serves as a labile organic matter pool that is a sensitive indicator and early predictor of changes in soil organic matter status (Powlson and Jenkinson, 1981; Powlson *et al.*, 1987) induced by changes in management practices such as residue management, tillage practice (Carter, 1986) and use of grass leys in rotation (Haynes *et al.*, 1991).

The amount of microbial biomass a soil can sustain depends on many factors including clay content, temperature, moisture, pH, soil organic matter content and soil management practice (Jenkinson and Ladd, 1981; Wardle, 1992). The microbial biomass is typically large under improved pastures (Yeates *et al.*, 1991; Sparling *et al.*, 1994; Haynes, 1999a) relatively low under conventional cultivation (Lynch and Panting, 1980; Haynes, 1999a) and increase in the surface layers under zero tillage (Gupta *et al.*, 1994).

Microbial biomass measurements are now commonly used in studies where the effects of land management on soil organic matter quality are being investigated (Haynes, 1999a). Indeed, many authors have suggested a strong link between soil microbial biomass, soil fertility and soil quality (Sparling, 1997).

#### **2.2.2.5 Microbial quotient**

The microbial quotient can be calculated by expressing microbial biomass C ( $C_{mic}$ ) as a percentage of total soil organic C [ $100(C_{mic} / C_{org})$ ]. By calculating this quotient, a more consistent trend in changes in organic matter during changes in land management can be calculated than by evaluating  $C_{mic}$  and  $C_{org}$  by themselves. Because the quotient is expressed as a percentage, it avoids the problems of working with absolute values and comparing across soils with different, organic matter contents (Sparling, 1997). Generally, if a soil is being used exploitively, then the microbial biomass will decline at a faster rate than the total organic matter, and

the microbial quotient will decrease.

#### **2.2.2.6 Basal respiration**

Soil respiration ( $O_2$  consumption or  $CO_2$  evolution) is often used as a measure of residue decomposition rate in soils. Nonetheless, soil respiration rates under field conditions often show wide fluctuations depending on substrate availability, moisture and temperature (Sparling, 1997). The great variability in values makes it very difficult to use them as measures of soil quality. For valid comparisons between soils, respiration rates are normally made in the laboratory under conditions where temperature and moisture are not limiting (Anderson, 1982). This measurement is generally termed as basal respiration and it provides an indication of organic matter quality and whether the soil environment is conducive to the decomposition process.

#### **2.2.2.7 Metabolic quotient**

The metabolic quotient ( $qCO_2$ ) is a measure of microbial respiration per unit of microbial biomass ( $\mu gCO_2-C$  evolved  $g^{-1}$  biomass C  $day^{-1}$ ) which incorporates both changes in microbial population size and respiratory rate in one value (Wardle and Ghani, 1995). Anderson and Domsch (1993) proposed the  $qCO_2$  as a measure of changes in microbial activity in response to disturbances (i.e. Rewetting of dry soil, herbicide application, acidification and substrate addition). In order to survive in such hostile environments, the microbial population puts up defense mechanisms by increasing its respiration per unit of biomass (Anderson and Domsch, 1993). Calculating the  $qCO_2$  thus gives an indication of what proportion of C is incorporated into cellular C, rather than respired as  $CO_2$ . Therefore, a high  $qCO_2$  indicate that the microbial community is stressed and is tending to respire metabolized C as  $CO_2$  rather than of incorporating it into their biomass. Conversely, in favorable conditions the microorganisms actively incorporate the C into their biomass, resulting in a larger population and a lower  $qCO_2$ .

### 2.2.2.8 Soil enzymes

Soil enzymes are proteins that are synthesized by plants and more particularly soil microorganisms, during metabolism (Dick, 1994). They are present in soils in living and dead cells or complexed with organic and mineral colloids. Soil enzymes mediate biochemical transformations, including those involved in organic residue decomposition and nutrient cycling (Skujins, 1978; Burns, 1978).

Various enzyme assays involving the measurement of the activities of a combination of enzymes have been developed. For example, assays such as arginine ammonification (Alef and Kleiner, 1987) and fluorescein diacetate hydrolysis (Adam and Duncan, 2001), are used as an index of overall microbial activity (Gregorich *et al.*, 1994). The activity of endocellular enzymes such as dehydrogenase is also used as indices of microbial activity (Tabatabai, 1994). Dehydrogenase activity reflects the total oxidative activities of soil microflora and has been used in many studies to show the effects of anthropogenic activities on soil quality (Haynes and Williams, 1992).

The activity of specific enzymes involved in mineralization of organic C, N, P and S are often assayed since they are important in relation to C, N, P and S cycling in soils (Dick and Tabatabai, 1993). These enzymes are predominantly extracellular and are released by microorganisms in order to breakdown organic compounds present in the soil matrix. The activities of exocellular enzymes are not necessarily directly related to soil microbial activity since these enzymes can be released in response to a shortage of a specific nutrient, can be subject to end product repression, they can persist in soils in active forms through sorption to organic and inorganic colloids, and their activity is affected by the nature of the soil environment (i.e. pH, ionic strength) (Dick and Tabatabai, 1993).

Methods used to measure the activity of soil enzymes are generally simple, rapid, precise and reproducible (Tabatabai, 1994). Although soil enzyme activities are

often roughly proportional to the content of soil organic matter (Skujins, 1967; Baligar and Wright, 1991; Baligar *et al.*, 1991; Haynes and Tregurtha, 1998) their activities have been used successfully to discriminate between a wide range of soil management practices such as fertilizer applications, crop residue and other organic inputs, changes in tillage practice, land use, and crop rotation (Dick and Tabatabai, 1993; Dick, 1994). Enzyme activities are now generally considered as appropriate and sensitive biochemical indicators of soil quality (Dick, 1994).

### **2.2.3 Microbial diversity**

Biodiversity is an expression of the variety of living things, at genetic, species and ecosystem levels (Harper and Hawksworth, 1994). The diversity of the microbial population is more extensive than that of any other group of organisms in the world. It has been estimated that between 1 and 5 % of all microorganisms on earth have been classified and named (Ward *et al.*, 1992). As a result of the large numbers of microorganisms in soils, their high diversity, the difficulties of culturing them in the laboratory and the problems of adequately defining species of different microorganisms, approaches to measuring biodiversity based on the qualitative description of communities rather than species have been developed. Understanding the full extent of the biological cycles and transformations in ecosystems requires knowledge of microbial communities and their functions (Kennedy and Smith, 1995).

#### **2.2.3.1 Phospholipid fatty acids analysis (PLFA)**

The living microbiological component of a soil community can be estimated by phospholipid fatty acid analysis (PLFA) because they are found only in the membranes of living cells, not as storage products of microorganisms (Zelles *et al.*, 1994). Phospholipid profiles also provide precise measures of total biomass numbers (Zelles *et al.*, 1992). Phospholipid fatty acids are extracted from the soil and analysed by gas chromatography. Specific peaks have been identified as

biomarkers for specific groups of microorganisms. The 10Me18:0 peak may indicate actinomycetes (Kroppenstedt, 1995), the 18:2w6 PLFA peak may indicate fungi (Federle, 1986) and 15:0 and 17:0 peaks may indicate bacteria (Cavigelli *et al.*, 1995). By exploiting the relationships of certain peaks further, we can not only obtain a fingerprint of the community structure, but we can monitor shifts in specific functional subsets of the community.

Biomarkers can be used to indicate a shift in community structure. Pennanen *et al.* (1996) showed a gradual change in soil microbial community along heavy metal pollution gradients. Similarly, Pankhurst *et al.* (1996) also revealed a shift in microbial communities with increasing salt concentrations (saline soils). Crop management practices may also cause a community shift by increasing resource heterogeneity (Wander *et al.*, 1995). This can provide valuable information on the functioning of soil microorganisms under disturbed conditions (Kennedy and Smith, 1995).

#### **2.2.3.2 Functional diversity**

Substrate utilization patterns have been used to obtain fingerprints of community structure (Zak *et al.*, 1994; Wunsche *et al.*, 1995; Bossio and Scow, 1995; Haack *et al.*, 1995; Garland, 1996; Degens and Harris, 1997). These measures may also provide indications of the metabolic potential of the microbial populations (Haack *et al.*, 1995). The soil bacterial communities is assessed by incubating soil suspensions in BIOLOG microtitre plates containing tetrazolium salts to detect microbial growth in 95 different C substrates (Garland and Mills, 1991; Zak *et al.*, 1994). However, there is conflict whether the BIOLOG technique provides an accurate assessment of the functional diversity of the whole microbial community in soils.

Generally, fewer than 1 % of microorganisms in soils can be cultured on agar plates (Domsch *et al.*, 1979) and the environment in microtitre wells is not greatly different from that on agar plates. It would, therefore, be unlikely that the microtitre

environment would encourage growth of a greater proportion of microorganisms in soils than can be grown on agar plates.

An alternative approach to assess the functional diversity of the soil microbial community was developed by Degens and Harris, (1997) based on a modification of the substrate induced respiration method. This is achieved by adding various substrates to the soil and measuring the CO<sub>2</sub> evolution after a short-term incubation (0 - 4h) to measure the response of the initial microbial community in the soil before growth of microorganisms occurs on the added substrate (Degens and Harris, 1997).

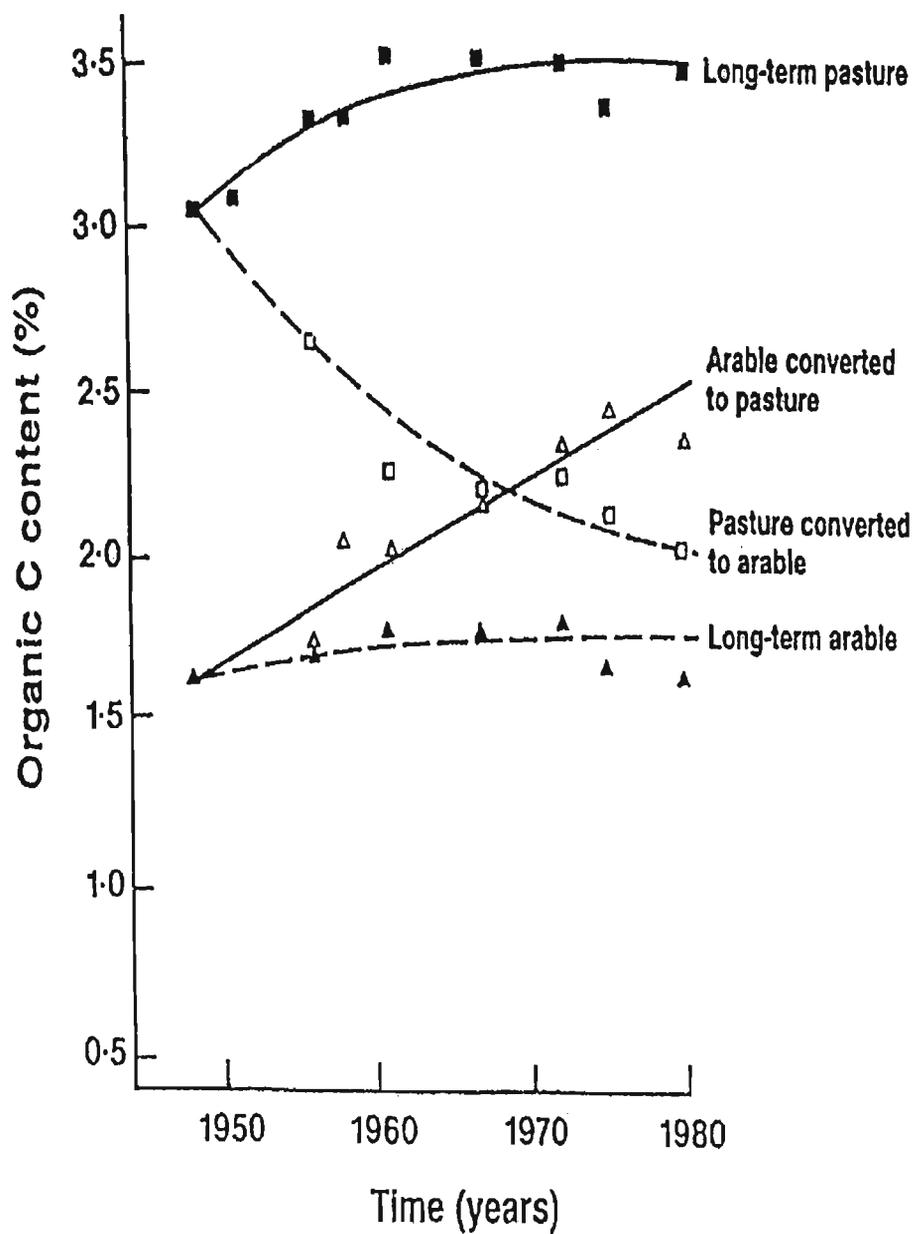
The use of substrate utilization to describe the functional diversity of microorganisms in the soil is increasing since it is a sensitive indicator of ecosystem functioning and for evaluating disturbed or contaminated systems (Kennedy and Smith, 1995). Functional diversity increases with increasing organic matter input and with rotation of crops (Degens *et al.*, 2000). However, more research in this field is needed.

#### **2.2.4 Effects of agricultural practice on soil organic matter content**

The organic matter content of soil is determined by the equilibrium between factors which determine its formation and those which promote its breakdown. The former includes the quantity and quality of the organic input to soil and the latter the complex of factors which determine the rate of oxidation of the organic matter. The formation of a new equilibrium organic matter content will therefore depend on management practices, particularly those affecting the inputs to and outputs of organic matter to and from the system. Soil organic matter content is generally high under natural or improved grasslands (Haynes and Beare, 1996). A typical temperate grassland may contain 5 - 6 % organic matter in the top 150 mm of soil. Organic matter inputs under grassland arise from large amounts of plant tops and roots, exudation of organic compounds from roots, and the turnover of the large microbial biomass in the rhizosphere (the area immediately surrounding the roots). The concentration of organic matter is highest near the surface and decreases steadily down the profile (Haynes, 1999b).

When native forest or grassland is brought under continuous arable cultivation there is appreciable breakdown of soil organic matter (Dalal and Meyer, 1986; Johnston, 1986; Haynes and Williams, 1992). The loss of organic matter with cultivation is usually exponential, declining rapidly during the first 10 - 20 years, then more slowly, and finally approaching a new equilibrium in 50 - 60 years (Haynes and Beare, 1996). Such a decline is the result of increasing the rate of organic matter oxidation during tillage operations by exposing organic matter that was previously protected by aggregate structure. Cultivation also increases the aeration status of the soil thus promoting decomposition. As a result, rapid decomposition and loss of native organic C and mineralization of organic N, P and S at parallel rates occurs following tillage. In addition, the amount of organic matter returned to the soil is generally also much lower. This reduction of organic matter input is due to the fact that the crop plants are usually spaced widely apart in rows resulting in less root and top material produced per unit of ground area and often much of the above-ground plant material is removed from the field with, or as, the harvested crop. Erosion of the topsoil can also contribute to the loss of soil organic matter.

Results presented in Figure 2.2 demonstrate such a decline in organic matter, when a long-term grassland site was put under arable cropping. One site had been under grass for at least 100 years and contained  $31 \text{ g C kg}^{-1}$ . When this long-term

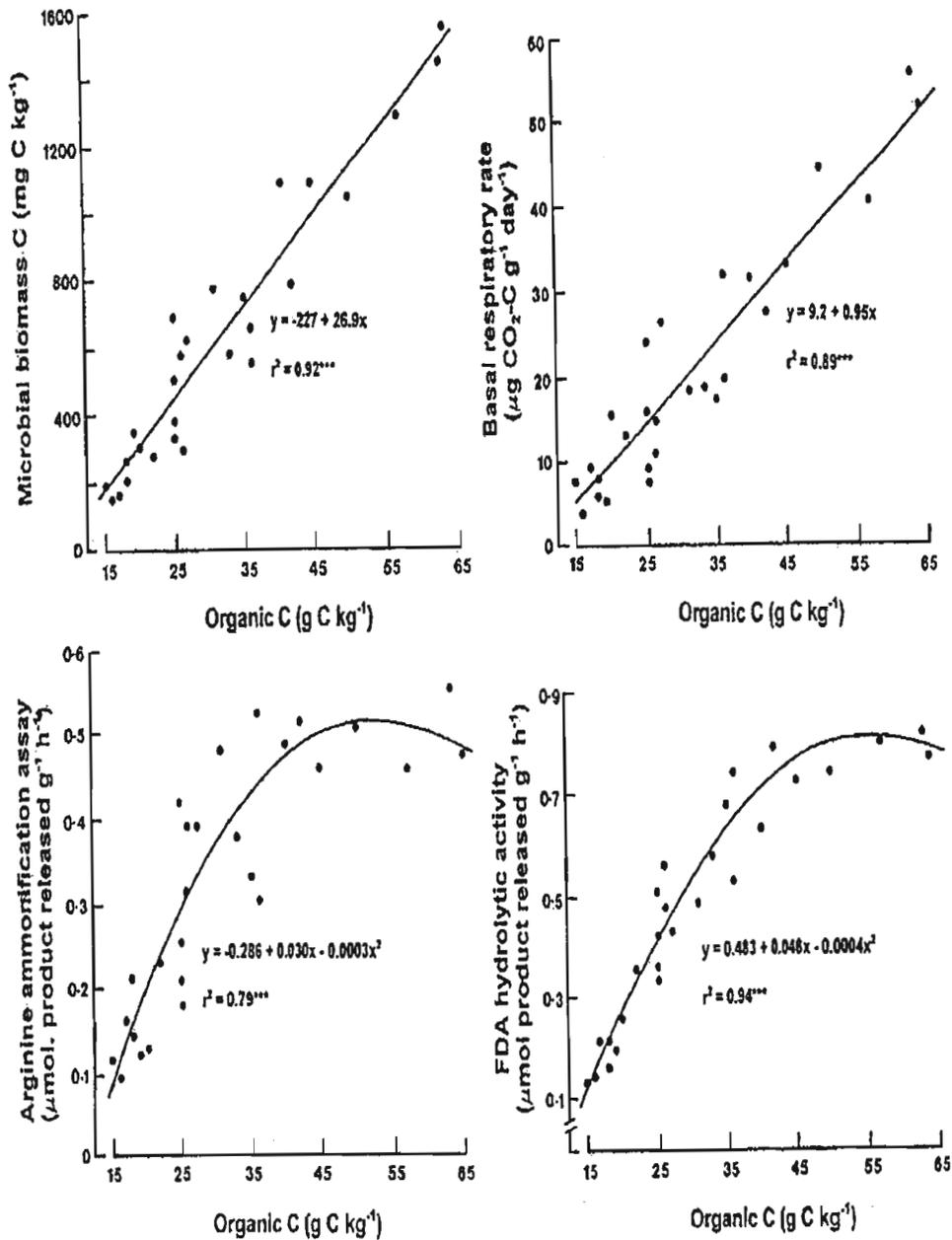


**FIGURE 2.2:** Organic C content of soils from the Rothamsted ley-arable experiment. Treatments consist of: long-term grassland, long-term grassland soil converted to arable, long-term arable and long-term arable soil converted to grassland (Johnston, 1986).

grassland site was put under arable cropping there was a rapid decline of  $9 \text{ g C kg}^{-1}$  during the first 15 years and a further slow decline of  $2 \text{ g C kg}^{-1}$  during the following 15 years.

A loss of soil organic matter content induced by arable cultivation generally results in a concomitant decrease in soil microbial activity. For example, Haynes and Tregurtha (1998) investigated the effects of long-term intensive vegetable production on soil degradation in the Pukekohe region of the North Island of New Zealand. The organic C content declined exponentially from  $65 \text{ g C kg}^{-1}$  to  $15 \text{ g C kg}^{-1}$  over a period of 80 years. The decline in organic matter content resulted in a linear decrease in the size of the soil microbial biomass and basal respiration and a curvilinear decline in microbial activity, measured as arginine ammonification and FDA hydrolysis rate (Figure 2.3).

As noted previously, changes in labile organic matter pools can often be detected in response to changes in soil management before any measurable changes in total organic C occur. The main reason for this is that the background level of soil organic matter is relatively large so that it is difficult to measure small changes in content that may occur in the short-term. For example, results from the South Island of New Zealand under an eight year rotation (four years of pasture followed by four years of arable crops) illustrates this point (Table 2.2). During the pasture phase of the rotation there is a development of a dense ramified root system and as a result, a large soil microbial biomass develops. The microbial biomass produces carbohydrates which act as binding agents, resulting in an increase in aggregate stability (Haynes *et al.*, 1991). When the field is returned to arable cultivation the dense pasture root system is replaced by a more sparse crop root system. As a result there is a rapid decline in soil microbial biomass and hot water extractable carbohydrates. The labile pools rapidly increase under short-term (4 year) pasture and decline under short-term arable management, yet total organic C content remains relatively unaffected.



**FIGURE 2.3:** Relationship between organic C content and microbial biomass C, basal respiration rate, arginine ammonification rate and fluorescein diacetate hydrolytic activity for a volcanic soil from New Zealand. Regression equation, line of best fit and statistical significance shown. \*\*P<0.01, \*\*\*P<0.001 ( Haynes and Tregurtha, 1998).

**TABLE 2.2:** Effect of previous cropping history on aggregate stability, organic C, acid hydrolysable and hot water extractable carbohydrate and biomass C in a New Zealand soil.

Previous cropping history	Aggregate stability (MWD,mm)	Organic C (%)	Acid-hydrolysable carbohydrate (%C)	Hot water-extractable carbohydrate ( $\mu\text{g C}^{-1}$ )	Biomass C ( $\mu\text{g C g}^{-1}$ )
18 year pasture	2.7	3.2	0.35	28	1018
4 year pasture <sup>1</sup>	2.5	2.5	0.26	169	....890
1 year pasture	2.0	2.4.....	0.25.....	152	....801
1 year arable	1.3	2.4	0.23.....	140	....738
4 year arable	1.2.....	2.4	0.23	134	....712
10 year arable	1.0.....	2.0.....	0.19.....	127	....610

<sup>1</sup> The 1 year and 4 year pasture and 1 year arable soils come from a cropping rotation of 4 years arable followed by 4 years pasture (Haynes *et al.*, 1991).

### 2.3 Sugarcane production and its effect on soil quality

In many tropical countries sugarcane (*Saccharum officinarum*) is an important plantation crop and its production has increased dramatically in the last few decades (Hartemink and Wood, 1998). Despite this, the sugar yield in many parts of the world, when expressed on a per hectare basis, has reached a plateau or even begun to decline. This phenomenon, which has become known as “sugar yield decline”, has been reported in Australia (Garside, 1997), USA (Coleman, 1974) and Barbados (Anderson *et al.*, 1995) as well as South Africa (Meyer *et al.*, 1996).

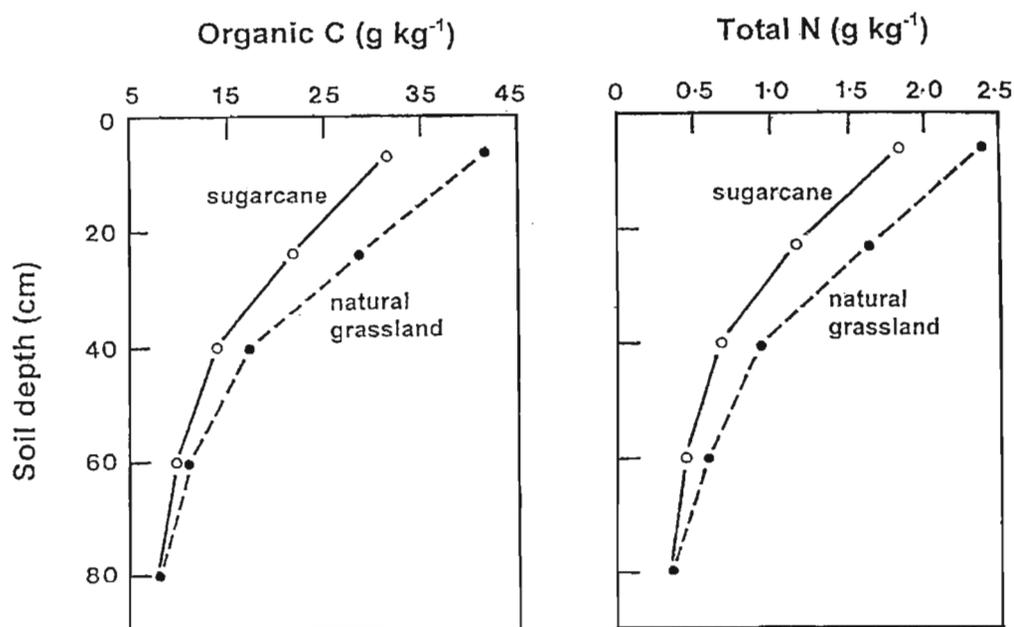
Initially this decline in Australia was associated with the root pathogen *Pachymetra chaunorhiza*. The isolation of *P. chaunorhiza* led to the engineering of resistant sugarcane varieties, increasing yield up to 40 % (Magarey, 1993; 1996). Nevertheless, a subsequent increase in yield was obtained when soil was fumigated, suggesting that factors other than *P. chaunorhiza* root rot were involved (Croft *et al.*, 1984). Limited success was obtained with isolation of other pathogenic fungi (Magarey *et al.*, 1995). Garside, (1996) suggested that a build up of root pathogens may simply have been the ultimate expression of other factors being out of balance

in the farming system. The focus shifted and "yield decline" was nominated as one of the major causes of an industry-wide productivity plateau. Yield decline is defined as the loss of productive capacity of soil under sugarcane monoculture (Garside, 1996). This realization drew attention to the importance of evaluating the effect that sugarcane monocropping systems have on soil quality (Haynes, 1997).

### 2.3.1 Soil organic matter content

As noted previously, decline in soil organic matter content when virgin sites are converted to arable cropping systems is common. When virgin soil is put under sugarcane production, an initial decrease in soil organic matter (i.e. organic C and total N content) is also observed (Figure 2.4). Such a decline has been observed in Fiji (Masilaca *et al.*, 1985), the Philippines (Alaban *et al.*, 1990), Swaziland (Henry and Ellis, 1995), Papua New Guinea (Hartemink and Kuniata, 1996; Hartemink, 1998a) and Australia (Wood, 1985) as well as South Africa (van Antwerpen and Meyer, 1996).

In a survey of soils in northern Queensland, Wood (1985) observed substantial losses of soil organic matter in the surface 10 cm of soil under sugarcane production. The mean values for soil organic C content for 19 paired sites were 1.5 % for virgin sites and 0.7 % for those under long-term sugarcane production. When three Oxisols were put under sugarcane production, in Fiji, there was a marked initial decrease in soil organic C in the topsoil (0 - 12 cm) (Masilaca *et al.*, 1985). The organic matter content reached a new equilibrium and stabilized at about two thirds of the original levels. Similarly in northern KwaZulu-Natal, in South Africa, van Antwerpen and Meyer (1996) observed a loss of organic matter under sugarcane relative to virgin sites on both dry-land and irrigated areas. In Swaziland, Henry and Ellis (1995) observed a loss of 2 g C kg<sup>-1</sup> between cultivated sugarcane soil and a paired virgin site after about 15 years.



**FIGURE 2.4:** Soil organic C and total N content down the soil profile after seven years of sugarcane production compared to under natural grassland on an Entisol in Papua New Guinea (Hartemink, 1998a).

To some extent such a marked decline in organic matter is surprising since sugarcane soils are usually cultivated every 5 - 10 years. That is, a planted crop is followed by 4 - 9 years of ratoon crops before the crop is removed, the ground is cultivated and a new crop is planted. Nonetheless, under sugarcane production using the conventional practice of pre-harvest burning (the common practice in South Africa) organic matter inputs to the soil are likely to be very small particularly in the inter-row area (Haynes and Hamilton, 1999).

In northern Queensland, over 70% of sugarcane is presently green cane harvested rather than pre-harvest burnt. Recent studies in Queensland, showed little change in soil organic C under long-term sugarcane production. Skjemstad *et al.* (1990) and Bramley *et al.* (1996) suggested that the relatively recent adoption of green cane harvesting with retention of a trash blanket may have improved depleted soil organic

matter levels.

### 2.3.2 Soil biological properties

Very little is known about the changes in soil biological properties that occur under sugarcane cultivation. In Queensland, Garside *et al.* (1997) observed, in a comparison between paired old and new land sites of sugarcane, that the soil microbial biomass was significantly lower in the older sugarcane land. Similarly McGarry *et al.* (1996) reported a 32% decline in microbial biomass when comparing sites that had been under sugarcane production for 10 years with an uncultivated site. They concluded that there is a rapid loss of soil microbial biomass C when sugarcane is established. These results coincide with the decrease in overall microbial activity reported by Henry and Ellis (1995) in Swaziland.

Current research on soil quality is centered very much on the use of biological indicators (Pankhurst *et al.*, 1997). The effect of sugarcane production under various crop residue management systems on soil biological activity is thus an area requiring considerable future research.

### 2.3.3 Soil physical conditions

Soil compaction is a common problem in sugarcane fields (Braunack, 1997). Wood (1985) compared the bulk density in the surface 8 cm of sugarcane soil to that of uncultivated land and found a significant increase of 0.15 to 0.18 Mg m<sup>-3</sup>. Generally, topsoil bulk density is significantly greater in the inter-row spaces than in the rows of the sugarcane fields. This inter-row compaction is generally caused by wheeled traffic during harvesting and field operations, and occurs to a greater extent during wet harvesting seasons when farmers are forced to harvest some of their sugarcane when the soil is very wet (Soane and Kershaw, 1987). Such compaction in the inter-row has been reported in Australia (Wood, 1985; McGarry *et al.*, 1996),

Fiji (Masilaca *et al.*, 1985), Papua New Guinea (Hartemink, 1998a), India (Srivastava, 1984), Hawaii (Trowse and Humbert, 1961), Colombia (Torres and Villegas, 1993) and South Africa (Johnston and Wood, 1971; Swinford and Boevey, 1984). The rutting and compaction of the wheeled harvesters in the inter-rows can also result in yield reduction in the following crop due to stool damage (Wood, 1985; Torres and Villegas, 1993; Meyer *et al.*, 1996). Each sugarcane row becomes straddled and this has an effect of lateral displacement of the soil, which causes stool compression, restricted infiltration and surface drainage.

Compaction and the consequent increase in bulk density result in a marked decrease in soil macroporosity. Van Antwerpen and Meyer (1996) reported that compaction can disturb the balance between water and air space and reduce plant available water. Together with increased penetration resistance, these two factors are often the cause for poor root proliferation in the inter-rows (Trowse and Humbert, 1961; Juang and Uehara, 1971; Swinford and Boevey, 1984). Limits at which bulk density restrict root growth of sugarcane vary depending on soil type (Trowse and Humbert, 1961). For Hawaiian Andosols and Ferralsols limits were found to be 1.8 and 1.52 Mg m<sup>-3</sup> respectively (Masilaca *et al.*, 1985).

#### **2.3.4 Soil nutrient availability**

During sugarcane cropping, large amounts of nutrients are extracted from the soil annually. For example, De Geus (1973) calculated that a crop of approximately 100 t ha<sup>-1</sup> removes about 120 kg N ha<sup>-1</sup>, 33 kg P ha<sup>-1</sup> and 125 kg K ha<sup>-1</sup> annually. Potassium removals during sugarcane cropping are notably high. This can lead to a rapid decline of exchangeable K levels in the soil. Declines in exchangeable K have been noted in a number of sugarcane producing areas including the Philippines (Alaban *et al.*, 1990), Fiji (Masilaca *et al.*, 1985), Swaziland (Henry and Ellis, 1995) and Papua New Guinea (Hartemink, 1998a). To ameliorate this effect, an adequate amount of fertilizer K needs to be applied (Meyer *et al.*, 1998). Soil phosphorus levels are affected in the same way, declining when no fertilizer is applied

(Hartemink, 1998a), and increasing when large amounts of fertilizer is applied regularly (Wood, 1985).

The loss of soil organic matter under sugarcane production (see section 2.3.1) means that the N-supplying capacity of the soil, through N mineralization, is much reduced. As a result, large applications of fertilizer N are often applied to sugarcane in order to balance the large N uptake by the crop and the large removals that accrue at cane harvest (Haynes and Hamilton, 1999).

### 2.3.5 Soil pH

In general, changes in soil chemical properties under sugarcane production are consistent, with soil acidification occurring. This means that a decline in soil pH is accompanied by an increase in exchange acidity, exchangeable and extractable Al and a decline in exchangeable bases (Ca, Mg and sometimes K) and a decrease in cation exchange capacity (Wood, 1985; Bramley *et al.*, 1996; van Antwerpen and Meyer, 1996; Hartemink, 1998a, b). Wood (1985), for example, compared 19 paired sites in the Herbert Valley in northern Queensland and demonstrated that sites under sugarcane had lower pH, lower exchangeable Ca and Mg, lower cation exchange capacity and higher exchange acidity than adjacent, uncultivated land.

In South Africa, soil acidification has been clearly demonstrated under sugarcane production. Schroeder *et al.* (1994) reported a general decline in pH values with an accompanying decrease in base cation concentrations and an increase in extractable Al on commercial sugarcane fields on the south coast of KwaZulu-Natal. They suggested that a decrease in pH is common irrespective of soil type although it was most apparent for sugarcane grown on recent sand deposits. More recently, Meyer *et al.*, (1998), using data collected from the Fertilizer Advisory Service of the South African Sugar Association Experiment Station, showed that in the last decade

there has been a marked increase in soil acidification. Average soil  $\text{pH}_{(\text{water})}$  values declined from 6.2 in 1980-81 to 5.6 in 1996-97. The extent of acidification was shown by an increase in the proportion of soil samples that were below pH 5.0 from 18% in 1980 to 43 % in 1997. The trend towards soil acidification was centered on samples originating primarily from the northern and southern coast of KwaZulu-Natal.

The main cause of acidification is generally thought to be the use of ammonium - containing or forming fertilizers which are routinely applied to sugarcane at rates of  $100 - 250 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  (Abruna-Rodriquez and Vicente-Chandler, 1967; Pierre *et al.*, 1971; Schroeder *et al.*, 1994). When  $\text{NH}_4^+$  is nitrified to  $\text{NO}_3^-$  through the process of nitrification, two  $\text{H}^+$  ions are released per unit of  $\text{NO}_3^-$  produced. If  $\text{NO}_3^-$  is then lost from the soil (e.g. by leaching) the  $\text{H}^+$  ions are left in the soil and acidification is essentially permanent (Wild, 1994).

### 2.3.6 Soil salinisation / sodification

Improper irrigation and drainage are the main causes of salinisation (Johnston, 1977; Workman *et al.*, 1986). In some areas, where low amounts of rainfall occur, excessive salt concentrations have been recorded. These areas include Australia (Ham *et al.*, 1997; Nelson and Ham, 1998), Egypt (Nour *et al.*, 1989), Iraq (Sehgal *et al.*, 1980), United States (Bernstein *et al.*, 1966), India (Tiwari *et al.*, 1997), Swaziland (Workman *et al.*, 1986) and South Africa (Maud, 1960; von der Meden, 1966). Soils with excessive salt concentrations (saline soils) are soils where soluble salt concentrations restrict plant growth. Due to a rise in the water table, salts dissolved in the ground water accumulate on the soil surface by an upward capillary movement of the water. This is induced through excessive irrigation and poor drainage. The use of saline water for irrigation can also contribute to soil salinity (von der Meden, 1966).

Many saline soils are also sodic, where the amount in exchangeable  $\text{Na}^+$  expressed as the exchangeable sodium percentage (ESP) is above 15% (Sumner, 1993;

Sumner and Naidu, 1997). Sodic soil tends to disperse under irrigation or rainfall, causing a deterioration of soil structure. Saline-sodic soils generally have a reasonable soil structure but once the excess salts are leached away, sodic soil conditions prevail and dispersion occurs.

Nelson and Ham (1998), recorded a significant decline in sugarcane yields due to soil salinity in northern Queensland. They recorded that sugarcane yield was reduced by approximately  $2.4 \text{ t ha}^{-1}$  for every 1 % increase in ESP in the surface soil. Similarly a number of other cases of yield decreases have been reported (Maud, 1960; Bernstein *et al.*, 1966; Sehgal *et al.*, 1980; Culverwell and Swinford, 1986; Nour *et al.*, 1989; Ham *et al.*, 1997). By controlling irrigation and drainage, soil salinity and sodicity can be ameliorated and controlled. In salinised areas, the excess salt can be leached away by periodic irrigation where appropriate drainage systems have been installed (Sumner, 1997). In cases of sodic soils, much of the exchangeable  $\text{Na}^+$  can be replaced with  $\text{Ca}^{2+}$  by adding gypsum (Johnston, 1977; McMahon *et al.*, 1996). Through irrigation the excess  $\text{Na}^+$  is then leached out.

#### **2.4 The effect of crop residue management on soil quality**

In this section, the emphasis will focus on general information regarding the removal (during burning) or addition of an organic matter source, in the form of crop residues (during mulching), on soil quality. Data from sugarcane research is included where it is appropriate.

Burning is traditionally used to clear land of crop residues quickly prior to establishing the next crop, ensuring that tillage operations are not restricted by the residues, to facilitate seedbed preparation, and also as a substitute for herbicides and pesticides in controlling weed, pest and disease carry-over from one crop to the next (Elliot and Papendick, 1984). However, burning residues is now banned in many parts of Europe and the USA, mainly due to public nuisance complaints, farm labour

health and environmental pollution. However, in South Africa, off-farm uses of crop residues are limited, therefore most residues have to be managed on the farm. Information suggests that the residues of most small grain cereals, maize and sugarcane are burnt.

The most practicable way of maintaining or improving soil organic matter under sugarcane production, is green cane harvesting with trash retention (Wood, 1985; Meyer *et al.*, 1996). Both Wood (1985) and Meyer *et al.* (1996) have advocated the practice as a long-term strategy to reduce soil organic matter depletion and improve soil quality. Green cane harvesting with the retention of a trash blanket is a residue management practice that has recently gained interest in the sugarcane industry and is now a common practice in Australia (Sutton *et al.*, 1996; Garside *et al.*, 1997), Mauritius (McIntyre *et al.*, 1996) and Brazil (Blair *et al.*, 1998).

The concept of green cane harvesting has been with the sugar industry from the start. However various pests, diseases amongst laborers as well as insufficient labour initiated burning of sugarcane fields before harvesting. The introduction of burning cane resulted in a reduction of labour cost, and damage due to pests was mitigated encouraging the practice. The re-birth of green cane harvesting in Queensland occurred during the mid 1970's, primarily as a response to the need for cane harvest in wet weather (when burning was not possible) in order to provide a continuous supply of sugarcane to the mill.

Burning of crop residues may have both short-term and long-term effect on soil properties. The short-term effects involve the direct effect of fire, in which heat and /or ash affect particular soil properties at the time of burning. This occurs at the soil surface and may include changes to soil organic matter (Biederbeck *et al.*, 1980; Haines and Uren, 1990; Carter and Mele, 1992) and nutrient levels (Biederbeck *et al.*, 1980), microflora and microfauna activity and numbers (Biederbeck *et al.*, 1980; Haines and Uren, 1990; Carter and Mele, 1992), aggregate stability (Unger *et al.*, 1973; Dormaar *et al.*, 1979; Carter and Mele, 1992), and soil hydraulic properties

(Biederbeck *et al.*, 1980; Valzano *et al.*, 1997). Since burning is a means of removing residues and only small amount of above ground organic material is returned to the soil it result in depletion of organic matter levels in the long-term. The inter-rows are left fallow and the soil surface is exposed to the effects of wind and water resulting in erosion and lower aggregate stability (Raison, 1979).

Crop residues are a potential resource that could assist in stabilizing agricultural ecosystems because they contain nutrients extracted from the soil and are a source of newly fixed C, that can push the equilibrium in the opposite direction and replenish that which is lost by oxidation. The retention of crop residues on the soil surface, as a mulch, can have multiple effects on soil microclimate, physical conditions, organic matter, nutrient mineralization, soil erosion, and the localization and activity of soil fauna and microflora. However, most farmers perception is that mulch controls soil loss and promotes plant growth, but the mechanisms underpinning these effects are complex and, in many cases, not well understood.

## **2.4.1 Soil organic matter**

### **2.4.1.1 Burning of crop residues**

During the burning of crop residues, combustion is generally incomplete. An estimated 50 - 70 % of the residue carbon is lost (Rasmussen and Collins, 1991) as CO<sub>2</sub> and CO. The ash is black and contains residual carbon (Raison, 1979), which is mostly biologically inactive (i.e. charcoal-C) (Raison, 1979). Consequently, burning can change both the quantity and the quality of organic matter returned to the soil. Biederbeck *et al.* (1980), for example, reported an accelerated loss of organic C and total N where cereal straw had been burned for over 20 years (Figure 2.5). On the experimental site, soil initially contained 54 g C kg<sup>-1</sup>. The organic C and total N content remained relatively unaffected during the first 10 years of burning and the major decline occurred after a period of 10 -20 years. The results of Haines and Uren, (1990) also showed that after a period of 10 years of burning crop residues,

the organic C was not significantly different from treatments with surface applied residues. Similarly, Thompson (1992) reported a decrease of only 1.1 g organic C kg<sup>-1</sup> after 11 years of burning. The root biomass is relatively unaffected during the burning process (Biederbeck *et al.*, 1980) and can contribute significantly in maintaining the organic C content (Whipps, 1990). Nevertheless, in the long-term (>10 years) the organic matter contribution from mainly below ground plant material is not sufficient to main the organic matter levels and as a result organic matter levels tend to decrease steadily (Biederbeck *et al.*, 1980).

As expected, the labile, easily mineralizable, organic matter fractions are rapidly lost under arable cultivation where crop residues are burnt. Feller *et al.* (1987) showed that burning of crop residues on a sandy soil, cultivated for 3 years with a millet/peanut rotation, led to a significant decrease in light fraction organic matter. In addition, the lower C mineralization rates encountered after long-term residue burning, reflect the decline in readily decomposable organic matter, and change in quality of the organic matter present (Biederbeck *et al.*, 1980; Thompson, 1992). Decreased C mineralization in two soils under long-term burning of crop residues is clearly demonstrated in Figure 2.6.

The potentially mineralizable N also decreases where burning of crop residues is practiced (Biederbeck *et al.*, 1980; Haines and Uren, 1990; Thompson, 1992). The conditions of burning determine the proportion and amount of N fractions remaining in the ash. Organic N is volatilized chiefly as N<sub>2</sub> or oxides of N, but some aqueous and volatile tar-like products are also lost (Raison *et al.*, 1985).

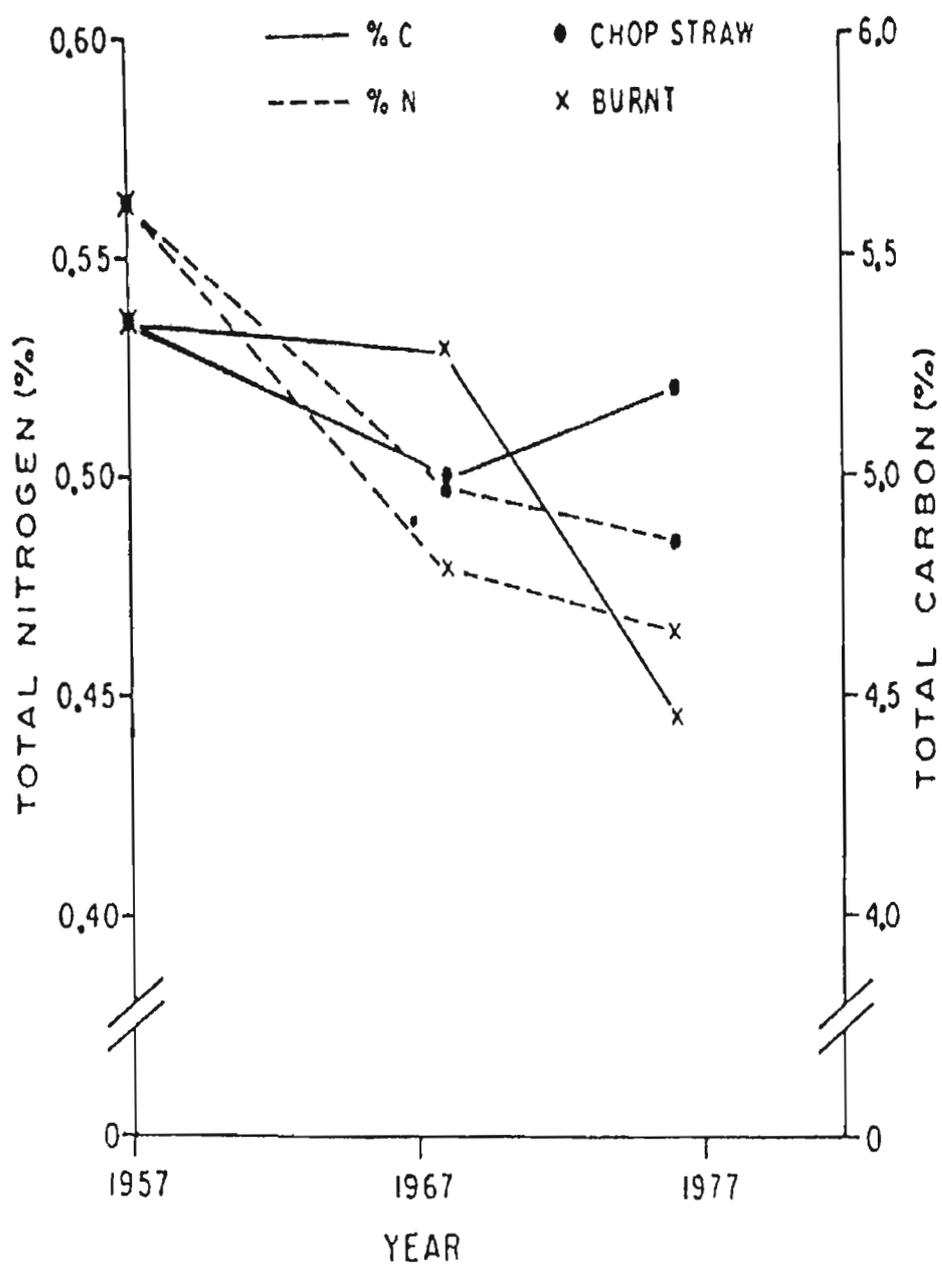
It is evident that burning of crop residues has a degrading effect on soil organic matter content and quality. This decline in organic matter content could be reversed or at least reduced by returning crop residues to the soil (Ladd *et al.*, 1994).

#### **2.4.1.2 Retention of crop residues**

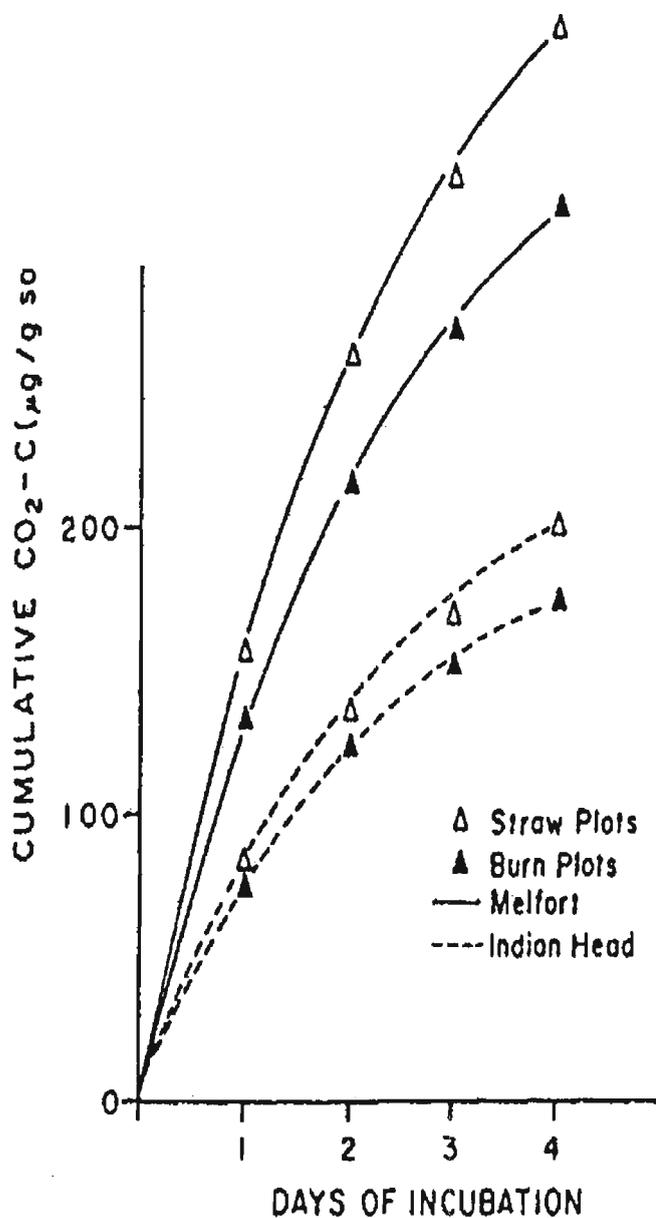
Residue input can play an important role in setting new, or maintaining organic matter equilibrium levels in soil. Several workers have shown that there is a linear relationship between carbon input to soil from above-ground crop residues and soil organic C content (Paustian *et al.*, 1992).

Figure 2.5 indicates that soil organic C levels can be more or less maintained over a period of 20 years, by the retention of crop residues on the soil surface (Biederbeck *et al.*, 1980). After a period of 10 years of crop residue management, Carter and Mele (1992) reported a tendency for the organic C content to be higher in the direct drill residues returned treatment compared to the direct drill residue burnt treatment.

Because the light fraction, is an intermediate state of decomposition between fresh plant organic material and soil humus, it increases significantly with the application of residues on the soil surface (Feller *et al.*, 1987). The increase in the light fraction and the large amounts of soluble organic material leaching from the mulch result in an accelerated C mineralization potential (Biederbeck *et al.*, 1980; Thompson, 1992).



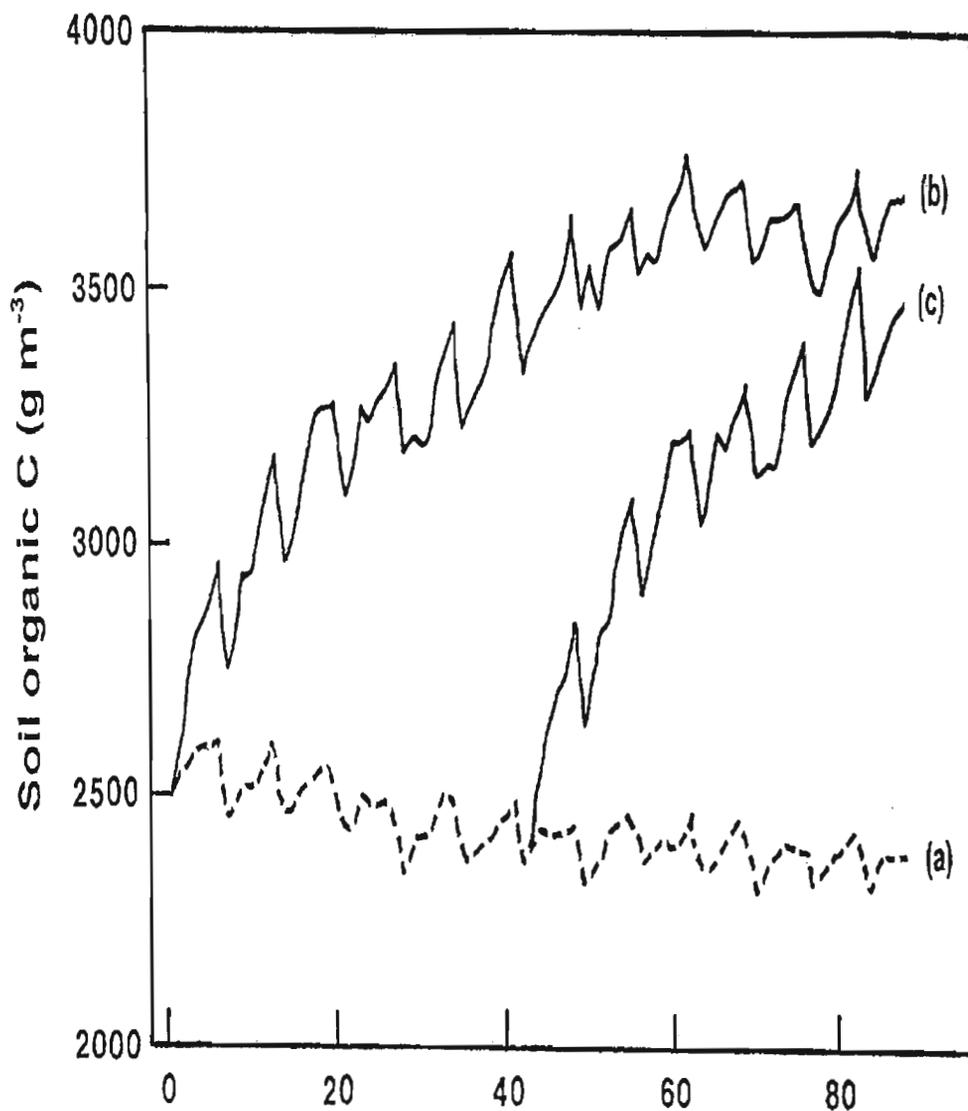
**FIGURE 2.5:** Change in soil C and N during 19 years of burning cereal straw at a site in Saskatchewan, Canada (Biederbeck *et al.*, 1980).



**FIGURE 2.6:** Effect of long-term burning compared to straw retention on soil respiration at two sites (Melfort and Indian Head) under wheat in Saskatchewan, Canada (Biederbeck *et al.*, 1980).

As noted earlier, in some recent studies in Queensland, little change in soil organic C content was measured under long-term sugarcane production (Skjemstad *et al.*, 1990; Bramley *et al.*, 1996). These workers suggested that the relatively recent adoption of green cane harvesting with retention of a trash blanket may have improved otherwise depleted soil organic matter levels. Indeed, a shift from burning to trash retention is a practice that can significantly increase soil organic matter levels (Wood, 1991; van Antwerpen and Meyer, 1998). Large amounts of organic matter are returned to the soil rather than lost during burning; up to 10 t ha<sup>-1</sup> of crop residues are left on the soil surface after harvesting (Ng Kee Kwong *et al.*, 1987). The CENTURY model was used to simulate long-term effects of sugarcane trash management on soil organic matter content (Vallis *et al.*, 1996). The model indicated that adoption of a trash blanket on old sugarcane producing soil would lead to an increase of approximately 40% in soil organic matter after 60 to 70 years and about half of this increase would occur during the first 20 years (Figure 2.7). Recent research by van Antwerpen and Meyer (1998) showed that 58 years of green cane harvesting, compared with burning and removal of tops, resulted in an increase in soil organic matter content from 5.1 to 6.2 % in the surface 5 cm layer. Blair *et al.* (1998) reported that the labile C fraction, measured by oxidation with KMnO<sub>4</sub>, increased significantly under green cane harvesting with trash retention, compared to that of burning after a period of 5 years.

Little is known regarding the rate of soil organic matter loss under South African sugarcane producing land (Meyer *et al.*, 1996). Green cane harvesting with trash retention would be a practicable way of increasing the soil organic matter content and soil biological activity over very large areas of agricultural land in the KwaZulu-Natal sugar belt under both small-scale and commercial farming practices.



**FIGURE 2.7:** Changes in soil organic C simulated using the CENTURY model in response to (a) continued burning, (b) green cane harvesting or (c) burning for 40 years followed by conversion to green cane harvesting under northern Queensland conditions (Vallis *et al.*, 1996).

## 2.4.2 Soil biological properties

In most agricultural soil systems available C limits soil microbial activity (Haynes, 1999). Reductions in microbial biomass under the burning regime, result from lower organic matter inputs and environmental stresses, created by this management practice. Biederbeck *et al.* (1984) observed a reduction in total heterotrophic microbial populations in soil where wheat residues were burnt. This was attributed to a decrease in easily-available labile C sources.

Biederbeck *et al.* (1980) also showed that the bacterial populations in the surface 2.5 cm of the burnt plots at the two long-term wheat sites in Saskatchewan, Canada, were permanently reduced by > 50 %. This appreciable decrease was as a result of the soil surface temperature reaching a maximum of about 338 °C. The high temperatures were shown to cause a decrease in the number of spore forming bacteria, resulting in a permanent loss of some of the bacterial populations. It is evident that burning crop residues decreases size and activity of the soil microbial biomass due to high temperatures and the loss of available C.

Systems with large organic matter inputs tend to have higher soil microbial biomass contents and activities because of the provision of high energy yielding material such as soluble carbohydrates, which are leached into the soil as the residue decomposes. Doran (1980), Biederbeck *et al.* (1984) and Gupta and Roper (1992) observed an increase in the populations of total heterotrophic microorganisms in the soil in response to the retention of crop residues (wheat, maize and barley respectively).

In addition, bacteria to fungi ratios have been reported to widen in response to residue retention (Gupta and Roper, 1992). Nonetheless, Holland and Coleman (1987) also observed that fungal biomass was significantly larger in the proximity of surface residue. The reason for this is that fungal hyphae link surface residues to

the soil below more readily than do bacteria.

Besides increasing the available carbon supply for microbial activity, retention of residue on the soil surface also provides a more favorable environment for biological activity because it reduces diurnal and seasonal fluctuations in soil temperatures (Van Doran and Allmaras, 1978), both through shading against incoming solar radiation and by reducing outgoing longwave radiation. In addition, surface mulch reduces evaporative losses of water and maintains a higher soil moisture content (Carter and Steed, 1992) in the surface soil. These conditions create microenvironments that favour microbial activity.

Very little information is available on the effect of green cane harvesting with the retention of a trash blanket on microbial biomass size and activity. Limited Australian research has shown that retention of a trash blanket, rather than burning, markedly increases the soil microbial biomass in the surface soil and also the size of the earthworm community (Wood, 1991; Sutton *et al.*, 1996). Since the microbial biomass and its activity are such important components in evaluation of soil quality, this topic deserves more detailed investigation.

### **2.4.3 Soil pH and nutrient availability**

Applications of ash to soils generally increase soil pH (Lerner and Utzinger, 1986) so that burning of crop residues rather than returning residues to the soils generally tends to raise soil pH (Raison, 1979). If combustion of plant material is nearly complete most of the C, H, O, N, S and organic P are volatilized (Raison, 1979) while most of the cationic (Ca, Mg, K and Na) components remain in the ash. When the ash is wetted, hydrolysis of the contained basic cations results in formation of alkaline residue which can have a pH exceeding 12 (Lerner and Utzinger, 1986). Additions of ash will also add basic cations and P to the soil but organic matter inputs are minimal (Biederbeck *et al.*, 1980; Prasad and Power, 1991). Where combustion

is not complete, the ash will be black and contain residual organic matter (Biederbeck *et al.*, 1980). Most of this organic matter will be of a charcoal nature and effectively inert when added to soils.

Most of these nutrients are present in water soluble components of ash and are therefore rapidly released in available forms. Most leaching of Na, K and Ca from ash residues occurs during the first 3 months after burning (Raison, 1979). Since these nutrients are in soluble form they are readily plant-available. Some nutrient losses could occur via surface run off of deposited ash and by leaching of mobile nutrients such as K (Raison, 1979). Other factors such as wind also play a role in removal of nutrients from the field, by blowing away large amounts of the ash that contain the residual nutrients.

Upon burning of residues, exchangeable  $\text{NH}_4$  tends to accumulate in the soil (Biederbeck *et al.*, 1980) as a result of proteolytic reactions (Laura, 1974). The  $\text{NH}_4$  is formed from organic nitrogenous compounds through purely chemical reactions, when they are heated. Accumulation of  $\text{NH}_4$  can also be attributable to ammonifiers that survive high temperatures. However, this available N is exposed to volatilization or nitrification to  $\text{NO}_3$ , which in turn can leach out of the profile before establishment of a new crop (Raison, 1979).

Crop residues contain a large amount of nutrients that are released upon decomposition. Results calculated from Wood (1991) indicate that a  $100 \text{ Mg ha}^{-1}$  crop of sugarcane produces approximately  $22 \text{ tons trash ha}^{-1}$ . The amount of nutrients present in 22 tons trash per ha amounts to about  $130 \text{ kg N ha}^{-1}$ ,  $113 \text{ kg K ha}^{-1}$ ,  $62 \text{ kg Ca ha}^{-1}$  and  $29 \text{ kg Mg ha}^{-1}$ . Nutrients are likely to be released slowly from the trash resulting in a slow but continuous supply to the crop. Beneficial effects of trash retention on soil fertility are likely to take a relatively long time to achieve (Haynes and Hamilton, 1999).

#### 2.4.4 Soil microclimate and physical conditions

The state of the soil physical environment is important for maintaining sustainable agronomic production. Generally, very little immediate alteration takes place during the burning process, unless heating has been very severe (Walker *et al.*, 1986). However, long-term effects could accrue from removal of protective residue by fire, which increases exposure of the soil to climatic conditions. The exposure of the soil surface, a decrease in organic matter and microbial biomass, results in a decrease of water stable aggregates near the soil surface (Biederbeck *et al.*, 1980). The strength of aggregates is closely related to the presence of organic matter, roots and fungal hyphae, and can predict the soils resistance to compactive loads (e.g. heavy machinery). Under these conditions the aggregates are exposed to the erosive effect of raindrops, which cause them to break, resulting in a concomitant decrease in macroporosity and infiltration rates (Greene *et al.*, 1990). The thin layer of ash covering the soil surface following a fire can also result in fine ash particles filling the soil macropores accentuating a decline in infiltration rates (Biederbeck *et al.*, 1980; Greene *et al.*, 1990; Valzano *et al.*, 1997). The amount of runoff subsequently increases, increasing the possibility of losing top-soil.

The impact of the frequent passage of agricultural vehicles on soil compaction can be effectively reduced by maintaining a surface mulch (Soane, 1990). The effective contact area of the wheels of machinery is increased, thus reducing contact stress. Organic material imparts on the soil a greater elasticity under compression forces and thereby reduces compactability by increasing resistance to deformation. The maintenance of a surface mulch can influence moisture availability in two ways. The first is the effect on the rate at which moisture is absorbed by the soil and the capacity of the soil to hold that absorbed moisture. On the other hand, the surface mulch maintains a higher soil moisture content so compaction can be increased with the retention of organic residues due to high moisture content during the time of harvesting, when heavy machinery is passing through the field (Wood, 1991; van Antwerpen and Meyer, 1998). Compaction occurs most easily at higher soil moisture

contents (Soane and Kershaw, 1987; Meyer *et al.*, 1996).

The use of mulches retards evaporative losses of soil water by protecting the soil from direct rays of the sun and from wind currents. Organic litter absorbs water to about 200 % of its dry mass, apart from the water it holds on the wetted surfaces. Therefore, even a relatively thin mulch layer ( $100 \text{ g m}^{-2}$ ) can absorb at least 0.2 mm per storm event. There is an almost linear relationship between a surface mulch and infiltration. The raindrop energy is reduced at the soil surface, resulting in less detachment of soil particles and disruption of soil aggregates.

The maintenance of a surface mulch encourages root growth. Higher moisture content, adequate temperatures and available nutrients promote lateral root growth from row crops in the inter-row area. Plant roots have two major effects on soil structure: by growing through the soil they make channels and some root tips exert sufficient pressure to push soil particles apart, thus changing the pore size distribution in their environment (Russell 1971). Plant roots also exert a stabilizing influence on soil structure, particularly through polysaccharide gums produced by the microbial population of the rhizosphere and by the exudation of mucigel from the roots (Tisdall, 1996), both of which have a binding action on soil aggregates. Thompson (1966) and van Antwerpen and Meyer (1998) reported an increase in aggregate stability where trash was retained as a blanket on the soil surface compared to burning the residues.

#### **2.4.5 Weed infestation**

Weed growth is known to be suppressed when plant residues are applied to the surface as a mulch. The main factors involved in this weed suppression are the presence of a covering over the soil surface and toxic substances, called allelochemicals, that are released from plant upon decomposition (Lorenzi *et al.*, 1989).

The suppression of weed under a trash blanket following green cane harvesting is well documented (Lorenzi *et al.*, 1989; Wood, 1991; McIntyre *et al.*, 1996). The best results are obtained when the trash is evenly distributed over the stool and inter-space. Lorenzi *et al.* (1989) reported that a trash blanket can suppress 83 to 92 % of weed infestation.

#### 2.4.6 Crop yield

Placement of organic material on the soil surface affects the soil water balance through increasing rainfall interception losses, increasing infiltration and reducing evaporation from the soil surface. These factors are regarded as the main factors responsible for an increase in sugarcane yield induced by green cane harvesting under non-irrigated fields (Thompson, 1966; de Beer *et al.*, 1996; McIntyre *et al.*, 1996). Mulching has many other effects: it protects the soil from erosion, returns organic matter and nutrients to the soil, insulates the soil, alters the surface reflectivity (albedo), increases the boundary layer for gaseous diffusion, particularly of water, and soaks up incoming rainfall. It is often very difficult to attribute plant responses following mulching to just one cause. Interactions between these factors result in mulches having either harmful or beneficial effects on plant performance.

In Australia, Wood *et al.*, (1991) reported that green cane harvesting yielded 10 tons cane ha<sup>-1</sup> more than that of burning cane. Octávio *et al.* (1994) showed that where a trash blanket was maintained, there was a consistently higher sugarcane yield; on average 24 % (12.5 tons cane ha<sup>-1</sup>) more than the burnt treatment. Where trash management was combined with contour planting, an increase of 28 Tones cane ha<sup>-1</sup> was observed (Yang, 1996). Yield increases are most pronounced during periods of low rainfall, as a result of water conservation under trash blankets (McIntyre *et al.*, 1996).

For trash retention to be a successful management practice, an essential pre-

requisite is good surface and subsurface drainage. Ponding of water in depressions or wheel ruts can cause cane stools to rot and result in a big reduction in ratoon yields (Wood, 1991).

Despite the increase in sugarcane yield  $\text{ha}^{-1}$ , the commercial cane sugar (CCS) levels tend to decrease slightly under trash management compared to the burnt treatments (Wood, 1991). This is mainly due to the fact that sugarcane that is produced under green cane harvesting grows for a longer time period compared to that under burning, due, at least in part, to slower release of nutrients from the trash.

This results in the sugarcane being less mature at harvest and it has lower sugar content (Wood, 1991).

## **2.5 Summary**

This chapter discussed the concept of soil quality and the important role of organic matter in evaluating the quality of soil. This key attribute to soil quality is partitioned into two major pools: stable and labile. The importance of the labile pools, as soil quality indicators were discussed in detail. It was noted that different crop residue management practices can influence these labile pools before they have any influence on the total organic matter content, emphasizing their sensitivity and therefore their role in predicting changes in agricultural systems due to management practices.

The effects of sugarcane production on soil chemical, physical and biological properties were also reviewed. Various concerns regarding soil degradation under sugarcane production were also discussed. Soil degradation under sugarcane production includes a loss of soil organic matter, soil acidification, soil compaction in the inter-rows and sometimes salinisation/sodification under irrigation. The loss of soil organic matter is particularly pronounced under the conventional method of production used in South Africa where the crop is pre-harvest burnt. Conversion to green cane harvesting with retention of a trash blanket would be a way of increasing

organic matter inputs to the soil.

Green cane harvesting results in large amounts of residue being returned to the soil compared to burning and this should cause soil organic matter levels to rise. Nevertheless, literature indicates that very little is known about the effect of green cane harvesting on soil quality and especially on soil biological properties. For this reason, there is a need to investigate the effects of these two management practices on soil quality, with emphasis on soil biological properties.

## CHAPTER THREE

### 3. Effects of green cane harvesting, burning and fertilizer application on soil fertility indices.

#### 3.1 Introduction

The annual cycling of plant nutrients through the plant-soil system is essential to maintain a productive and sustainable agricultural enterprise. Under agricultural production large quantities of nutrients are annually removed from the soil with the harvested crop. Annual removals of nutrients in a 100 t ha<sup>-1</sup> sugarcane crop amount to approximately 120 kg N ha<sup>-1</sup>, 33 kg P ha<sup>-1</sup> and 125 kg K ha<sup>-1</sup> (De Geus, 1973).

The most practical way to replace the removed nutrients, is by the application of chemical fertilizers. Whether available soil nutrient levels increase or decline under sugarcane depends largely on whether fertilizer inputs exceed or are less than nutrient removals in harvested cane plus other losses (e.g. leaching). However, returning crop residues to the soil can result in the recycling of a proportion of the plant nutrients, removed from the soil by the crop. A 100 Mg ha<sup>-1</sup> sugarcane crop produces approximately 22 Mg trash ha<sup>-1</sup> (Wood, 1991). The amount of nutrients present in 22 Mg trash ha<sup>-1</sup> approximates about 130 kg N ha<sup>-1</sup>, 113 kg K ha<sup>-1</sup>, 62 kg Ca ha<sup>-1</sup> and 29 kg Mg ha<sup>-1</sup>. Return of crop residues, rather than burning or removing them is an important management strategy to consider with regards to nutrient cycling within the system (Schoenau and Campbell, 1996; Dormaar and Carefoot, 1998).

A decrease of soil organic matter content under long-term sugarcane production is well documented (Masilaca *et al.*, 1985; Wood, 1985; Alaban *et al.*, 1990; Henry and Ellis, 1995; Hartemink and Kuniata, 1996; van Antwerpen and Meyer, 1996; Hartemink, 1998a). However, results from Wood (1991) indicated that a 100 Mg ha<sup>-1</sup> sugarcane crop produced approximately 22 Mg trash ha<sup>-1</sup> (containing about 11 Mg C

ha<sup>-1</sup>). Green cane harvesting, with retention of a trash blanket over the soil surface, is common practice in many parts of the world (Hudson, 1984; Ng Kee Kwong *et al.*, 1987; Wood, 1991). Recent studies in Queensland showed little change in soil organic C content under long-term sugarcane production. It was suggested by Bramley *et al.* (1996) and Skjemstad *et al.* (1999) that the reason for this was that the relatively recent adoption of green cane harvesting in the locality, with retention of a trash blanket, may have improved otherwise depleted soil organic matter levels.

Nevertheless, the usual practice in the South African sugar industry is to burn standing cane and dispose of post-harvest crop residues. During burning, large amounts of C, N and S present in crop residues are lost as a result of volatilization (Raison, 1979). The ash typically has a high basic cation (Ca, Mg and K) and P content, is alkaline and its nutrient content is generally released rapidly to the soil (Raison, 1979). Thus, returns of ash to the soil, rather than unburnt crop residues, tend to lead to a decline in organic matter content and a rise in soil pH (Raison, 1979; Biederbeck *et al.*, 1980; Rasmussen and Collins, 1991).

There is also concern regarding soil acidification under long-term sugarcane monoculture (Bramley *et al.*, 1996; Garside *et al.*, 1997; Moody and Aitken, 1997). The most common cause of acidification in established sugarcane plantations is thought to be the routine use of ammonium containing or forming fertilizers [e.g. ammonium sulphate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and urea CO(NH<sub>2</sub>)<sub>2</sub>] (Sumner, 1997; Hartemink, 1998a). Two fundamental factors limit the fertility of acid soils; nutrient deficiencies (e.g. P, Ca and Mg) and the presence of phytotoxic substances (e.g. soluble Al and Mn) (Foy, 1988). Return of crop residues showed varied effects of soil pH, (Hoyt and Turner, 1975; Lungu *et al.*, 1993; Schjøning *et al.*, 1994; Wild, 1994; Noble *et al.*, 1996; van Antwerpen and Meyer, 1998), however the formation of Al-organic matter complexes during residue decomposition has been shown to render soluble Al<sup>3+</sup> non-phytotoxic (Kretzschmar *et al.*, 1991; Bessho and Bell, 1992; Wong *et al.*, 1995; Noble *et al.*, 1996).

Thus there is great potential for the use of green cane harvesting with the retention of a trash blanket on the soil surface as a source of plant nutrients, organic matter and complexing agent for toxic substances. In addition to this, increased organic matter returns tend to improve soil physical conditions (i.e. aggregate stability). However, there is very little information on the effects of long-term green cane harvesting on soil fertility. The long-term trash management trial at the South African Sugar Association Experiment Station at Mount Edgecombe provides an opportunity to investigate these effects. The experiment has continued for 61 years and, compares burning with green cane harvesting with half the plots fertilized and the others unfertilized. We investigated the changes in soil chemical properties that have occurred under these treatments.

### **3.2 Materials and Methods**

The experimental site, referred to as BT1, is a burning and trashing trial situated at the South African Sugar Association Experiment Station on the outskirts of Durban, in the South African province of KwaZulu-Natal (longitude 31° 04' 29" and latitude 29° 43' 20"). Mean annual rainfall at the site is approximately 950 mm. The soil is classified as Arcadia form, Lonehill family (Soil Classification Working Group, 1991) or as a Chromic Vertisol (FAO). The A-horizon depth is approximately 500 mm and the clay content of the upper 200 mm is about 58 % (+/- 2 %). Its mineralogy is dominated by interstratified smectite-vermiculite with some kaolinite and mica also being present (Johnston *et al.*, 1999).

The experiment was established in 1939 (Thompson, 1965; 1966) and is believed to be the oldest sugarcane residue management trial in the world. Sugarcane fields are often pre-harvest burnt and all the trash (dead leaves around the stems) is burnt off leaving stems and green leaves at the top of the plants (tops) intact. With green cane harvesting both trash and tops are returned to the soil. When burning is employed, there is no trash but tops are returned to the soil surface. Tops may be left on the surface as a partial mulch or raked off. The experimental treatments are:

(i) green cane harvested with retention of a trash blanket (100% cover on the soil surface) (T) (Plate 3.1), (ii) burnt with tops left scattered on the soil surface (67 % cover on the soil surface) (Bt) (Plate 3.2) and (iii) burnt with tops removed (Bto) (Plate 3.3). The treatments are either (a) unfertilized (Fo) or (b) fertilized annually with 140 kg N ha<sup>-1</sup>, 20 kg P ha<sup>-1</sup> and 140 kg K ha<sup>-1</sup> (F). The experiment is replicated four times in a randomized block design with trash management treatments as main plots. An aerial view of the experimental site is shown in plate 3.4. Experimental plots are 18 m long and 8.4 m wide and sugarcane is planted in rows 1.4 m apart. A schematic layout of the experimental trial is presented in figure 3.1.

Sugarcane has been grown on the experimental trial for an average of about eight years (a planted crop plus seven ratoon crops) before being replanted. For the first 20 years conventional tillage was used at replanting but since then a minimum tillage system has been used (old ratoons are ripped from rows and replanting occurs within these rows). 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 (0 - 30 cm) using a 50 mm diameter soil sampler (10 samples per plot taken from the inter-row area) 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.

Rows between the experimental blocks that have been under grass for the duration of the experiment (no fertilizer applied) were also sampled. These were considered to be as close to the condition under undisturbed grassy vegetation as it is possible to find in the vicinity of the trial. Soil samples were air-dried and sieved (< 2 mm) and a proportion was finely ground (<150  $\mu$ m). Ground soil was used for analysis of organic C, organic P, P fractionation, construction of P adsorption isotherms and extraction of non-exchangeable K.

Organic C was determined by a dichromate wet oxidation method (Yeomans and Bremner, 1988). Total N was determined by semi-micro Kjeldahl digestion with colorimetric determination of the liberated ammonium (Foster, 1995). Soil pH was

determined using a glass electrode in 1:2.5 soil : water suspension following equilibration for 16 hr. Exchangeable acidity was determined by extracting the soil with 1M KCl and an aliquot of the extract was then titrated with 0.01 M NaOH to a pH of 6.8 (Thomas, 1982).



PLATE 3.1: Green cane harvesting results in a 100% cover consisting of leaves and tops on the soil surface.



PLATE 3.2: The sugarcane in burnt and only the tops of the cane are left on the soil surface (67 % cover on the soil surface).



PLATE 3.3: The cane is burnt and after harvesting only the ash remains on the soil surface.

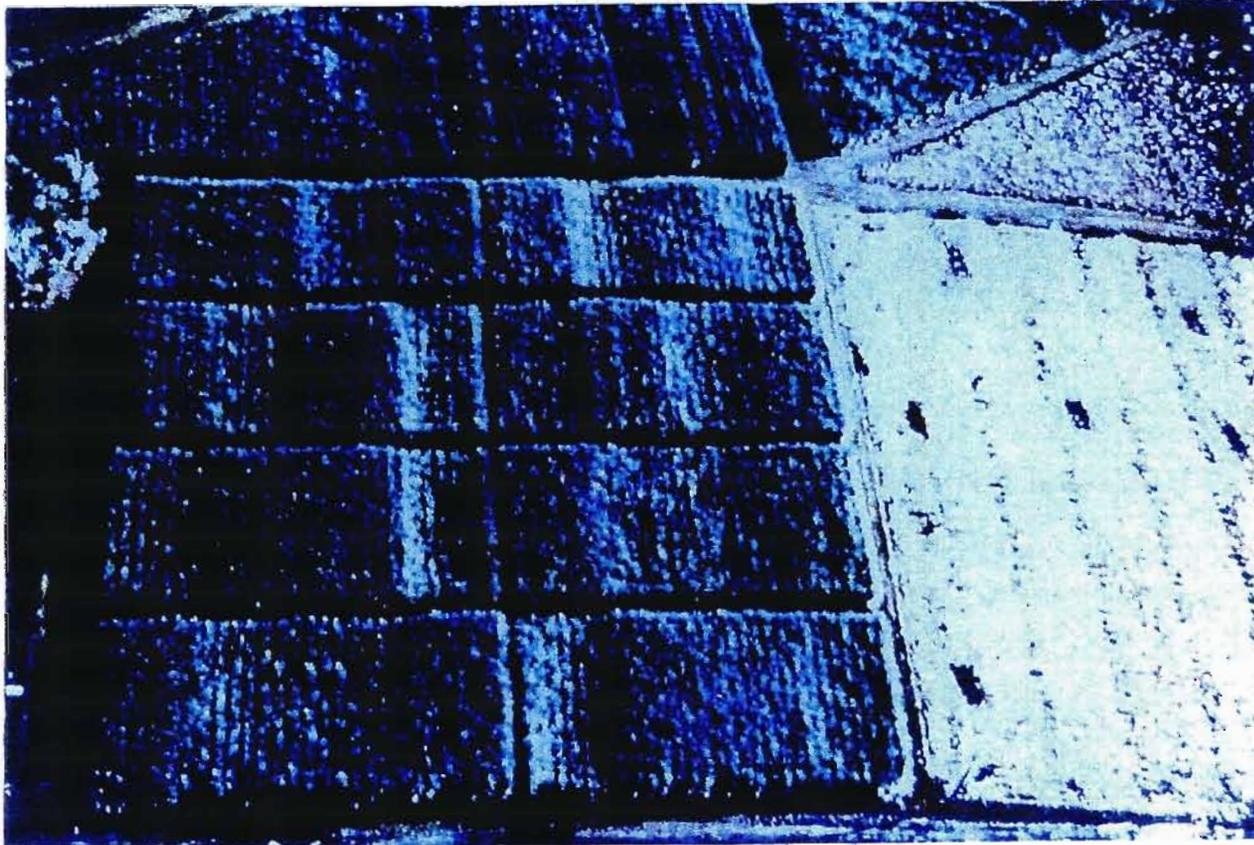


PLATE 3.4: An aerial view of the experimental site, BT1, indicating the four replicates and the grass rows between the replicates

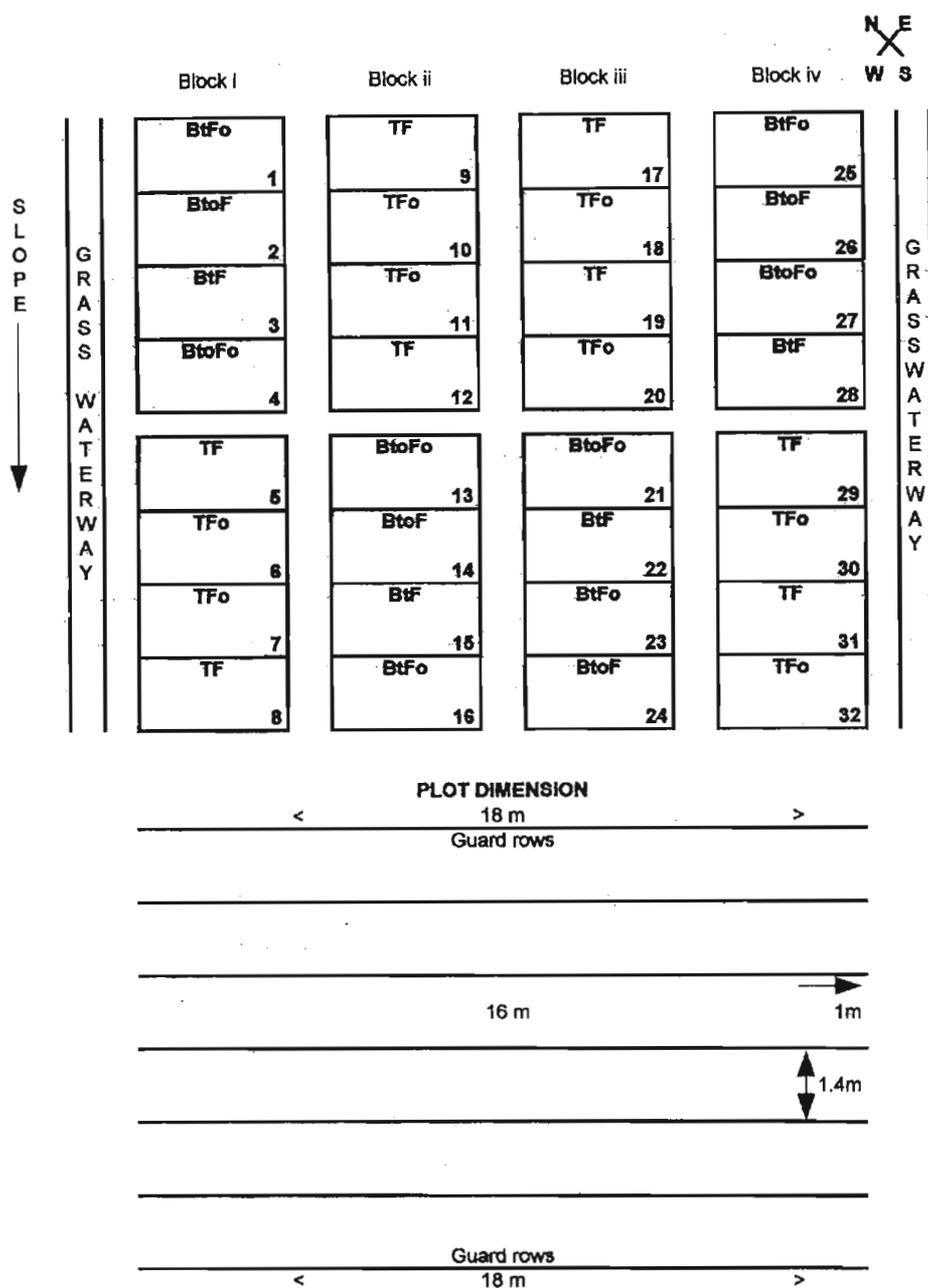


FIGURE 3.1: Schematic diagram of the experimental site, BT1, at the South African Sugar Association Experiment Station at Mount Edgecombe.

Exchangeable K, Ca, Mg and Na were extracted with 1N ammonium acetate by shaking 50 g of soil with 200 ml of ammonium acetate for 2 hours (Beater, 1962) and K, Ca and Mg were determined by atomic absorption and Na by atomic emission spectrophotometry.

Non - exchangeable K was extracted with 1.0 N boiling HNO<sub>3</sub> over a period of 25 minutes (Helmke and Sparks, 1996). The extract was filtered and analyzed by atomic absorption spectrophotometry. The non-exchangeable K values were calculated as the difference between HNO<sub>3</sub> - extractable K and the exchangeable K.

Extractable "available" P was determined by extraction with Truog reagent (0.05 N H<sub>2</sub>SO<sub>4</sub>) (Beater, 1962). Total organic P was determined by an ignition method (Olsen and Sommers, 1982). To further investigate the accumulation of inorganic and organic P induced by fertilizer additions, samples from the Grass, BtoFo, BtoF, TFo and TF treatments from the 0 - 2.5, 2.5 - 5 and 5 - 10 cm layers were fractionated following the method described by Condon and Goh (1989), in which soil is sequentially extracted with (a) 0.5 M sodium bicarbonate (b) 0.1 M sodium hydroxide, (c) 1 M hydrochloric acid and (d) 0.1 M sodium hydroxide. The extracts were analyzed for inorganic P (Pi) and also total P (following digestion with nitric and perchloric acids with the addition of MgCl<sub>2</sub>) and organic P (Po) content was calculated by difference. The fractions were designated as NaHCO<sub>3</sub> - Pi and Po (labile Pi and Po sorbed to the soil surface plus some microbial P); NaOH(I) - Pi and Po (Pi more strongly bound to Fe and Al hydrous oxides and Po associated with humic compounds); HCl - Pi (relatively insoluble basic calcium phosphate minerals); and NaOH(II) - Pi and Po (insoluble "occluded" forms of Pi and more stable Po forms). Phosphorus in all the extracts was analyzed by the molybdenum blue method (Olsen and Sommers, 1982). For construction of P adsorption isotherms, duplicate soil samples (equivalent to 10 g air-dry weight) were mixed with varying amounts of KH<sub>2</sub>PO<sub>4</sub>, a drop of toluene and enough CaCl<sub>2</sub> solution and water to bring the final volume to 30 ml and the final concentration of CaCl<sub>2</sub> to 0.01M. The suspensions were shaken for 96 h and phosphate adsorbed was calculated from the difference

between the phosphate added and that remaining in the solution.

To further investigate the nature of Al solubilized by soil acidification in fertilizer- and trash-amended soils, samples from the Grass, BtoFo, BtoF, Tfo and TF treatments from the 0 - 2.5, 2.5 - 5 and 5 - 10 cm layers were subjected to an Al fractionation and, in addition, total and monomeric Al in soil solution were measured. Soils were re-sampled in July 1998, after several heavy rainfall events had re-wetted the surface 20 cm layer to about 30 % w/w and sieved (< 2 mm). Soil Al was fractionated (Soon, 1993) by sequentially extracting air-dried soil with (a) 1M potassium chloride (b) 0.1M copper chloride (c) 1M ammonium acetate (d) 0.2 M ammonium oxalate. These extracts were designated as exchangeable, organically - bound, sorbed and amorphous Al fractions respectively (Soon, 1993). Soil solution was obtained by centrifuging a sub-sample of moist soil and collecting the filtered soil solution in the bottom of the centrifuge tube (Eikhatib *et al.*, 1987). Total soluble Al ( $Al_T$ ) was determined by transferring an aliquot of soil solution into a centrifuge tube and adding 0.02 ml of 0.5 M La and 0.01 ml of 0.05 M Fe reagent. After a reaction time of 16 hours an aliquot of the supernatant was transferred to a vial and 0.5 ml iron interferent was added. Following a reaction time of 1 minute, 0.2 ml of PCV reagent and 1.0 ml of indazole buffer were added and the absorbency measured at 578 nm after 20 minutes (Menzies *et al.*, 1992). For the determination of monomeric Al ( $Al_{Mono}$ ), 3 ml of the filtered soil solution was transferred to a vial and 0.5 ml of iron interferent, 0.2 ml PCV reagent and 1.0 ml hexamine buffer were added. The absorbency was measured 585 nm after 1 minute (Kerven *et al.*, 1989).

Aggregate stability was measured using a wet sieving technique as described by Haynes (1993). Thirty grams of air-dried aggregates (2 - 6 mm) were transferred to the uppermost of a set of three sieves with aperture sizes of 2.0, 1.0 and 0.50 mm. The water content was adjusted so that the aggregates on the upper sieve were just submerged at the highest point of oscillation. The oscillation rate was 25 cycles  $min^{-1}$ , the amplitude of the sieving action was 35 mm and the period of sieving was 15 min. The soil remaining on each sieve was oven dried and weighed. Mean weight diameter (MWD) was calculated as the sum of the fraction of soil remaining on each

sieve after sieving for the standard time multiplied by the mean diameter of the adjacent sieve apertures. The upper and lower limits of mean weight diameter in this case were 3.0 and 0.25 mm respectively.

A subsidiary experiment was conducted to establish the effect that divalent and monovalent cations have on aggregate stability in the study soil. The reason for this was that in the fertilized treatments, large amounts of K (monovalent cation) had been applied whilst acidification had resulted in considerable leaching of Ca and Mg, (divalent cations) out of the topsoil. This was suspected to be having an effect on aggregation. A 300 g sample of air-dried, sieved (2 - 6 mm) soil from the 0 - 5 cm layer of the TF treatment (pH 5.0) was treated with  $\text{Ca}(\text{OH})_2$  or KOH to give a pH of about 5.8 (i.e. , that of unfertilized treatments). A quantity of K (as  $\text{K}_2\text{SO}_4$ ), equivalent to that applied as KOH to the fertilized treatments, was added to samples from the unfertilized (TFo) treatment (pH 5.8). Samples were rewetted to a moisture content of 30 % w/w, incubated for three weeks and then air-dried. This drying and rewetting cycle was repeated twice and at the end of the incubation period aggregate stability was determined on soil samples as outlined above.

In order to calculate the quality of nutrients present in the soil profile to a depth of 30 cm, per unit area, nutrient concentrations were first converted to volumetric content. For this purpose, bulk density was measured in quadruplet in the 0 - 10, 10 - 20 and 20 - 30 cm layers for each plot using the core method.

### 3.3 Results

Data for mean concentrations of key chemical properties of all the experimental treatments are presented for the surface 10 cm. To demonstrate the effect of trash retention and fertilizer applications on the distribution of various properties within the soil profile, data for the Bto (burnt with tops raked off) and T (trash and tops retained), fertilized and unfertilized treatments are shown in the profile to 30 cm. Data for the Bt treatment showed values intermediate between the Bto and T

treatments and are not presented with depth. However, all the data in the profile for all treatments are presented in the appendices. For the same reason, where more specialized analysis was carried out to explain treatment effects (e.g., soil P and Al fractionations) these were performed on the BtoFo, BtoF, TFo and TF treatments only.

Concentrations of organic C in the surface 10 cm increased with increasing amounts of crop residue returned in the order Bto < Bt < T (Appendices 3.1; 3.2; 3.3; Figure 3.2). Below 10 cm, there were no significant treatment effects (Appendices 3.4; 3.5; Figure 3.3). Fertilizer application also resulted in accumulation of organic C (Figure 3.2) and this accumulation was also evident to a depth of 10 cm. When compared to grass rows, there was a net loss of organic C in the soil profile on a per-hectare basis in all treatments (Table 3.1); the loss was more pronounced on unfertilized plots.

Concentrations of exchangeable and non-exchangeable K are shown in Appendices 3.1; 3.2; 3.3; 3.4; 3.5. Exchangeable K in the surface 10 cm soil layer increased in the order BtoFo < BtFo ≤ BtoF = BtF < TFo < TF (Figure 3.2). The fertilized treatments (BtoF, BtF and TF) and the unfertilized trashed treatment (TFo) had higher non-exchangeable K levels than the grass row whilst the two unfertilized burnt treatments (BtoFo and BtFo) both had lower values. A decrease in exchangeable and non-exchangeable K (Figure 3.4) in the profile was evident to a depth of 30 cm.

As expected, Truog - extractable P concentrations were much higher in fertilized (BtoF, BtF and TF) than unfertilized (BtoFo, BtFo and TFo) treatments in the 10 cm soil layer (Figure 3.5). There was also a tendency for the amount of Truog - extractable P to increase with an increasing amount of crop residues returned to the soil surface. A decrease in extractable P with soil depth was evident (Appendices 3.1; 3.2; 3.3; 3.4; 3.5 and Figure 3.6) and there was a marked accumulation of P in the surface 10 cm for the fertilized treatments (BtoF, BtF and TF). There was a net accumulation of extractable P in fertilized plots to 30 cm (Table 3.1) but no

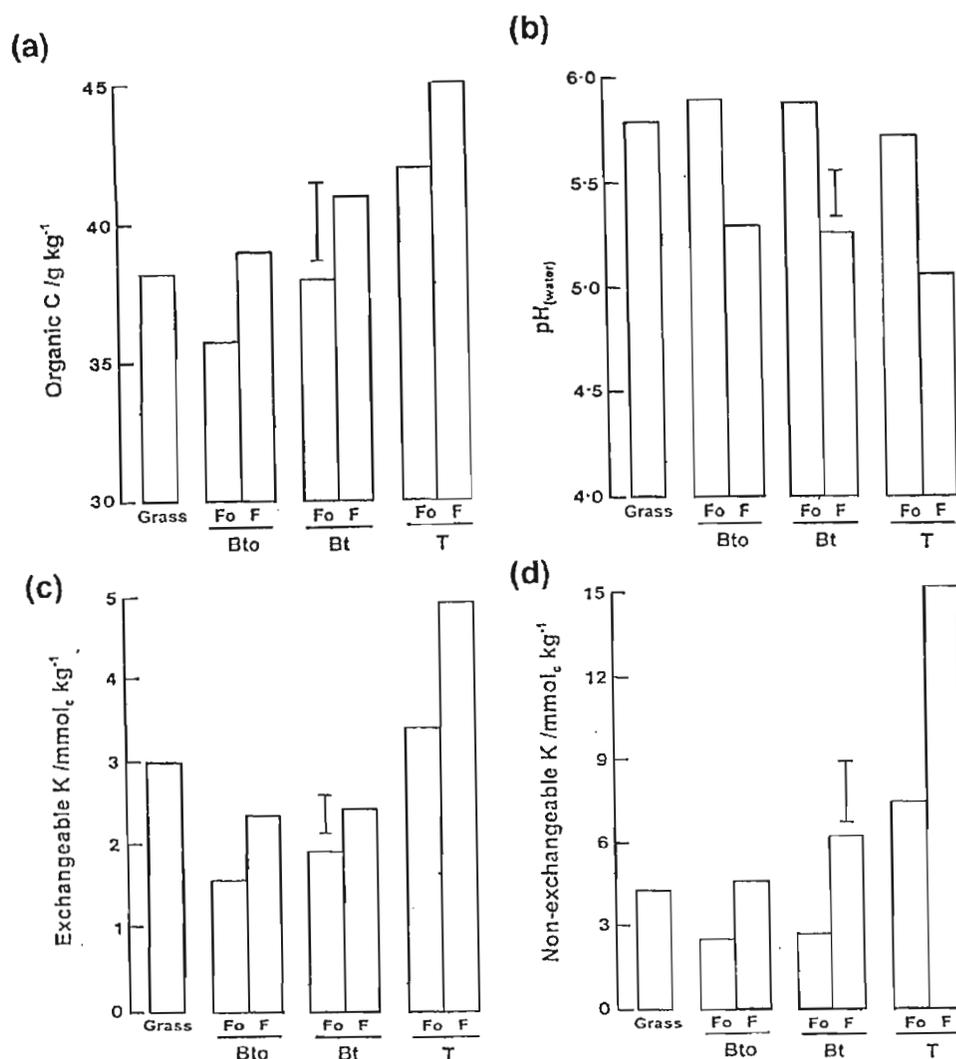
measurable change in unfertilized ones.

There was a net loss of organic P from the soil profile in all treatments although this loss was less for fertilized than unfertilized treatments (Table 3.1). Even so there was a distinct accumulation of organic P in the surface 10 cm of soil as a result of both fertilizer and trash retention (Figure 3.5 and Figure 3.6).

**TABLE 3.1:** Losses (-) or gains (+) of organic C, and P and available nutrients ( $\text{kg ha}^{-1}$ ) to a depth of 30 cm under the various treatments after 59 years in comparison with grass rows.

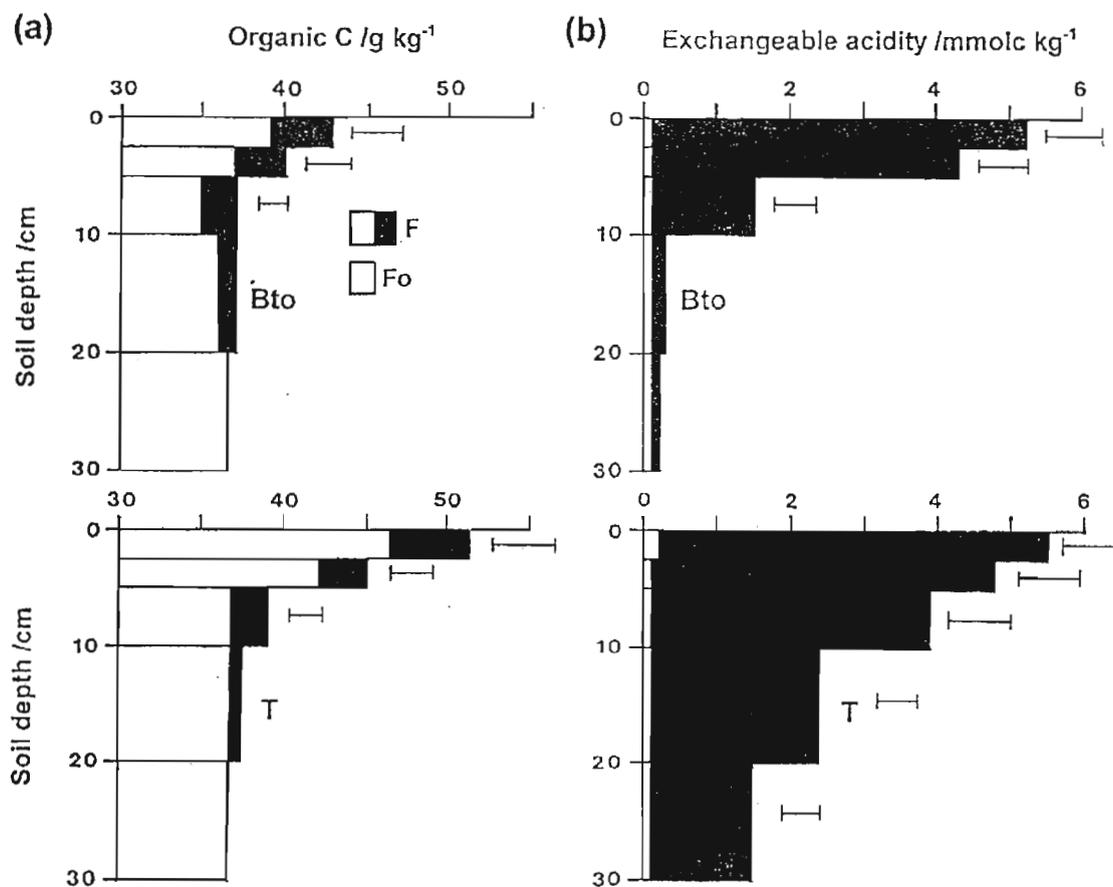
Measurement	Management treatments <sup>1</sup>						LSD
	BtoFo	BtoF	BtFo	BtF	TFo	TF	
Organic C	-6500	-5900	-4700	-2300	-4100	-800	508
Organic P	-150	-108	-135	-123	-138	-102	27
Truog P	+0.2	+16	+1.2	+19	+1.5	+24	2.6
Exchangeable Ca	-1020	-1740	-600	-1740	-480	-1860	193
Exchangeable Mg	-133	-997	-288	-1166	-410	-1044	127
Exchangeable K	-110	-44	-96	+21	-5	+129	15
Non-exchangeable K	-23	+48	-69	+94	+431	+1169	11

<sup>1</sup> T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) for comparison between treatments shown.

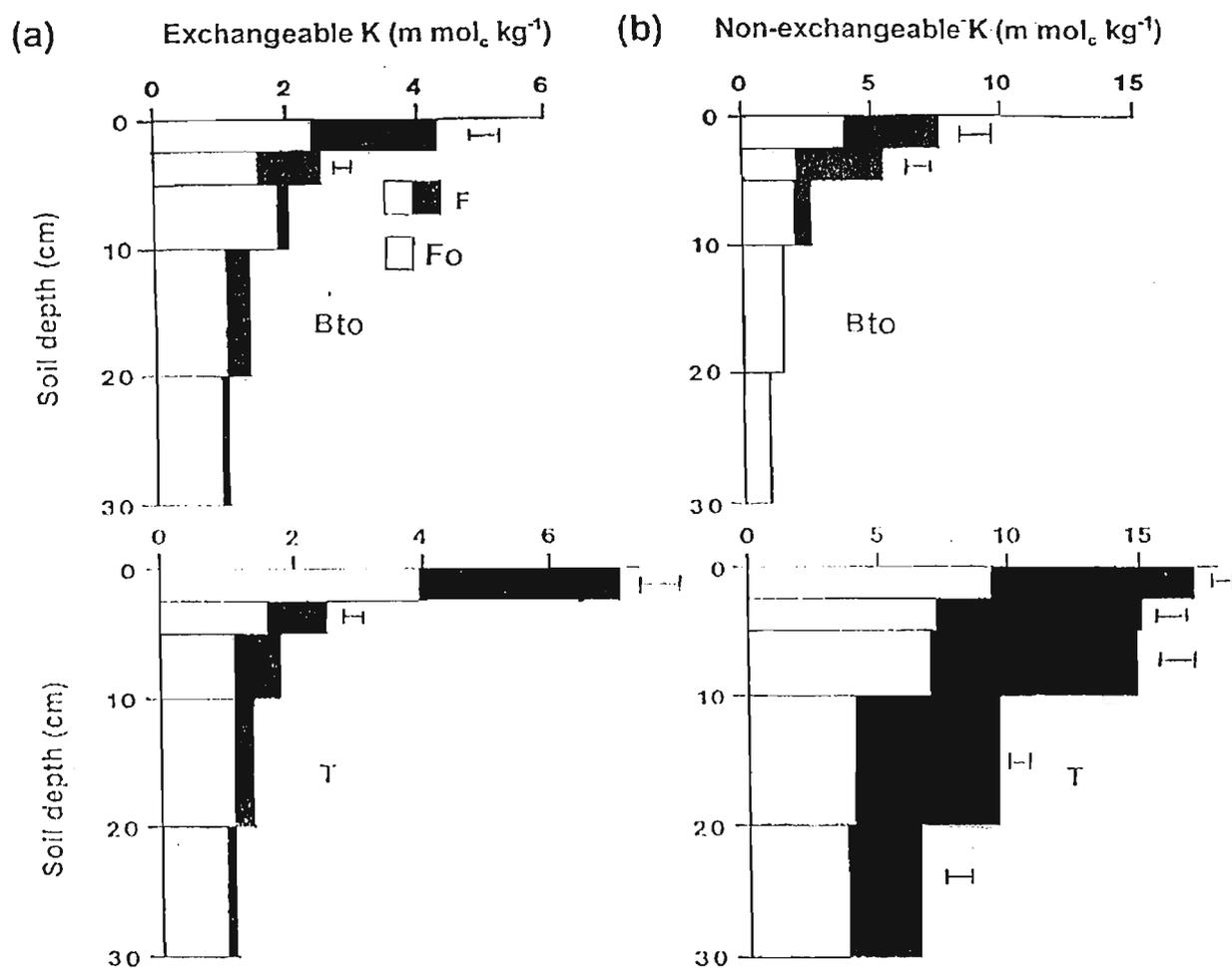


**FIGURE 3.2:**

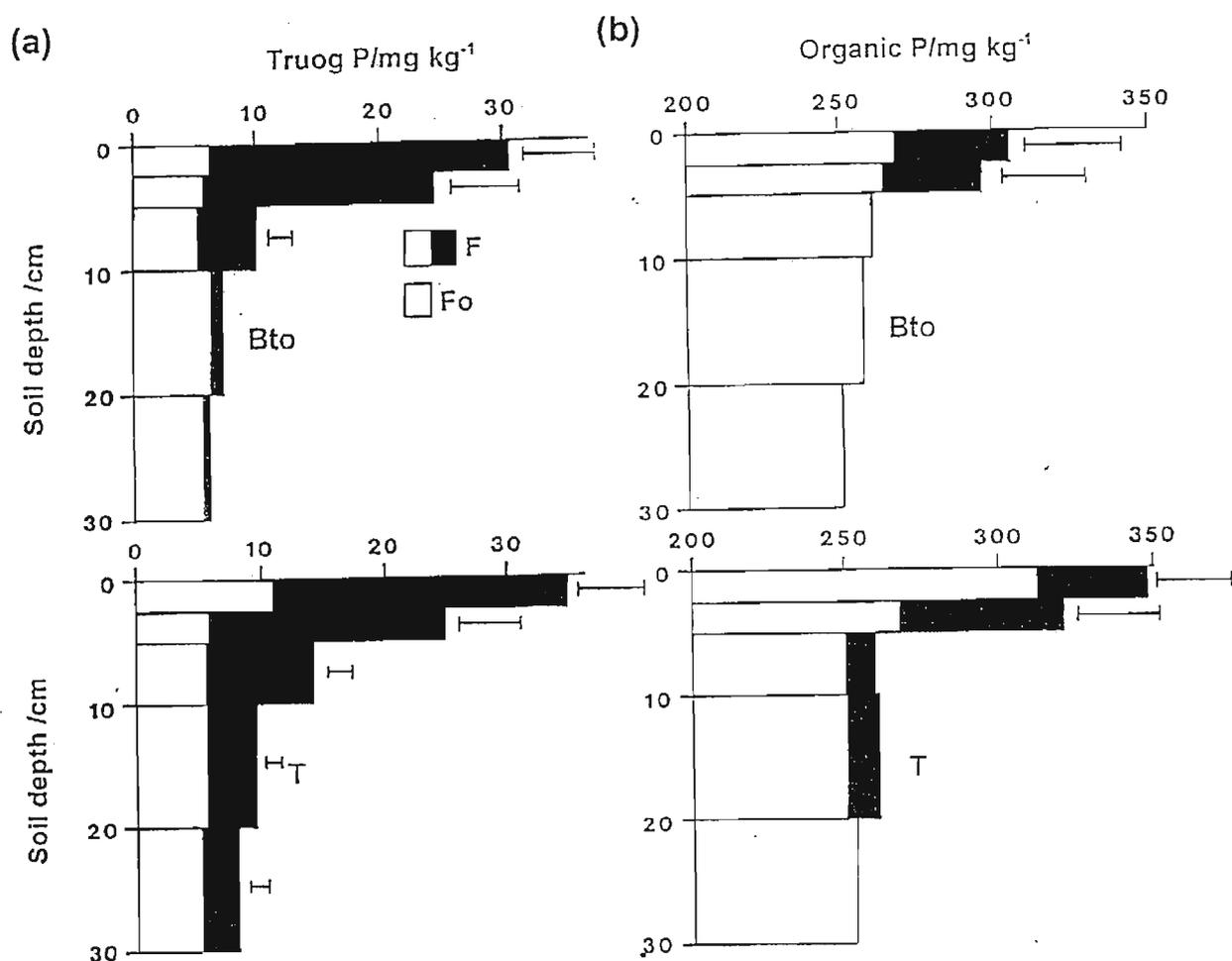
Quantities of (a) organic C, (b) pH<sub>(water)</sub>, (c) exchangeable and (d) non - exchangeable K in the surface 10 cm layer as affected by the various experimental treatments. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) for comparison between treatments.



**FIGURE 3.3:** Effect of burning or green cane harvesting with trash retention on fertilized and unfertilized plots on (a) organic C and (b) exchangeable acidity in the soil profile. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.



**FIGURE 3.4:** Effect of burning or green cane harvesting with trash retention on fertilized and unfertilized plots on (a) exchangeable and (b) non-exchangeable K in the soil profile. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.



**FIGURE 3.6:** Effect of burning or green cane harvesting with trash retention on fertilized and unfertilized plots on the (a) Truog-extractable and (b) organic P content in the soil profile. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.

Fertilizer application induced marked increases in Pi in the available ( $\text{NaHCO}_3$ ), adsorbed [ $\text{NaOH(I)}$ ] and occluded [ $\text{NaOH(II)}$ ] P fractions to a depth of 10 cm in the soil (Appendices 3.6; 3.7; 3.8). The biggest differences were in the 0 - 2.5 cm soil layer and data for this layer are shown in Figure 3.7. Fertilizer addition also resulted in some accumulation of Po in the  $\text{NaHCO}_3$  fraction (Figure 3.8). The absolute and percentage increases in the Pi and Po fractions as affected by fertilizer application in the 0 - 2.5 cm soil layer are presented in Table 3.2. The largest percentage increase occurred in the  $\text{NaHCO}_3$  - Pi fraction and the largest absolute increase occurred in the  $\text{NaOH(II)}$  - Pi fraction. In comparison with burning, trash retention resulted in a general accumulation of Pi in the available form ( $\text{NaHCO}_3$ ) and of Po particularly in the recalcitrant  $\text{NaOH(II)}$  fraction. Accumulation of Po was particularly notable in the trashed fertilized (TF) treatment where there was an additional accumulation of Po in the  $\text{NaHCO}_3$  and  $\text{NaOH(I)}$  fractions (Figure 3.8).

P adsorption capacity for the BtoFo and BtoF and Tfo and TF treatments respectively were not significantly different and only results for the fertilized treatments are presented in Figure 3.9. Trash retention caused a decrease in P adsorption capacity.

Along with unpublished data on mean cane yields (1978 - 1990) the amounts of trash potentially produced in various treatments is presented in Table 3.3. The amount of trash potentially produced and the nutrients held in the trash were calculated from data collected by Thompson (1965).

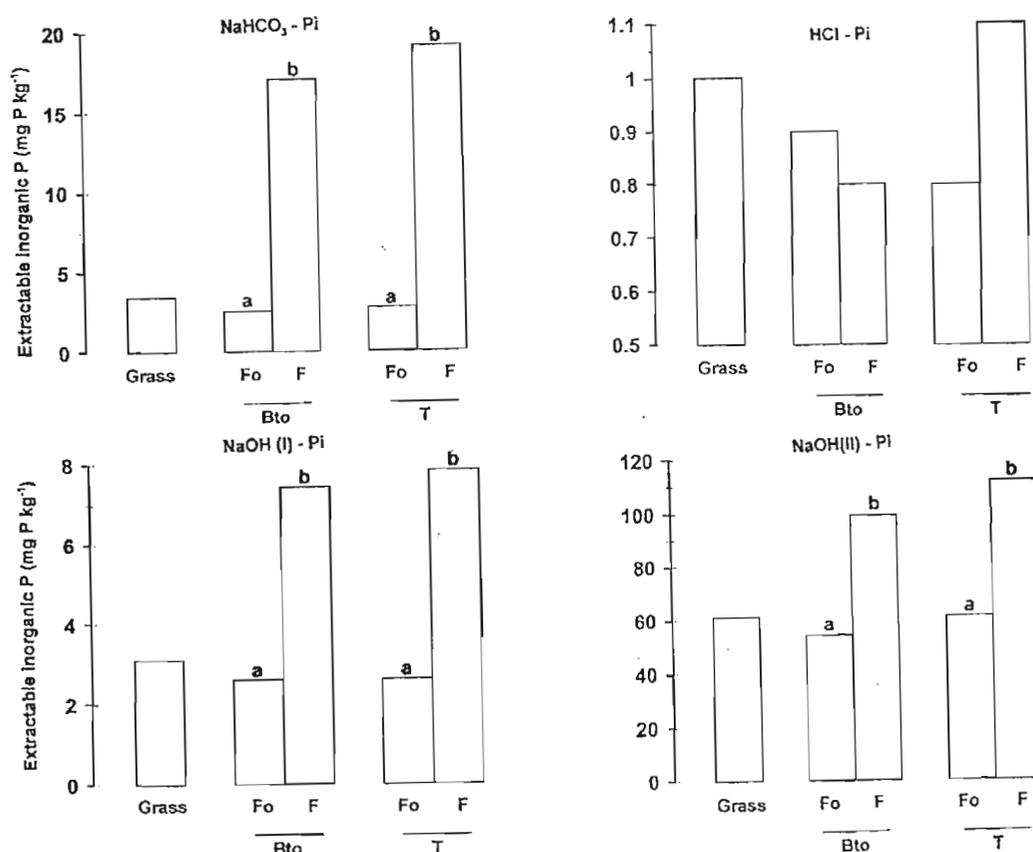
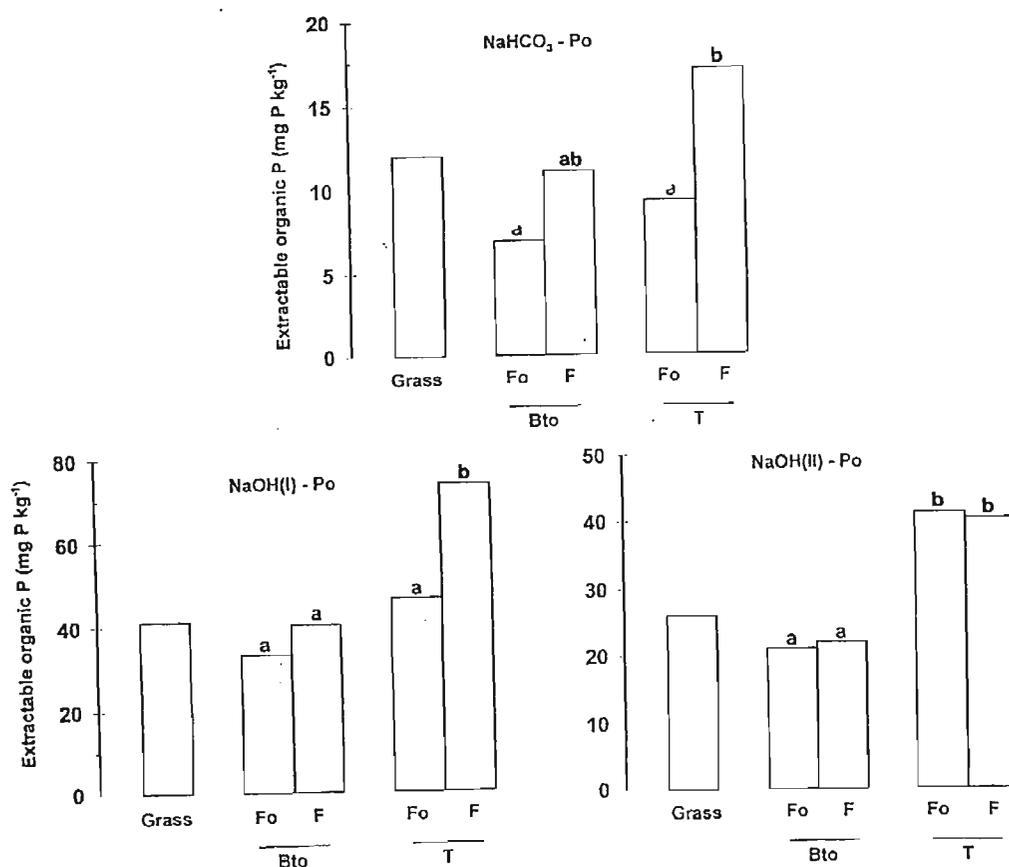


FIGURE 3.7:

Quantities of (a)  $\text{NaHCO}_3 - \text{Pi}$ , (b)  $\text{HCl} - \text{Pi}$ , (c)  $\text{NaOH(I)} - \text{Pi}$  and (d)  $\text{NaOH(II)} - \text{Pi}$  in the 0 - 2.5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).

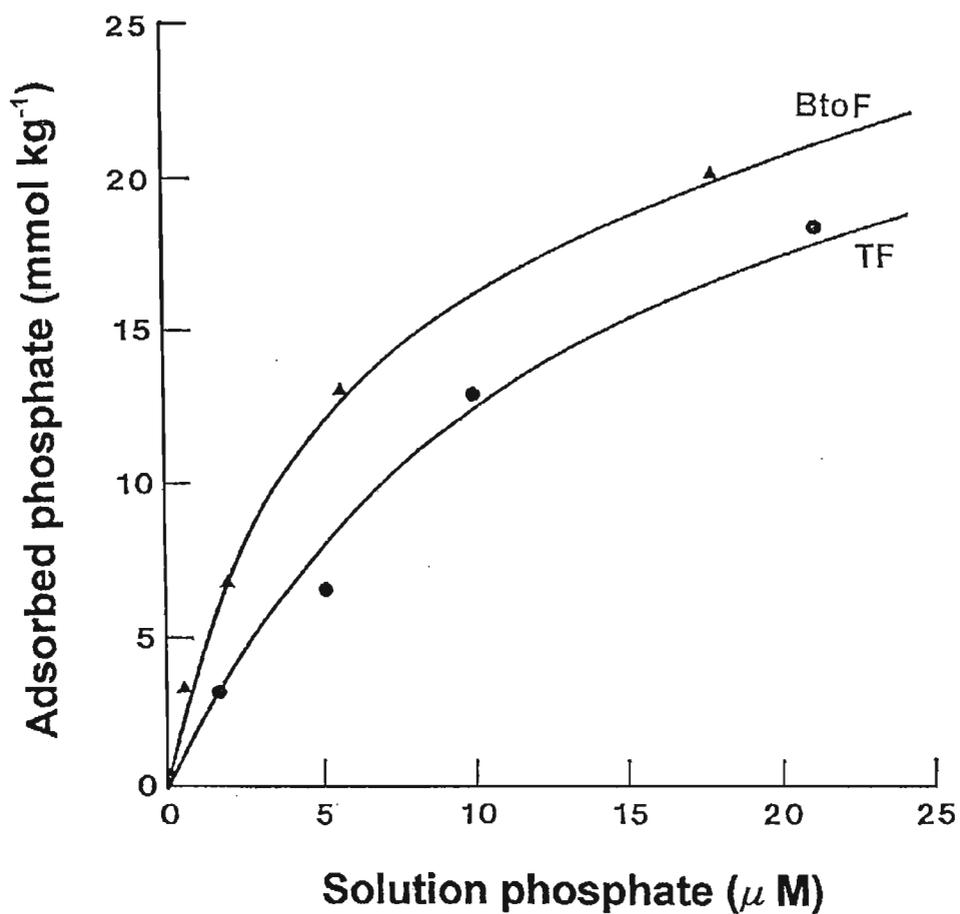


**FIGURE 3.8:** Quantities of (a)  $\text{NaHCO}_3 - \text{Po}$ , (b)  $\text{NaOH(I)} - \text{Po}$  and (c)  $\text{NaOH(II)} - \text{Po}$  in the 0 - 2.5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).

**TABLE 3.2:** Absolute and percentage increases in extractable inorganic (Pi) and organic P (Po) fractions by sequential extraction from the 0 - 2.5 cm soil layer.<sup>1</sup>

Extractable P fraction	Absolute increase (mg kg <sup>-1</sup> )	Increase (%)
NaHCO <sub>3</sub> – Pi	15	576
NaOH(I) – Pi	5	192
NaOH(II) – Pi	48	83
NaHCO <sub>3</sub> - Po	6	74
NaOH(I) - Po	17	43

<sup>1</sup> Increase calculated from the mean of fertilized treatments compared with that of unfertilized ones.



**FIGURE 3.9:** Phosphate adsorption isotherms for soil sample (0 – 2.5 cm) from the BtoF and TF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K.

**TABLE 3.3:** Mean annual cane yields (1978 - 1990), estimated quantities of trash produced and amounts of nutrients held in the trash

Management treatments <sup>1</sup>	Cane yields Mg ha <sup>-1</sup>	Trash produced Mg ha <sup>-1</sup>	Nutrients in trash (kg ha <sup>-1</sup> )		
			N	P	K
BtoFo	41	6.3	24	2.6	25
BtoF	97	15	56	6.2	60
BtFo	43	6.6	25	2.7	27
BtF	103	16	59	6.6	64
TFo	54	8.3	31	3.4	33
TF	106	16	61	6.7	65

<sup>1</sup> T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Data for pH, exchangeable acidity and exchangeable Ca, Mg and Na are presented in Appendices 3.1; 3.2; 3.3; 3.4; 3.5. Soil pH was decreased by both fertilizer and trash additions in the surface 10 cm soil layer (Figure 3.2 and Figure 3.10). Fertilizer induced acidification was measurable to a depth of 30 cm and was particularly pronounced in the TF treatment (Figure 3.10). Exchangeable Ca and Mg levels increased in the order BtoF < BtF < TF < BtoFo ≤ BtFo < TFo in the surface 10 cm soil layer (Figure 3.10 and Figure 11). Soil acidification induced by fertilizer and trash retention was accompanied by a decrease in exchangeable Ca and Mg concentrations (Figure 3.10 and Figure 3.11). In comparison with grass rows, all fertilized treatments contained significantly lower exchangeable Ca and Mg concentrations, whilst all unfertilized treatments contained higher concentration. The effective cation exchange capacity (ECEC) (e.g., sum of exchangeable bases plus Al) increased in the same order as soil organic C content: Bto < Bt < T (Figure 3.11). Nevertheless, ECEC was decreased appreciably by fertilizer addition even though soil organic C concentrations were increased.

There was a concomitant increase in exchangeable acidity (Figure 3.11) with fertilizer induced acidification. As soil pH increased with depth, exchangeable acidity decreased greatly (Figure 3.3) Exchangeable acidity was negligible in

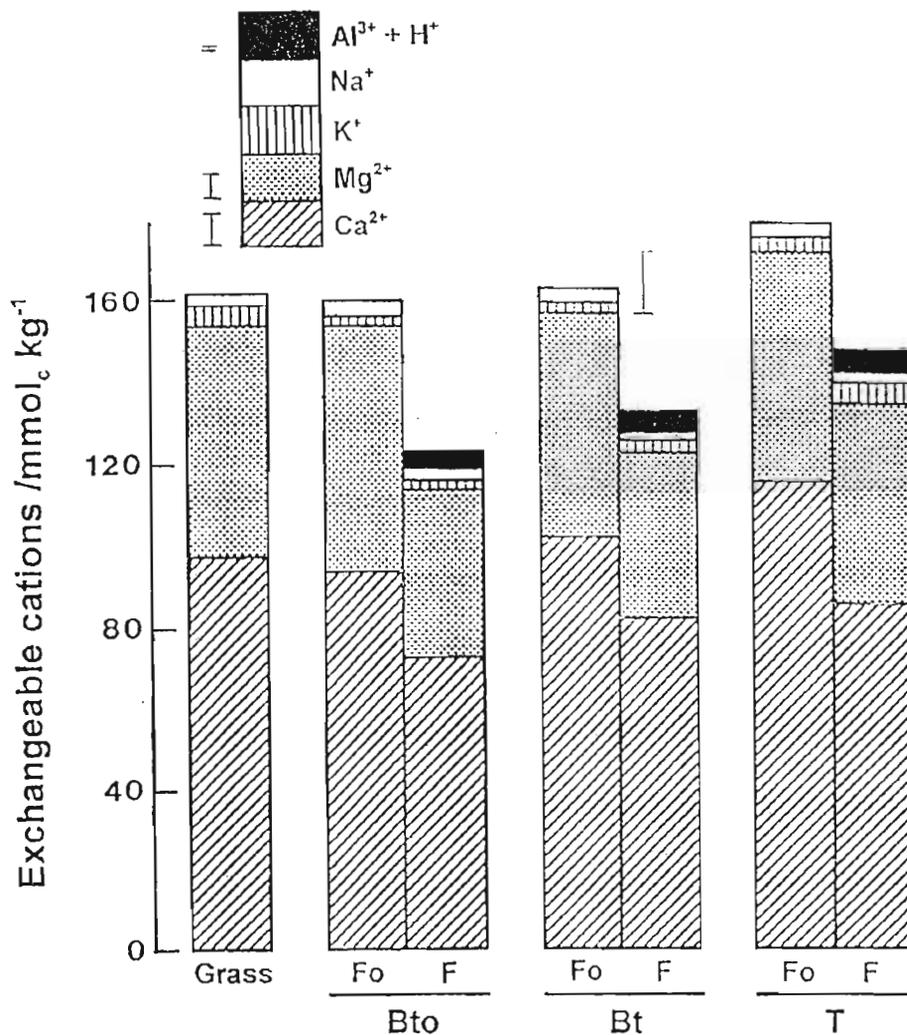
unfertilized treatments since soil pH values were above 5.5.

Results of the Al fractionation are presented in Appendices 3.6; 3.7; 3.8 and Figure 3.12. Fertilizer application increased exchangeable, organic - bound and sorbed hydroxy-Al in the 0 - 2.5 cm layer (Figure 3.12). Exchangeable Al concentrations were negligible in the unfertilized (BtoFo, BtFo and TFo) and grass rows. The fertilized trash treatment (TF) had higher oxalate - extractable Al concentrations than the other treatments.

The concentration of total aluminium in soil solution ( $Al_T$ ) in the 0 - 2.5 cm soil layer increased in the order Grass < BtoFo < BtoF = TFo < TF (Table 3.4). Fertilizer applications caused an increase in the concentration of both  $Al_T$  and  $Al_{Mono}$  in the soil solution as well as the proportion of  $Al_T$  present as monomeric form (Table 3.4). Trash retention also tended to increase concentrations of  $Al_T$  and  $Al_{Mono}$ . There was also a tendency for the concentration of  $Al_T$  and  $Al_{Mono}$  as well as the proportion of  $Al_T$  present as monomeric form to decrease down the profile to a depth of 10 cm.

As shown in Figure 3.13 a, aggregate stability (MWD) was greatest for soils from the grass rows. It increased in the order Bto < Bt < T and values for fertilized plots were less than those for unfertilized ones. It is evident from Figure 3.13 a that fertilized plots had a lower proportion of aggregates in the size class 2 - 6 mm and a greater proportion in classes less than 2 mm diameter. Absolute values for aggregate stability in the subsidiary experiment (Figure 3.13 b) are lower and not comparable with those in Figure 3.13 a. This is because aggregates were subjected to three wetting and drying cycles so that some of the aggregates were broken up. This is demonstrated by the larger proportion of 1 - 2 and 0.5 - mm size classes recovered in the subsidiary experiment. In the subsidiary experiment, additions of  $Ca(OH)_2$  to the TF treatment markedly increased the percentage of aggregates greater than 2mm diameter (and MWD) while adding KOH decreased those parameters. For the TFo treatment, addition of  $K_2SO_4$  greatly decreased MWD and the proportion of aggregates greater than 2mm diameter.





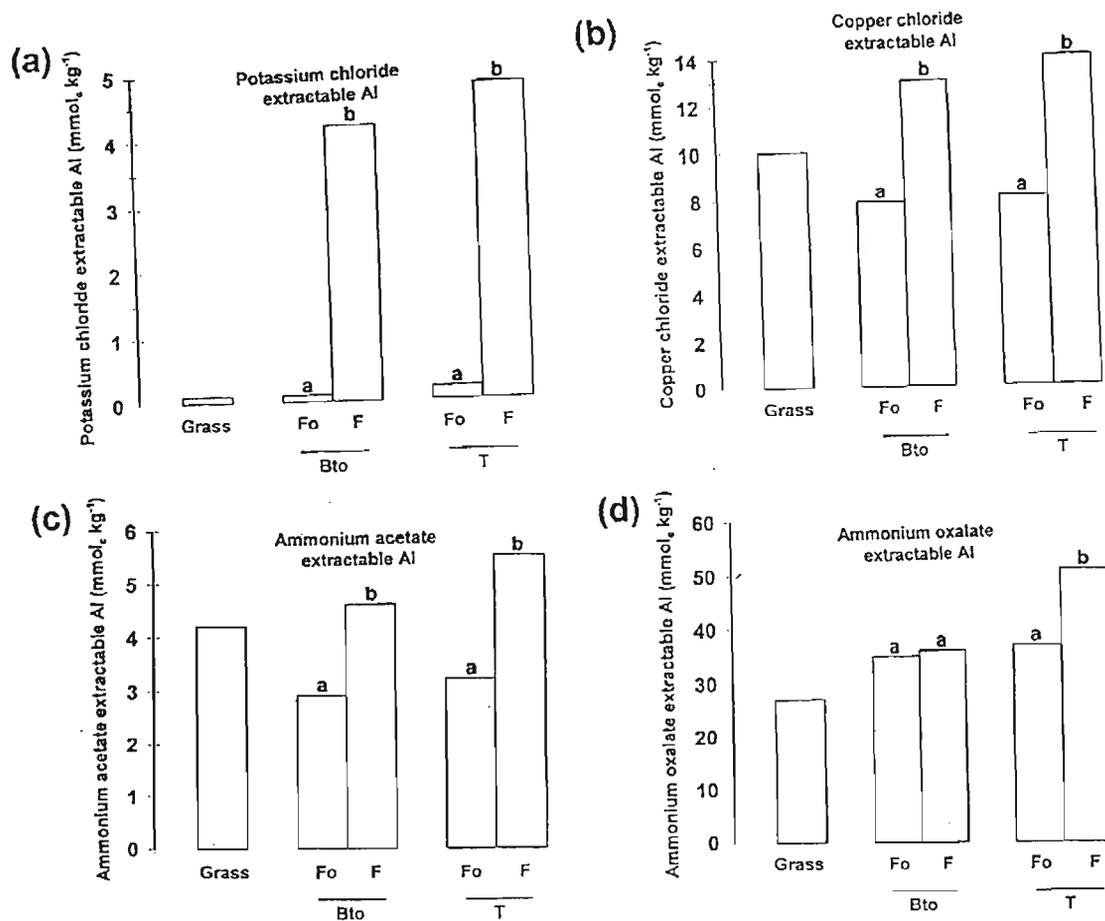
**FIGURE 3.11:**

Long-term effects of burning versus green cane harvesting with or without annual fertilizer applications under sugarcane on the concentrations of exchangeable Ca, Mg, K, Na and Al in the surface 10 cm. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) for comparison between treatments.

**TABLE 3.4:** The effects of different management practices on the mean concentration of total Al ( $Al_T$ ), monomeric Al ( $Al_{Mono}$ ) and the proportion of  $Al_T$  present as  $Al_{Mono}$  in soil solution in the profile to a depth of 10 cm.

Management treatments <sup>1</sup>	Depth								
	0 - 2.5 cm			2.5 - 5 cm			5 - 10 cm		
	$Al_T$	$Al_{Mono}$	Proportion of $Al_T$ present as $Al_{Mono}$	$Al_T$	$Al_{Mono}$	Proportion of $Al_T$ present as $Al_{Mono}$	$Al_T$	$Al_{Mono}$	Proportion of $Al_T$ present as $Al_{Mono}$
Grass	9.1	0.2	2.1	8.8	0.3	3.4	8.6	0.3	3.4
BtoFo	10 a <sup>2</sup>	0.3 a	3.0	12 a	0.2 a	1.6	12 a	0.2 a	1.6
BtoF	15 a	0.8 b	5.3	15 a	0.4 b	2.6	14 a	0.3a	2.1
TFo	14 ab	0.2 a	1.4	11 a	0.2 a	1.8	12 a	0.2 a	1.6
TF	19 b	1.3 b	6.8	15 a	0.5 b	3.3	14 a	0.3	2.1
Fertilizer effect <sup>3</sup>	*	*	*	ns	ns	*	ns	ns	**

<sup>1</sup> T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. <sup>2</sup> Mean values within one column followed by the same letter are not significantly different for comparison between BtoFo, BtoF, TFo TF at  $P \leq 0.05$ . <sup>3</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$  \*\* =  $P \leq 0.01$ ; ns = no significant difference).



**FIGURE 3.12:** Quantities of (a) potassium chloride-, (b) copper chloride-, (c) ammonium acetate- and (d) ammonium oxalate extractable Al in the 0 - 2.5 cm layer as affected by the various experimental treatments. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).

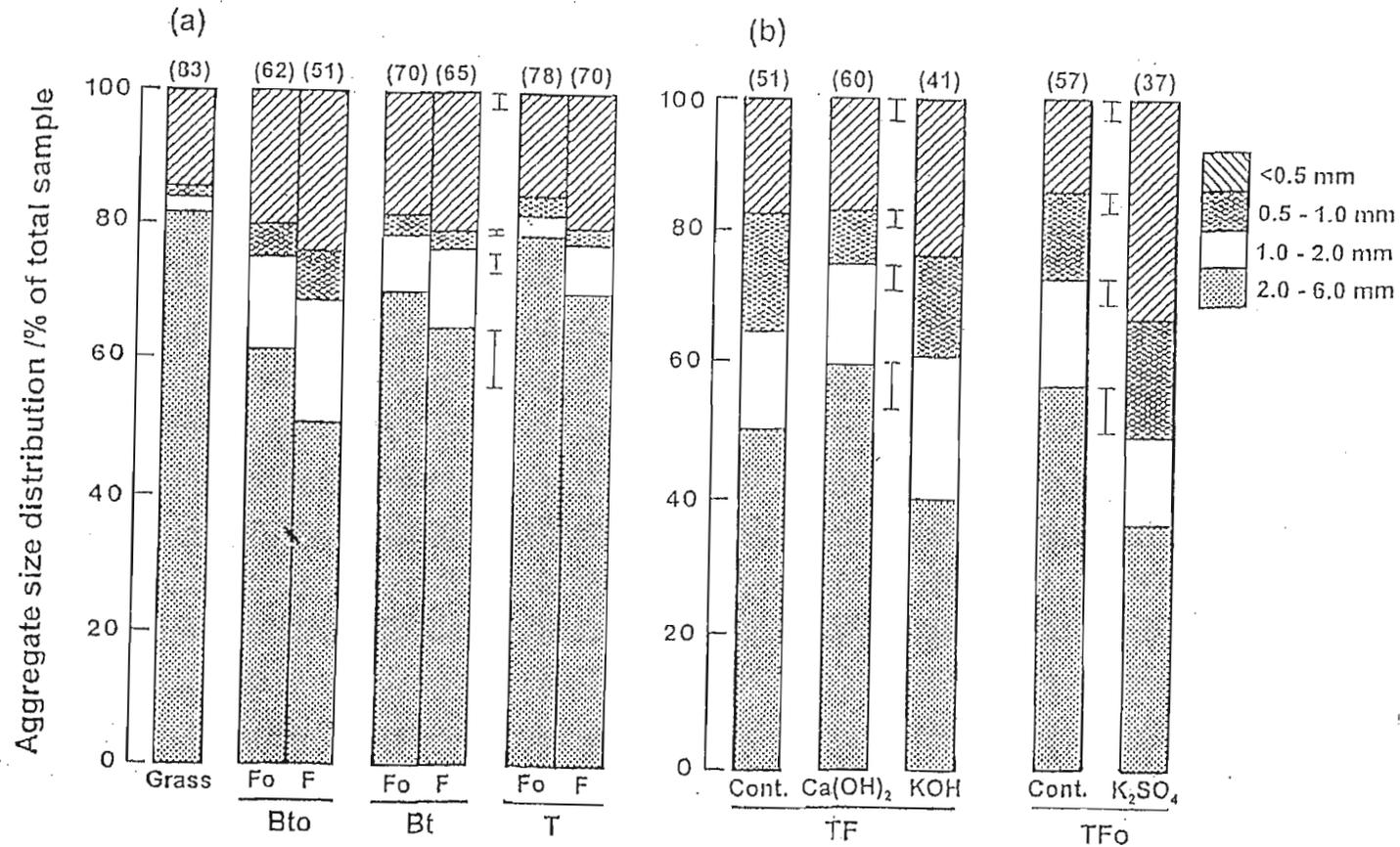


FIGURE 3.13a: Long-term effects of soil management under sugarcane on the size distribution of aggregates following wet sieving in the surface 0 - 2.5 cm layer.

b: Size distribution of aggregates (0 - 5 cm layer) following wet sieving after a six-week incubation period of the TF treatment with  $\text{Ca}(\text{OH})_2$  or KOH to raise the pH to that of the TFo treatment and for the TFo treatment with  $\text{K}_2\text{SO}_4$  added at the same rate of K as that added to the TF treatment as KOH. Percentage of aggregates remaining in the 2 - 6 mm class shown in brackets and the addition of monovalent and divalent cations. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.  $\text{LSD}(P \leq 0.05)$  shown for comparison of treatments for each size class.

### 3.4 Discussion

An appreciable increase in organic matter can be achieved by returning crop residues to the soil. For example, in Australia, retention of cereal stubble in long-term experiments (8 - 10 years) has resulted in significant increases in soil organic carbon content (Dalal and Meyer, 1986; Saffigna *et al.*, 1989; Chan *et al.*, 1992; Gupta *et al.*, 1993). However, experimental data on the long-term effects of green cane harvesting with the retention of residues as a trash blanket on soil fertility are scarce (Haynes and Hamilton, 1999). For this reason, the CENTURY model (which models organic matter cycling) was adapted to study the long-term effects of sugarcane trash management on soil organic matter levels in northern Queensland (Vallis *et al.*, 1996). Results predicted that an increase of approximately 40 % in soil organic matter would occur 60 - 70 years after the adoption of trash blanketing on old cultivated soil. Compared to the traditional practice of burning crop residues, in this study retaining crop residues on the soil surface for 59 years resulted in a 26.5 % increase in organic C content in the surface 10 cm (Figure 3.2).

The decomposition of surface-applied residues is often slow because of large fluctuations in surface applied residues due to greater fluctuations in surface temperature and moisture and reduced availability of nutrients to microorganisms colonizing the surface residues (Douglas *et al.*, 1980; Schomberg *et al.*, 1994). In addition, crop roots can sometimes be more abundant in the surface soil layer where crop residues are surface applied, rather than burnt (see chapter 6). This further enhances the organic matter concentration in the surface soil layer. Indeed, if the soil at the grass row is considered similar to that of undisturbed grassy vegetation, then long-term sugarcane production with trash retention has, in fact, increased the soil organic matter content to above that of the base-line value in the surface 2.5 cm layer. For the two trashed treatments (TFo and TF), the biomass returned (8.3 and 16 Mg ha<sup>-1</sup>) is equivalent to about 4 000 and 7 700 kg C ha<sup>-1</sup> yr<sup>-1</sup> respectively (Table.3.1).

This is a particularly important finding in relation to the effects of sugarcane monoculture on soil quality. Hartemink and Wood (1998) for example, concluded that loss of soil organic matter is one of the most important negative effects that sugarcane has on soil quality. Such diminution of soil organic matter is likely to result in a reduction in soil biological activity, nutrient supplying power and soil structural condition (Haynes and Hamilton, 1999). Indeed, a substantial reduction in soil organic matter content under sugarcane production has been observed previously in both South Africa (van Antwerpen and Meyer, 1996) and worldwide (Wood, 1985; Masilaca *et al.*, 1986; Alaban *et al.*, 1990; Henry and Ellis, 1995; Hartemink and Kuniata, 1996; Hartemink, 1998a). Results of this study suggest that the main reason for loss of organic matter under sugarcane production is burning of crop residues prior to harvest.

Burning of crop residue prior to harvest, particularly where tops are removed (Bto), has caused a notable loss of soil organic matter over the 59 - year period compared to the grassed rows. During burning, 50 - 70% of the residue carbon is commonly volatilized to CO<sub>2</sub> and CO (Rasmussen and Collins 1991), although losses of up to 90% have been measured when combustion of residues is almost complete (Raison and McGarity, 1979). This organic C volatilization during burning contributed to the depletion of organic matter in the burnt treatments over the 59 - year period.

Fertilizers are applied to soils in order to maintain or increase crop yields. In the long-term, increased crop yields result in increased organic matter returns (from both roots and tops) and thus a higher soil organic matter content (Haynes and Naidu, 1998). Indeed, in this study mean cane yields 1978 - 1990 were increased with fertilizer application for all treatments (Table 3.3). An accumulation of organic matter induced by fertilizer applications has been observed on several other long-term field trials (Johnston, 1986). For example, the results of a long-term manuring experiment at Rothamsted on a flinty silt loam (Batcombe series) demonstrated that plots which received annual N, P, K fertilizers have equilibrated to an organic matter content that is 15 % higher than the unfertilized plots (Johnston, 1986). When compared to the

grass rows, the loss of organic C in the soil profile on a per-hectare basis was less pronounced for the fertilized treatments (Table 3.3).

The fact that the experimental site was conventionally tilled prior to replanting every 7 - 10 years for about the first 20 years, after which minimum tillage has been used, is reflected in the distribution of organic matter in the soil profile. For example, the accumulation of organic matter in the surface 2.5 cm of all the experimental treatments (even Bto where only ash is returned) is presumably the result of the minimum tillage system now used. Surface accumulation of organic matter is a characteristic of minimum tillage systems since there is little or no downward redistribution of organic matter (Haynes and Beare, 1996).

On the other hand, the higher concentration of soil organic C in all the treatment plots, compared with the grass rows, in the 10 - 30 cm soil layer probably reflects the conventional cultivation and downward redistribution of organic matter which occurred in the initial stages of the trial (Figure 3.3). There is, however, other evidence of accumulation of organic matter in the subsurface horizons under cane in comparison with undisturbed sites (Masilaca *et al.*, 1985; McGarry *et al.*, 1996; Skjemstad *et al.*, 1999). Although this can be partially attributed to a downward redistribution of topsoil due to cultivation, it has also been suggested that the deep-rooting nature of sugarcane facilitates accumulation of organic matter at depth through continual turnover of root material (Masilaca *et al.*, 1985).

The relatively high organic C content found in this soil, even after 59 years of cultivation, can be attributed to organic matter being strongly bound to smectitic clays. In general, the organic matter held in Vertisols is extremely stable (Ahmad, 1988). Microaggregates in Vertisols are held together strongly by clay-polyvalent metal - organic matter bonds (Wilding and Tessier, 1988). The organic matter within these microaggregates is physically inaccessible to microorganisms and hence, not subjected to rapid decomposition (Tisdall, 1996).

Since the decomposition of carbon and nitrogen from plant residues generally follows a very similar pattern (Haynes, 1986), the effect of residue management on organic C and total N are also very similar. Total soil N generally increased in the order of Bto < Bt < T as was the case for soil organic C. During the burning of crop residues the estimated loss of N to the atmosphere is in the order of 30 - 90 %, depending on the extent of combustion (Raison and McGarity, 1979; Biederbeck *et al.*, 1980). Organic N is volatilized chiefly as N<sub>2</sub> or oxides of N (Norman and Wetselaar, 1960; Lloyd, 1971) but some aqueous and volatile tar-like products are also lost. In comparison with commonly reported C/N ratios for soil organic matter of 10 - 12 (Schlesinger, 1995), values for this soil were rather wide (15 - 19). This probably reflects the protective effect of the high content of smectitic clays present in the study soil.

Due to the immobility of orthophosphate in soils, accumulation of Truog-extractable P, induced by fertilizer, was most pronounced in the surface 2.5 cm soil layer. Nevertheless, significant accumulation was also detected in the 5 - 10 and 10 - 20 cm soil layers (Figures 3.4 and 3.5). It is interesting that increases in Truog extractable P induced by fertilizer applications were evident in the 5 - 10 and even the 10 - 20 cm soil layers. Such downward movement of P is surprising since phosphate is known to be rather immobile in soils and it characteristically accumulates near the soil surface as noted in this study. However, this probably reflects the downward redistribution of applied P, which would have occurred in the earlier years of the trial at replanting when the site was conventionally cultivated. As expected, fertilization, induced the greatest percentage increase in extractable P the labile, easily extractable (NaHCO<sub>3</sub> - Pi) fraction (Figures 3.7 and 3.8, Table 3.2).

Nevertheless, the greatest absolute increase of inorganic P was in the insoluble "occluded" P (NaOH (II) - Pi) form reflecting that over time residual Pi becomes increasingly less labile as slow reactions between orthophosphate and soil colloids continue (Barrow, 1980). The mechanisms of the slow reaction and the nature of the firmly held phosphate are uncertain. However, this firmly held phosphate is less

available to plants than the newly adsorbed phosphate, especially in the short-term (Barrow, 1980).

The extractable P levels also tended to increase with the increase in crop residue retention. A number of studies have demonstrated that addition of organic amendments to soil can significantly increase the availability of P to plants and/or decrease the P adsorption capacity of soils (Easterwood and Sartain, 1990; Hue *et al.*, 1994; Iyamuremye *et al.*, 1996). The reduced P adsorption and increased P availability following applications of organic amendments to soils is thought to be the cumulative result of several mechanisms (Iyamuremye and Dick, 1996). Organic material generally contains significant quantities of P and during its decomposition P is released into soil solution thus increasing P availability. In addition to this, a wide range of organic compounds is released from residues during their decomposition and/or synthesized by the decomposer microflora. The two most important groups of organic compounds in relation to P availability are soluble humic molecules and low molecular weight aliphatic organic acids. Both these groups of substances can be adsorbed onto Al and Fe oxide surfaces through both electrostatic bonding (anion exchange) and specific adsorption (ligand exchange) (Earl *et al.*, 1979; Violante and Gianfreda, 1993; Stevenson, 1994; Jones and Brassington, 1998). These substances will block potential P adsorption sites and tend to increase the availability of P originating from both the organic materials and subsequently added fertilizer P (Figure 3.9).

As expected, accumulation of organic P was stimulated by fertilizer additions since it also favoured accumulation of soil organic matter. The highest percentage increase in organic P in the 0 - 2.5 cm layer occurred in the labile  $\text{NaHCO}_3$  - Po fraction although highest absolute increases were in the more stable  $\text{NaOH}$  (I) - Po fraction (Figure 3.8, Table 3.2). The labile Po may be transformed to more stable forms (e.g.  $\text{NaOH}$ (I) - Po) during humus formation (Haynes and Williams, 1992) and evidently added fertilizer P accumulated in both forms.

Accumulation of P in organic forms due to long-term annual applications of fertilizer

P has been observed in other long-term experiments (Condrón and Goh, 1989) and is not surprising where an accumulation of soil organic matter has also occurred. As expected, this accumulation of P in organic form was more pronounced in the trashed than burnt treatments due to greater organic matter content. Fertilizer applications and trash retention both induced a pronounced increase in labile ( $\text{NaHCO}_3$  - Po) and NaOH (I) - Po fractions (Figure 3.8). The distinct accumulation of P into the NaOH (II) - Po fraction under trash retention suggests that over time, there has been an accumulation of P into the humified, recalcitrant fraction of soil organic matter.

Potassium removals in harvested cane are characteristically large; for a  $100 \text{ Mg ha}^{-1}$  crop removals of K commonly ranges from  $100 - 200 \text{ kg K ha}^{-1}$  (Meyer and Wood, 1985; Haynes and Hamilton, 1999). Thus, the substantial depletion of exchangeable and non-exchangeable K in both the BtoFo and BtFo treatments is not surprising, indicating that a net removal of K occurred in unfertilized treatments (Figures 3.2 and 3.4). Vertisols contain 2:1 clay minerals which contain K as part of their mineral structure (Wilding and Tessier, 1988) and when soil solution K concentrations become low, the clay lattice may partly open. Non-exchangeable K is released to exchangeable form in order to supply the high demands for K by the sugarcane crop (Sparks and Huang, 1985). Similarly, Naidu *et al.* (1992) demonstrated a depletion of both exchangeable and non-exchangeable K under sugarcane production on smectitic soils in Fiji. The large accumulation of K in both exchangeable and non-exchangeable forms in the TF treatment reflect the large amount of K being annually cycled in the trash plus the effect of annual application of fertilizer K (Table 3.1). Results demonstrate that in this Vertisol, non-exchangeable K can act as both a source and sink for plant-available K.

The lower exchangeable and non-exchangeable K concentrations on the burnt, compared with trashed, plots indicates a loss of K due to burning (Figures 3.2 and 3.4, Table 3.1). This is surprising since K is not lost via volatilization during burning. However, visual observations at the site (van Antwerpen, personal communication,

1999) suggest that much of the returned ash is often blown off the plots by strong coastal winds. This would result in significant losses of K, Ca, Mg and P from the burnt plots.

A positive interaction between trash retention and fertilizer K addition was evident with the TF treatment having the highest exchangeable and non-exchangeable K concentrations recorded. Indeed, for South African soils, Meyer and Wood (1985) suggest a critical exchangeable K level of 2.9 for light and medium textured soils and 5.8 mmol<sub>c</sub> kg<sup>-1</sup> for heavy textured soils. This heavy textured soil therefore has low K levels for all of the treatments, except for the trashed, fertilized (TF) treatment.

Soil acidification is an aspect of soil degradation, which commonly occurs under sugarcane production (Abruna-Rodriguez and Vicente Chandler, 1967; Wood, 1985; Masilaca *et al.*, 1986; Schroeder *et al.*, 1994; Coale, 1993; Hartemink and Kuniata, 1996; Hartemink, 1998 a,b) and is clearly evident on fertilized plots. The main cause of such acidification is likely to be nitrification of ammonium sulphate, which is applied at 140 kg N ha<sup>-1</sup> yr<sup>-1</sup>. During nitrification of ammonium sulphate fertilizer two H<sup>+</sup> ions are released per unit of NH<sub>4</sub><sup>+</sup> - N applied. Such acidification will only be permanent if the nitrate is lost from the system (e.g., by nitrate leaching) effectively leaving the H<sup>+</sup> ions produced behind (Wild, 1994). Thus, the substantial acidification that was observed to a depth of 30 cm suggested there is a considerable loss of nitrate from the soil via leaching. Hartemink (1998b) also observed a rapid decline in pH under sugarcane on a Papua New Guinea plantation associated with the use of ammonium sulphate fertilizer.

Data regarding the effects of returns of organic residues to soils on soil pH are conflicting with both increases and decreases being reported (Hoyt and Turner, 1975; Lungu *et al.*, 1993; Schjønning *et al.*, 1994; Wild, 1994; Noble *et al.*, 1996; van Antwerpen and Meyer, 1998). In this study, trash retention tended to reduce soil pH compared to burning and this may be attributed to nitrification during mineralization as the trash blanket decomposes (Figure 3.2) (Hoyt and Turner, 1975). This

suggests that greater quantities of N are cycled by the practice of trash retention probably causing greater nitrate leaching and more acidification. By contrast, under burning any acidifying effect of nitrification would tend to be countered by the return of ash, which is generally an alkaline, base - rich material (Raison, 1979).

A decrease in soil  $\text{pH}_{(\text{water})}$  below about 5.5 can cause solubilization of Al from the crystal lattice of clay minerals, resulting in the formation of amorphous Al compounds and the movement of Al ions into exchange positions and into soil solution. Thus, in the fertilized treatments there was an increase in concentrations of  $\text{Al}_T$  and  $\text{Al}_{\text{Mono}}$  in soil solution, in exchangeable Al, and also in the buffering reserve of non-exchangeable Al complexed with organic matter (i.e., copper chloride extractable) (Table 3.4). Both  $\text{H}^+$  and  $\text{Al}^{3+}$  ions have a strong affinity for negatively charged soil surfaces and displace exchangeable base cations (e.g. Ca, Mg, K and Na) from exchange sites. These base cations move into soil solution and leach to lower soil layers; essentially in the form of calcium and magnesium nitrates (Haynes and Swift, 1986). This process results in considerable losses of exchangeable Ca and Mg and to a lesser extent, K and Na concentrations from the surface soil (Figures 3.3, 3.10 and 3.11).

The decrease in soil pH in fertilized treatments resulted in the surface charge conferred on the variable charge surfaces on soil colloids becoming less negative and as a result there was a decrease in ECEC. Acidification in fertilized treatments not only increased exchangeable Al but also the buffering reserve of non-exchangeable Al; both that complexed with soil organic matter (copper chloride - extractable) and that present as hydroxy - Al associated with mineral colloids (ammonium acetate - extractable ). For the TF treatment there was also a detectable increase in amorphous and oxalate - extractable Al (Figure 3.12). Due to the high CEC of organic matter (Stevenson, 1994) the increased organic matter content under trash retention resulted in an increase in ECEC. This enabled the soil to retain greater amounts of Ca, Mg and K, which were returned to the soil in the trash (Figure 3.11).

As expected, concentrations of  $Al_T$  in soil solution increased with decreasing pH (Table 3.2). However, in relation to the phytotoxicity of Al in soil solution, it is the concentration of  $Al_{Mono}$  that is important (Wright, 1989). Several workers have demonstrated that additions of decomposing organic residues to soils can reduce both  $Al_T$  and  $Al_{Mono}$  in soil solution (Kretzschmar *et al.*, 1991; Bessho and Bell, 1992; Wong *et al.*, 1995; Noble *et al.*, 1996). More particularly, soluble organic matter produced during crop residue decomposition can complex with  $Al_{Mono}$  and markedly reduce the proportion of  $Al_T$  present in monomeric form (Kretzschmar *et al.*, 1991; Bessho and Bell, 1992; Berek *et al.*, 1995; Haynes and Mokolobate, 2001). Aluminium forms strong complexes with soluble organic matter (e.g., soluble humic compounds and organic acid anions) (Kretzschmar *et al.*, 1991; Bessho and Bell, 1992; Berek *et al.*, 1995) and studies have shown that such Al is not toxic to plants (Tan and Binger, 1986; Kochian and Jones, 1996).

In this study trash retention tended to increase the concentrations of both  $Al_T$  and  $Al_{Mono}$  in soil solution. Other workers have also shown that  $Al_T$  in soil solution can be unaffected or even increased during organic residue decomposition (Berek *et al.*, 1995; Slattery and Morrison, 1995). It is thought that soluble organic matter, originating from residue decomposition, complexes with Al and maintains it in the solution phase. In addition, in this study the trashed treatments tended to have a lower soil pH than other treatments and this would also tend to increase the concentration of  $Al_T$  and particularly  $Al_{Mono}$  in solution.

Whilst the effects of fertilizer N and trash management on soil acidification and Al chemistry discussed above are of scientific interest, they are of little agronomic significance for sugarcane. This is for two reasons. Firstly, sugarcane is characteristically tolerant of soil acidity and high concentrations of exchangeable and soluble Al (Hetherington *et al.*, 1988; Hartemink, 1998c). Secondly, due to the lack of amorphous clay minerals in the study soil, the concentrations of exchangeable and soluble Al reported in this study (even at pH values of 5.0 and

below) are very low (Hetherington *et al.*, 1988; Bramley *et al.*, 1996). However, the marked acidification to a depth of 30 cm in the fertilized treatments is of potential concern since subsoil acidity is characteristically difficult to ameliorate, due to the immobility of lime, and the use of other amendments such as gypsum needs to be considered (Sumner, 1997). Sugarcane can also be sensitive to Ca deficiency (Baver and Ayres, 1962). Thus, the substantial loss of exchangeable Ca that accompanies acidification is also a potential problem.

Acidification was particularly severe where a combination of N fertilization and trash retention (TF) was applied. As noted previously, this acidification is attributable to nitrification and subsequent leaching of nitrate. Reducing fertilizer N application rates where trash retention is practiced (and N returns are much greater than for burning) seems desirable. Indeed, strategies to improve fertilizer N use-efficiency by the sugarcane crop, and therefore reduce nitrate-leaching losses (e.g., split N applications) would be desirable and should reduce soil acidification considerably.

Soil organic matter contributes significantly to the formation and stabilization of soil structure so that maintenance of soil organic matter and stabilization of soil structure often go hand-in-hand. For example, there is often a close correlation between soil organic matter content and aggregate stability (Haynes, 1999b). Micro-aggregates (< 250  $\mu\text{m}$  diam) act as the building blocks of soil structure and through the cementing actions of polysaccharides and humic substances, they can become linked with one another and with other components such as fragments of decomposing organic material and sand particles to form macro-aggregates (Tisdall and Oades, 1982). The sharp decline in aggregate stability after plowing-in a long-term grassland is characteristic (Low, 1972) and is often more pronounced than the initial decline in soil organic matter content. This is because grass has positive effects on aggregate stability in addition to those associated with increased organic matter content. These additional effects include production of large quantities of polysaccharide binding agents by the microbial biomass in the pasture rhizosphere and the enmeshing effects of fine grass roots and associated mycorrhizal hyphae (Haynes and Beare, 1996). Thus, aggregate stability in the grass rows was greater

than that under trash retention even though  $C_{org}$  was, in fact, higher under trash (Figure 3.13).

That aggregate stability followed the general order burnt with harvest residues removed < burnt with tops left on the soil surface < green cane harvested with trash retention reflects the importance of increasing soil organic matter content in stabilizing soil aggregates. Stabilization is favoured through the binding and glueing actions of humic substances and polysaccharides and the enmeshing effect of fungal hyphae (Haynes and Beare, 1996). Nonetheless, fertilized plots had notably lower aggregate stability than unfertilized ones, despite the tendency for the former to have higher  $C_{org}$  values. Van Antwerpen and Meyer (1998) suggested that acidification in the fertilized plots indirectly contributed to this decline in stability. That is, the leaching of Ca and Mg out of the acidified surface soil in association with annual additions of K resulted in an increase in the proportion of monovalent to divalent cations present and this favoured dispersion and a decrease in aggregate stability in fertilized plots. In agreement with this assertion, raising the pH of samples of the TF soil using  $Ca(OH)_2$  increased aggregate stability. Furthermore, addition of K (as  $K_2SO_4$ ) to samples of TFO soil caused a marked reduction in stability. Whilst soil pH *per se.* may influence dispersion / flocculation phenomena (Haynes and Naidu, 1998), in this study it appeared to be the relative quantities of K and Ca that were important since additions of KOH and  $Ca(OH)_2$  had opposing effects (Figure 3.13).

In a review of aggregation in Vertisols, Dalal and Bridge (1996) observed that although significant relationships exist between aggregation and  $C_{org}$  content, exchangeable cations exert a dominant role. Other workers (Coughlan and Loch, 1984; Dalal, 1989; Cook *et al.*, 1992) have also noted that aggregate stability in Vertisols is primarily associated with exchangeable cations; especially Na even though it is usually present in moderate amounts (< 10 % of total exchangeable cations). At the experimental site, exchangeable Na concentrations were small and unaffected by fertilizer applications. Nevertheless, the mean percentage monovalent

exchangeable cation content (K plus Na as a percentage of ECEC) in the surface 0 - 2.5 cm layer was 3.5 % in unfertilized plots and 6.7 % in fertilized ones.

Apparently, dispersion/flocculation phenomena are particularly important in determining aggregate stability in the study soil, which has a clay mineralogy that is dominated by smectites. Cations near the negatively charged clay surfaces are subject to an electrostatic attraction toward the surface as well as a tendency to diffuse into bulk solution. As a result, the concentration of cations diminishes exponentially as a function of distance from the clay surface. The partition of cations between surface sorption in the diffuse layer and solution is termed the electrical double layer. The formation of the double layer leads to mutual repulsion of opposing clay surfaces in dilute electrolyte solutions. As the valency of cation (e.g. divalent cations replace monovalent ones) and / or solution ionic strength increases, the diffuse layer is compressed and the repulsive force decreases. Attractive forces between clay particles (e.g. Van der Waals forces) diminish rapidly with increasing separation distance. Thus when the double layer is compressed, attractive forces become more important. Where the net forces are attractive, the clay particles remain close together and are described as flocculated. Conversely, if the net force is repulsive the particles move further apart and may exist as separate entities in a dispersed state.

Evidently, both soil organic matter content and the composition of exchangeable cations present are important determinants of aggregate stability in the study soil. This means that management of soil physical properties in such soils requires not only management of soil organic matter content but also management of K fertilization and the balance between exchangeable K and that of Ca and Mg.

### **3.5 Conclusions**

The most negative effect that sugarcane has on soil quality is the loss of soil organic matter. One of the main contributing factors is management practice. The common

practice of burning standing cane to dispose of post-harvest crop residues is the main reason for the loss of soil organic matter. Large amounts of C and N are volatilized during the burning process. Such diminution of soil organic matter is likely to result in a reduction in soil biological activity, nutrient supplying power and soil structural condition. Therefore, soil organic matter is considered a key attribute to soil quality. However, green cane harvesting with the retention of a trash blanket over the soil surface, results in large amounts of crop residues being returned to the soil surface. These residues decompose slowly and result in an accumulation of organic matter in the surface soil. This management practice thus has a positive effect on one of the most important aspects of soil quality.

Crop residues also play an essential role in the cycling of nutrients through agricultural ecosystems. The accumulation of P in labile organic fractions and K in exchangeable form under trash retention, underlines the large amounts of nutrients that are recycled under green cane harvesting. The nutrients added to soil in organic residues have the advantage of being released gradually and are less sensitive to leaching, volatilization or fixation. The additional accumulation of P in more stable organic matter fractions and K in non-exchangeable forms also indicates a build-up of nutrient reserves associated with trash blanketing. However, in the long-term, recommended fertilizer rates are likely to be lowered if there is a shift from burning to green cane harvesting.

Soil acidification is an aspect of soil degradation, which commonly occurs under sugarcane production. The main cause of such acidification is the use of ammonium containing fertilizers as was the case in this study. Although sugarcane is tolerant to low pH levels and increased levels of Al in soil solution (associated with soil acidification) this acidification results in an increase in exchangeable acidity and a concomitant displacement of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  that is leached from the surface layer of soil essentially in the form of calcium and magnesium nitrates. To improve fertilizer N use efficiency, split applications of fertilizer N should be considered in order to reduce the opportunity for  $\text{NO}_3^-$  leaching and thus permanent soil acidification.

Both soil organic matter content and the composition of exchangeable cations present are apparently important determinants of aggregate stability in the study soil. This means that management of soil physical properties in such soils requires not only management of soil organic matter content but also management of K fertilization and the balance between exchangeable K and Na and that of Ca and Mg.

## CHAPTER FOUR

### 4. The effects of long-term crop residue management and fertilizer application on soil organic matter quality.

#### 4.1 Introduction

An important objective for any sustainable agricultural system is to maintain and improve the soil organic matter content (Gregorich *et al.*, 1994). Soil organic matter is important as a reservoir of nutrients such as N, S and P and, upon mineralization, plant available forms are released. It also contributes significantly to the formation and stabilization of soil structure. For these reasons soil organic matter is considered a key attribute to soil quality. Because crop residues are primary sources of organic matter in agricultural systems, crop management and fertilizer regimes can exert a significant influence on soil organic matter quality (Campbell *et al.*, 1991). Sustaining or enhancing soil productivity therefore depends, at least partly, on soil and crop management practices that maintain or increase soil organic matter levels (Havlin *et al.*, 1990). Management practices such as no tillage and crop residue retention, crop rotation and fertilizer application may influence organic matter levels in soil by influencing the quantity and quality of crop residues (tops and roots) which are returned to the soil as well as the rate of decomposition of both added residues and native soil organic matter (Angers *et al.*, 1993; Gregorich *et al.*, 1994; Haynes and Beare, 1996).

In many parts of the world, including South Africa, the normal practice is to burn standing sugarcane prior to harvest to removed dead leaves (trash) clinging to the harvested stems. During burning, large amounts of C, as well as N and S, present in the plant residues are lost via volatilisation (Raison, 1979). Furthermore, approximately every 7 years, the sugar fields are conventionally cultivated and then replanted and this promotes soil organic matter decomposition (Haynes and Beare, 1996). It is, therefore, not surprising that many workers have measured an

appreciable decrease in soil organic matter content under long-term sugarcane production (Blair *et al.*, 1998; Blair, 2000).

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. Short-term (< 5 years) effects of trash retention include a small increase in labile, and sometimes total organic matter content, in the surface soil and increased earthworm activity (Wood, 1991; Blair, 2000). Nevertheless, the effects of conversion from burning to green cane harvesting on soil organic matter content and quality are likely to be cumulative and become more pronounced in the longer term (e.g. > 50 years). Whilst these effects can be modelled (Vallis *et al.*, 1996), lack of long-term data means the results of such exercises are somewhat speculative.

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 rates 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 various tillage methods, straw incorporation and use of grass leys in rotation (Carter, 1986; Powlson *et al.*, 1987; Haynes, 1999b). 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 size of labile fractions may also fluctuate over the season due to changes in organic matter inputs and/or decomposition rates whilst total soil organic

matter content remains unchanged (Gregorich *et al.*, 1994).

There is very little information available regarding labile organic matter fractions under sugarcane production. With the recent interest in organic matter quality and the concern regarding the organic matter decline under sugarcane production, Bramley *et al.* (1996) and Skjemstad *et al.* (1999) presented groundwork quantifying various labile organic matter fractions under sugarcane. The long-term crop residue management trial, at the Sugar Experiment Station at Mount Edgecombe, is an ideal site to investigate the effect of crop residue management and fertilizer application on soil organic matter quality.

## 4.2 Materials and Methods

The experimental site at the South African Sugar Association Experiment Station at Mount Edgecombe was sampled in 1998 to a depth of 30 cm and sectioned into the 0 - 2.5, 2.5 - 5, 5 - 10, 10 - 20, and 20 - 30 cm layers (refer to site description in chapter 3). Samples from each plot were bulked. A field-moist sub-sample was sieved (<2 mm) and stored at 1 °C for not more than 72 h for microbiological and other analyses. Part of this sample was subsequently air-dried and finely ground (< 150  $\mu\text{m}$ ). Organic C and total N were determined on air-dried samples (see chapter 3) whilst the other analyses were performed on field-moist ones.

A labile C fraction was extracted with 333mM  $\text{KMnO}_4$  according to the method of Blair *et al.* (1995). Microbial biomass C ( $C_{\text{mic}}$ ) and N ( $N_{\text{mic}}$ ) were estimated based on the difference between organic C and total N, respectively, extracted with 0.5 M  $\text{K}_2\text{SO}_4$  from chloroform-fumigated and unfumigated soil samples using a  $K_c$  factor of 0.38 (Vance *et al.*, 1987) and a  $K_N$  factor of 0.54 (Brookes *et al.*, 1985). The quantity of  $\text{K}_2\text{SO}_4$  - extractable C and N extracted from unfumigated soil was used as a measure of labile soil C and N. The  $C_{\text{mic}}/C_{\text{org}}$  and  $N_{\text{mic}}/N_{\text{org}}$  ratios were calculated by expressing microbial biomass C and N as a percentage of total soil organic C and total N respectively.

Light fraction (LF) was isolated from sieved (<2 mm) field - moist soil by the method of Gregorich and Ellert (1993) using NaI solution (SG = 1.70). Two successive extractions of 60 min were used (1:2 soil : extractant ratio) and the isolated LF was oven - dried at 70 °C and weighed. Organic C and total N content of the LF were determined as outlined above.

Mineralizable C and N were determined from closed aerobic incubations at 25°C over 10 days. Thirty grams oven dry soil equivalent to field - moist soil was placed in 50 ml beakers and incubated in darkness, in 1L air - tight jars. The CO<sub>2</sub> evolved, was trapped in a container of 10 ml of 0.5 M NaOH and determined by titration on two occasions (5 and 10 days) during the incubation (Anderson, 1982). Inorganic soil N (NH<sub>4</sub><sup>+</sup> - and NO<sub>3</sub><sup>-</sup> - N) was determined by distillation (Keeney and Nelson, 1982) at the commencement and end of the incubation after extraction of 10 g of soil with 50 ml 2 M KCl.

The statistical significance of experimental treatments was determined by subjecting the data to analysis of variance and least significant differences (LSD) were calculated at the 5% level.

### 4.3 Results

For completeness, data for organic C (C<sub>org</sub>) and total N (N<sub>tot</sub>) from chapter 3 are included here. In the surface 5 cm, concentrations of C<sub>org</sub> (Appendices 3.1; 3.2; Figure 4.1) and N<sub>tot</sub> (Appendices 3.1; 3.2) generally followed the order BtoFo ≤ BtFo ≤ BtoF ≤ BtF < Tfo < T. Concentrations of C<sub>mic</sub> (Appendices 4.1; 4.2; Figure 4.1) and N<sub>mic</sub> (Appendix 4.1; 4.2) showed broadly similar trends to those for C<sub>org</sub> and N<sub>tot</sub> being increased by both increasing returns of crop residues and the addition of fertilizers. The order was BtoFo < BtoF ≤ BtFo < BtF < Tfo < Tf. Since changes in N<sub>tot</sub> and N<sub>mic</sub> with treatments were considerably more marked than those of C<sub>org</sub> and C<sub>mic</sub>, the C/N ratio of both the total soil organic matter and of the microbial biomass was decreased by both increasing crop residue returns and fertilizer additions (Appendices 3.1; 3.2; 4.1; 4.2; Figure 4.1).

Significant increases in  $C_{org}$  (refer to chapter 3, figure 3.3) and  $N_{tot}$  due to trash retention (and fertilizer additions) were observed only in the surface 10 cm of soil. By contrast, significant increases in  $C_{mic}$  were observed to a depth of 30 cm in response to trash retention (Appendices 4.1, 4.2, 4.3, 4.4, 4.5; Figure 4.2). The fertilizer-induced increases in  $C_{mic}$  and  $N_{mic}$  were also evident to a depth of 30 cm (Appendices 4.1, 4.2, 4.3, 4.4, 4.5; Figure 4.2). Since  $C_{mic}$  was considerably more influenced by treatments than  $C_{org}$ , the microbial quotient increased in the order  $BtoFo < BtoF \leq BtFo \leq BtF < Tfo < TF$  (Figure 4.3). The microbial quotient decreased with increasing soil depth but was markedly increased by trash retention to a depth of 30 cm (Figure 4.2).

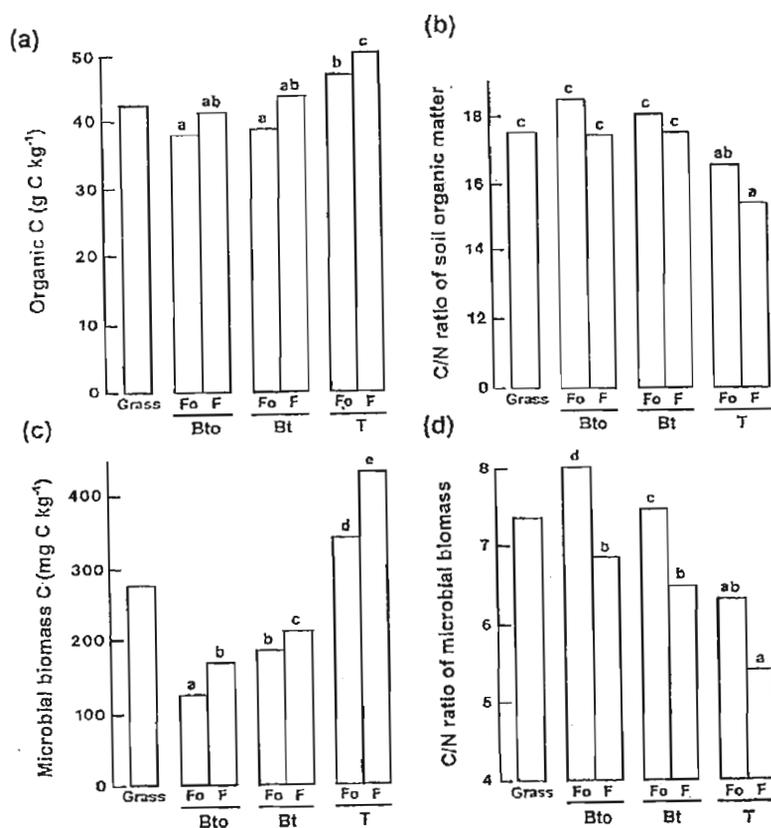
In the surface 5 cm,  $K_2SO_4$ -extractable C,  $KMnO_4$ -extractable C, and readily mineralizable C and N all increased in response to increasing amounts of crop residues being returned and to fertilizer additions (Appendices 4.1; 4.2; Figure 4.4 and Figure 4.5). Mineralizable N was notably increased by both factors and, as a result, the ratio of mineralizable C/N ratio declined in response to both crop residue and fertilizer additions (Figure 4.4).

Concentrations of  $K_2SO_4$ - extractable C (Figure 4.6), light fraction C (Appendices 4.6, 4.7, 4.8, 4.9, 4.10; Figure 4.8) and mineralizable C and N (Appendices 4.1, 4.2, 4.3, 4.4, 4.5) were all higher under trash retention than burning to a depth of 30 cm. In contrast, concentrations of  $KMnO_4$  -extractable C were higher under trash retention only to a depth of 10 cm (Figure 4.6). Light fraction dry matter, C and N showed a general trend for increasing concentrations with increasing returns of crop residues (i.e.  $Bto < Bt < T$ ) (Appendices 4.6, 4.7, 4.8, 4.9, 4.10 and Figures 4.7). However, lower values were recorded for F than Fo for light fraction dry matter (Bt and T) and C (T). The C/N ratio of the light fraction was higher for the sugarcane soils than in the grass row but was less for TF than the other treatments.

When quantities of  $C_{org}$ ,  $N_{tot}$  and various labile fractions of organic matter were

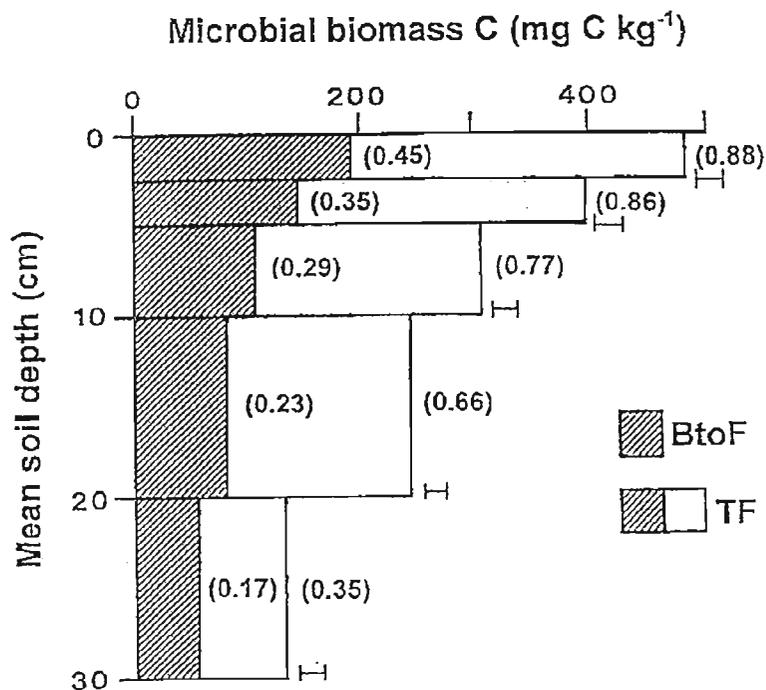
calculated on a per-hectare basis to a depth of 30 cm (Table 4.1), there were no significant treatment differences for  $C_{org}$  (also refer to chapter 3, table 3.1). The reason for this is that significant differences in  $C_{org}$  content were observed only in the top 10 cm. In fact, on a per hectare bases, the quantity of  $C_{org}$  present in the surface 10 cm in TFo and TF was significantly larger than that in the BtoFo and BtoF treatments respectively (data not shown). However, the quantity of  $C_{org}$  present in the surface 10 cm represented only about 35% of that present to a depth of 30 cm.

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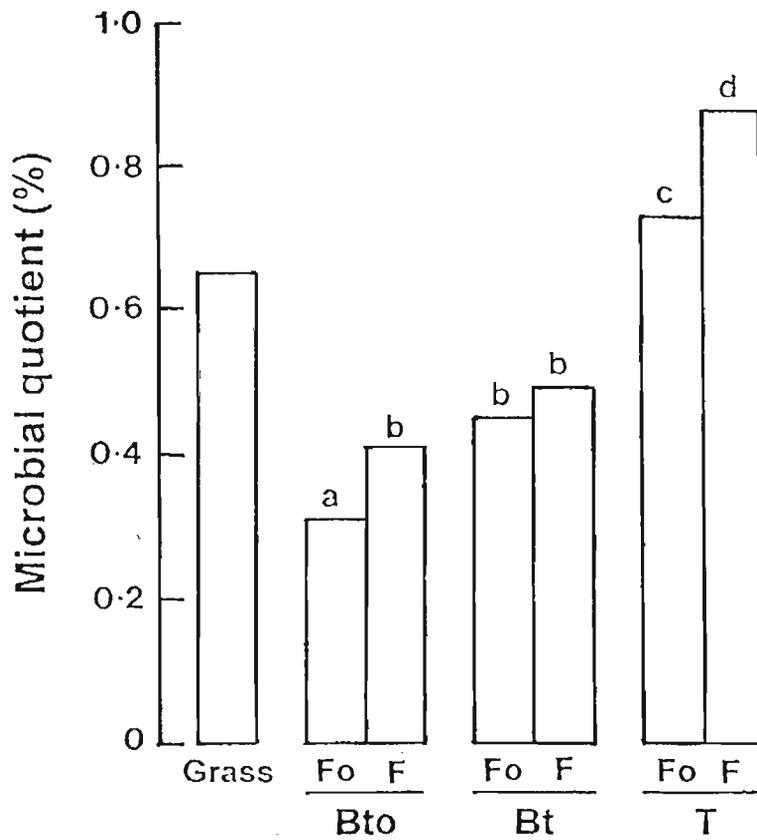
**FIGURE 4.1:**

Effects of long-term residue management practices and fertilizer applications on (a) organic C (redrawn from chapter 3), (b) microbial biomass C and the (c) C/N ratio of the organic matter and the (d) microbial biomass in the 0 - 5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



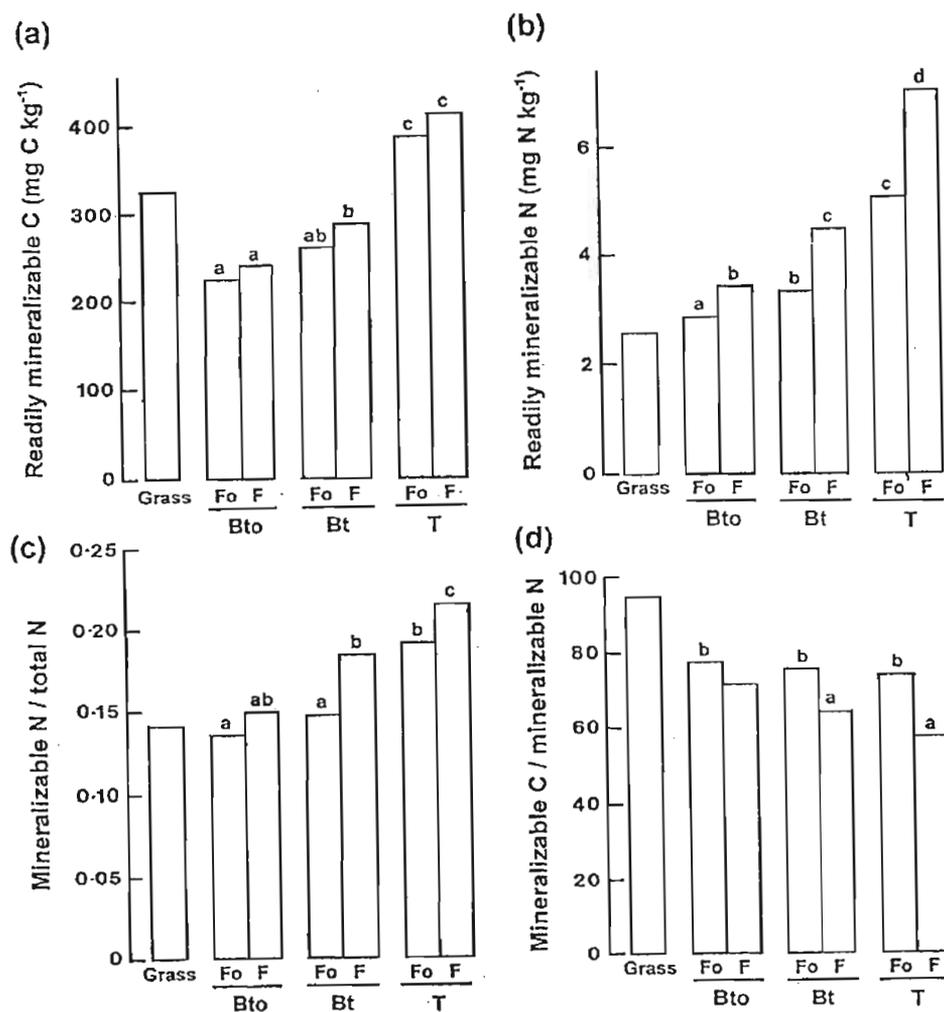
**FIGURE 4.2:**

Distribution of 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. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.



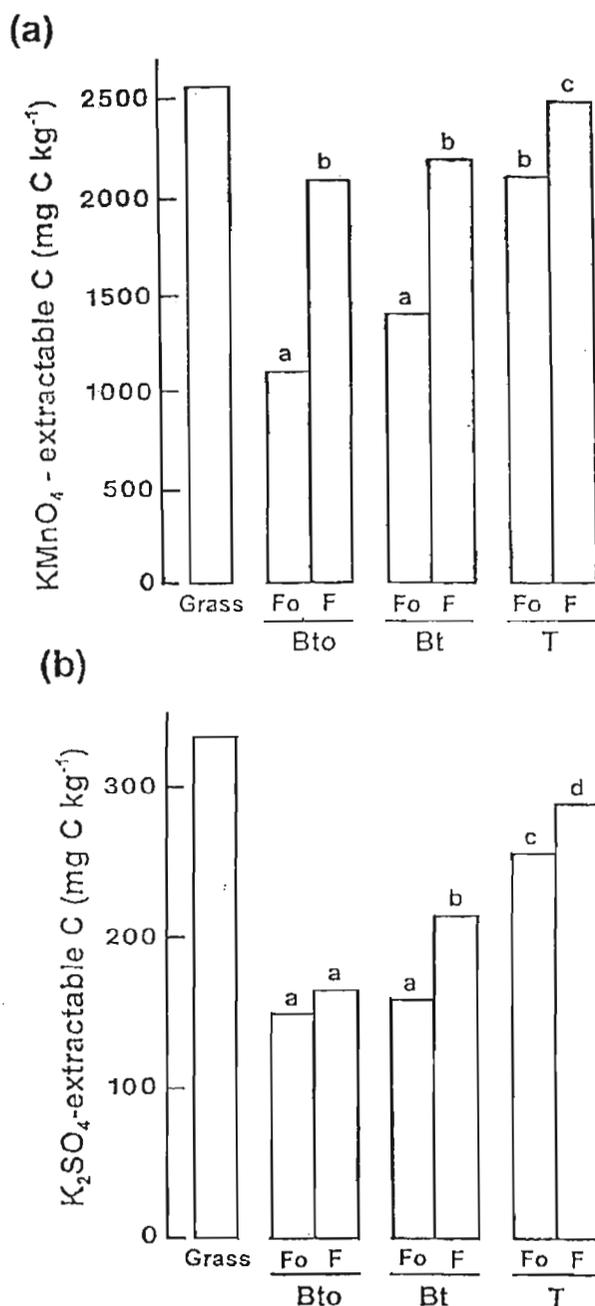
**FIGURE 4.3:**

Effects of long-term residue management practices and fertilizer applications on the microbial quotient in the 0 - 5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



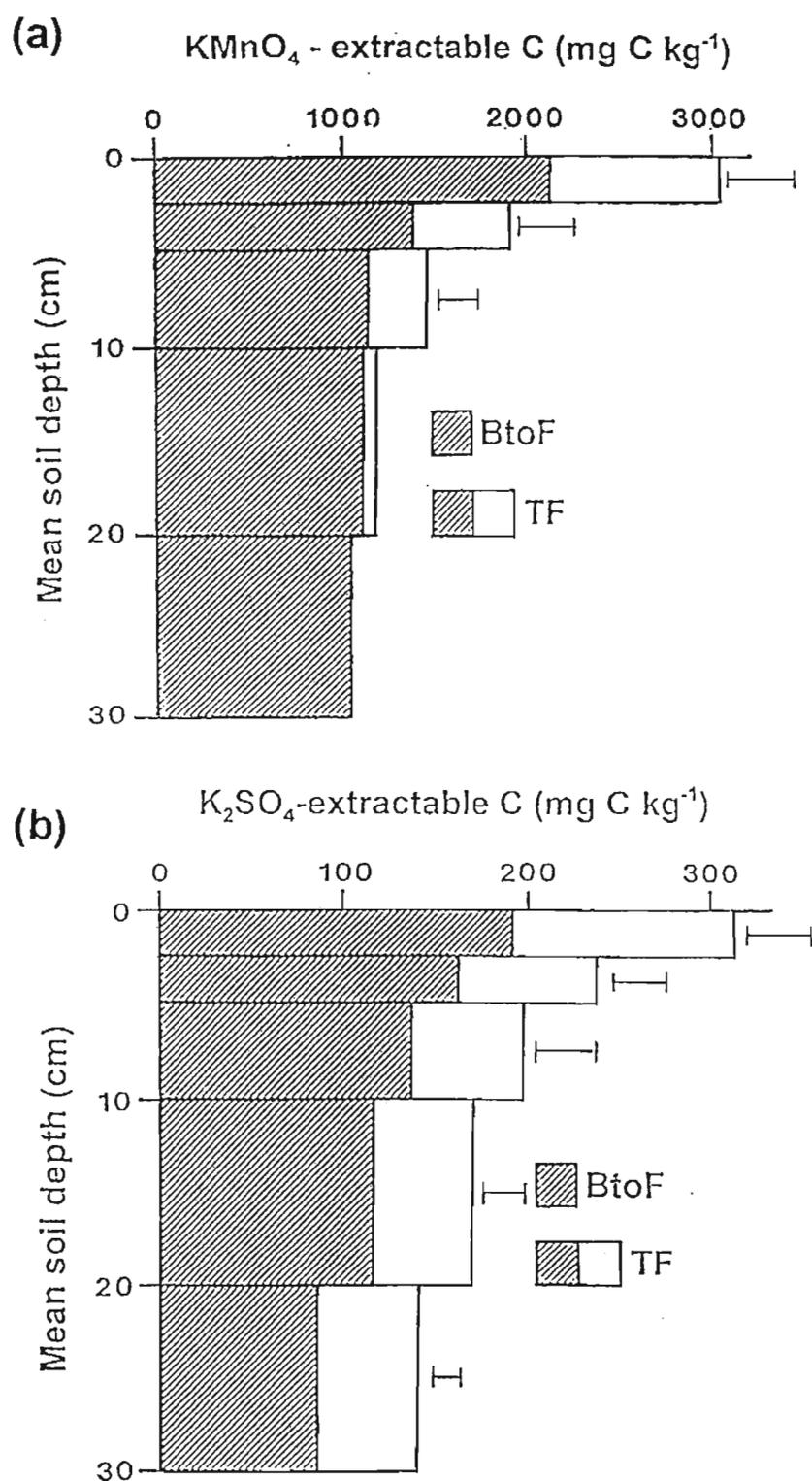
**FIGURE 4.4:**

Effects of long-term residue management practices and fertilizer applications on (a) readily mineralizable C and (b) N, (c) the mineralizable N/total N ratio and the (d) mineralizable C/mineralizable N ratio in the 0 - 5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



**FIGURE 4.5:**

Effects of long-term residue management practices and fertilizer applications on (a) KMnO<sub>4</sub>-extractable C and (b) K<sub>2</sub>SO<sub>4</sub>-extractable C in the 0 - 5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means in the 0 - 5 cm layer associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



**FIGURE 4.6:** Distribution of (a)  $\text{KMnO}_4$ -extractable C and (b)  $\text{K}_2\text{SO}_4$ -extractable C in the soil profile in the BtoF and TF treatments. T = green cane harvested with trash retention; Bto = burnt with

harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.

Thus, when calculated to 30 cm, no significant differences were observed. As already noted, changes in  $N_{\text{tot}}$  with treatment (in the surface 10 cm) were considerably more marked than those for  $C_{\text{org}}$  and, as a result, significant with increasing additions of crop residues. For mineralizable C and N,  $K_2SO_4$ -extractable C, light fraction C and  $C_{\text{mic}}$ , highly significant increases were observed in the soil profile to 30 cm in response to increased returns of crop residues and fertilizer additions. Such increases were not surprising since for these parameters, treatment effects were generally significant to a depth of 30 cm.

Compared to BtoF, the percentage accumulation of organic matter in the profile due to trash retention (TF) to 30 cm amounted to 3.8 % for  $C_{\text{org}}$ , 153 % for  $C_{\text{mic}}$ , 46 % for  $K_2SO_4$  - extractable C, 66 % for readily mineralizable C and 91 % for light fraction C. This demonstrates that trash retention caused much larger increases in the size of labile C pools in the soil than those for  $C_{\text{org}}$ . Nonetheless, the corresponding increase for  $KMnO_4$  - extractable C was only 11.5 % and this reflects the lack of significant differences in this pool of C below 10 cm soil depth. Compared to Tfo, accumulation of  $C_{\text{org}}$  in response to annual fertilizer application (i.e. in TF) amounted to only 2.2% whilst for  $C_{\text{mic}}$  the corresponding value was 23 %.

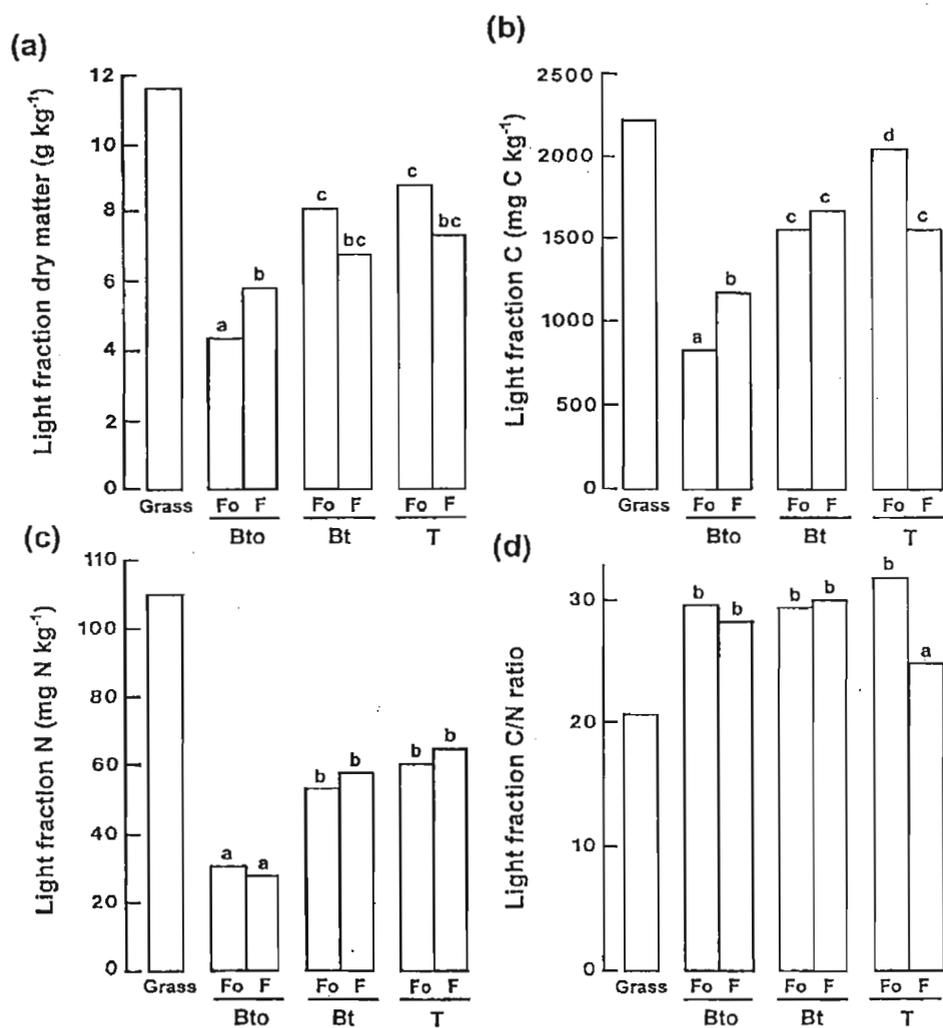
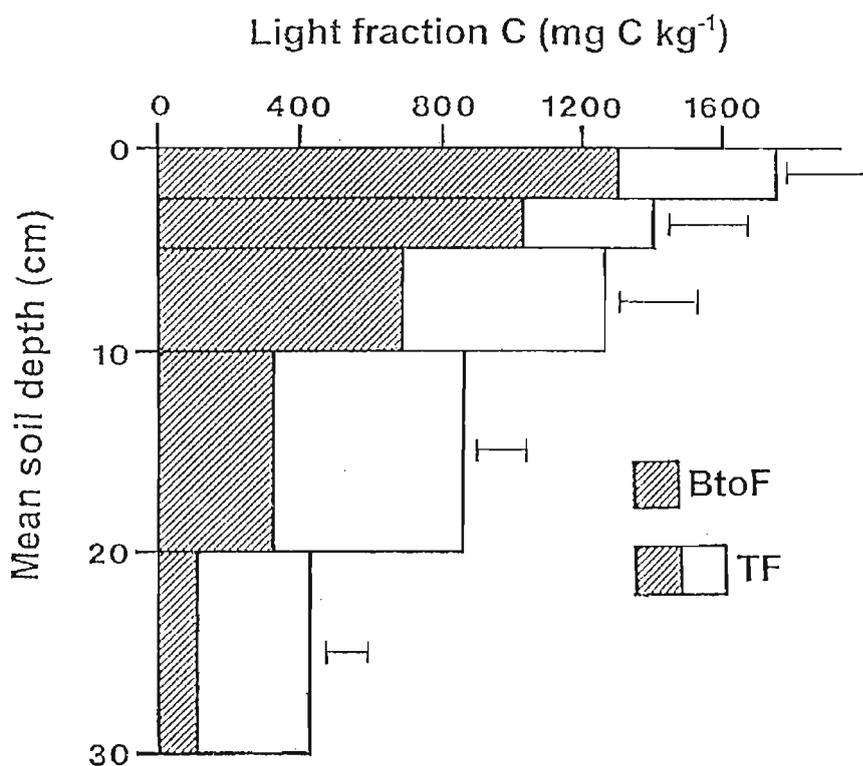


FIGURE 4.7:

Effects of long-term residue management practices and fertilizer applications on the (a) dry matter, (b) C and (c) N content and (d) C/N ratio of the light fraction in the 0-5 cm soil layer. Grass = unfertilized grass, T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



**FIGURE 4.8:** Distribution of light fraction C in the soil profile in the BtoF and TF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.

**TABLE 4.1:** Quantities of organic C, total N, mineralizable C and N, microbial biomass C, K<sub>2</sub>SO<sub>4</sub>- and KMnO<sub>4</sub> - extractable C and light fraction C in the surface 30 cm of soil. Mean cane yields (1978 – 1990) are also shown.

Management treatments <sup>1</sup>	Mean sugarcane yield (Mg ha <sup>-1</sup> )	Organic C (Mg ha <sup>-1</sup> )	Total N (Mg ha <sup>-1</sup> )	Mineralizable C (kg ha <sup>-1</sup> )	Mineralizable N (kg ha <sup>-1</sup> )	Microbial biomass C (kg ha <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> - extractable C (kg ha <sup>-1</sup> )	KMnO <sub>4</sub> - extractable C (kg ha <sup>-1</sup> )	Light fraction C (kg ha <sup>-1</sup> )
Grass	-	141.8	6.62	643	5.9	479	548	6556	3772
BtoFo	41	132.0	6.54	482	7.3	248	408	3760	998
BtoF	97	132.6	6.60	495	8.1	338	419	4270	1606
BtFo	43	133.8	7.44	580	8.5	401	455	3800	2257
BtF	103	136.2	7.68	643	11.0	478	495	4540	2406
TFo	54	134.4	7.79	774	12.1	695	468	4390	3575
TF	106	137.7	8.42	822	18.0	858	614	4760	3060
LSD ( $P \leq 0.05$ ) <sup>2</sup>	-	14.0	0.89	51	0.9	46	46	774	486

<sup>1</sup> Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

<sup>2</sup> LSD ( $P \leq 0.05$ ) for comparison between treatments shown.

#### 4.4 Discussion

Because of the extremely important properties of soil organic matter that greatly influence soil fertility, soil biological and physical properties, it is undoubtedly a key attribute of soil quality (Gregorich *et al.*, 1994). The amount of organic material returned to the soil under arable cropping is generally low, therefore the management of the crop residues will have a great impact on the soil organic matter content and also on other important soil properties (i.e. soil physical and biological properties).

In chapter 3 the accumulation of  $C_{org}$  with the retention of crop residues during green cane harvesting was demonstrated and discussed in detail. The  $C_{org}$  had the highest concentration in the 0 - 2.5 cm layer decreasing steadily to a depth of 10 cm and below (Figures 3.2 and 3.3). There was also a tendency for fertilized treatments to have a higher  $C_{org}$  than their unfertilized counterparts. However, as already mentioned, it is the more sensitive labile pools of soil organic matter with their dynamic nature that can provide information concerning the quality of the organic matter present. Such pools include microbial biomass C and N, light fraction organic matter, readily mineralizable C and N and easily extractable C and N.

The decomposition of soil organic matter to mineral forms is often measured by mineralization of soil organic N-compounds and a simultaneous evolution of  $CO_2$ . Microbial decomposition of organic N involves the release of ammonium (ammonification) and the microbial conversion of part of the ammonium first to nitrite and then to nitrate (nitrification). The ammonifying microorganisms are mainly heterotrophic whereas nitrification is mediated almost entirely by autotrophic organisms. The resynthesis of inorganic constituents ( $NH_4^+$  and  $NO_3^-$ ) into organic compounds by heterotrophic microflora (N-immobilization) occurs simultaneously and constantly in opposition to mineralization. Net mineralization with the release of mineral N will only occur if the organisms do not need the inorganic ions for cell synthesis. Thus, mineralizable N reflects a balance between gross mineralization

and immobilization by the soil microbial biomass.

Since readily mineralizable N was more affected by experimental treatments than  $N_{\text{tot}}$ , mineralizable N, as a percentage of  $N_{\text{tot}}$ , showed the trend burnt with harvest residues removed < burnt with only tops left on the soil surface < green cane harvested with trash retention and was further increased by fertilizer applications. This indicates that N inputs originating from either organic (trash) or inorganic (ammonium sulphate fertilizer) sources are preferentially accumulated into a readily mineralizable pool of soil organic N. In general, recently immobilized N is more readily mineralizable than the bulk of the native soil organic N (Haynes, 1986).

In comparison with commonly reported C/N ratios for soil organic matter of 10 - 12 (Schlesinger, 1995), values for this soil were rather wide (15 - 19). This was thought to be due to the high content of smectitic clays present in Vertisols as discussed in chapter 3. Similarly it is thought that values for the microbial quotient (0.3 - 0.9%) are notably lower than those commonly quoted in the literature (i.e., 1 - 2%, Sparling, 1997). The protective effect of the high clay content probably reduced the proportion of readily metabolizable C present and thus the relative size of  $C_{\text{mic}}$  that could be supported (Hassink and Whitmore, 1997).

The larger N inputs originating from increasing inputs of crop residues (Bto < Bt < T) and fertilizer N resulted in decreases in the C/N ratio of the total and readily mineralizable pools of soil organic matter and the microbial biomass. This effect was particularly conspicuous for the microbial biomass because of its labile nature and rapid rate of turnover.

Because of the dynamic nature of  $C_{\text{mic}}$ , 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  $C_{\text{org}}$  content can be detected. In a similar way, in this study, changes in  $C_{\text{mic}}$  and  $N_{\text{mic}}$  in the surface 5 cm in response to long-term trash retention and fertilizer application

were much more pronounced than those for  $C_{org}$  and  $N_{tot}$ . (Figures 4.1 and 4.2). For example, the proportional increases in  $C_{mic}$  (2.67-fold) and  $N_{mic}$  (2.95-fold) in trashed (T) compared with the burnt (Bto) treatments were much greater than those for  $C_{org}$  (1.23-fold) and  $N_{tot}$  (1.36-fold).

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 and light fraction C. 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 are located principally at, or near, the soil surface. Due to the decline in proportion of readily metabolizable C with increasing depth, there was also a decline in the microbial quotient.

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  $C_{org}$  (refer to chapter 3) or  $N_{tot}$  below 10 cm yet  $C_{mic}$ ,  $K_2SO_4$  - extractable C, light fraction and mineralizable C and N 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 mulching affect of the trash blanket favoured a crop root distribution more concentrated in the interrow spaces and nearer the soil surface (see chapter 6). This would have resulted in a greater turnover of root material in these layers and greater deposition of light fraction material. From a practical viewpoint, the increases in labile organic matter in the 10 - 30 cm layer resulted in proportionally greater treatment effects being evident for labile C fractions than  $C_{org}$  when compared on a per-hectare basis to a depth of 30 cm.

It is interesting that the effect of trash management on  $KMnO_4$  - extractable C was only evident in the surface 10 cm. The  $KMnO_4$  - oxidizable C fraction contains a

significant portion of polysaccharide and humic C (Conteh *et al.*, 1999) and it is an order of magnitude greater than that of the  $K_2SO_4$ -extractable (mainly dissolved organic C; Bolan *et al.*, 1996) or microbial biomass C fractions. The increasing proportions of total organic C present in relatively recalcitrant humic forms with increasing soil depth meant that the relatively harsh  $KMnO_4$  oxidation could not differentiate between the small differences in labile C present at depths below 10 cm. By contrast, in the surface layers, the  $KMnO_4$  fraction was clearly increased by both crop residue and fertilizer additions.

Despite the considerable soil acidification that occurred in this study [mean pH (0 - 5 cm) was 5.8 for unfertilized plots and 5.1 for fertilized ones] (refer to chapter 3), both  $C_{mic}$  and the microbial quotient were increased by fertilizer applications. This suggests that the increase in  $C_{org}$  and labile organic matter (e.g.  $K_2SO_4$ -extractable and mineralizable C) induced by fertilizer applications effectively nullified any negative effects (Grace *et al.*, 1994) that acidification may have had. In addition, fertilizer N, P and K applications presumably created conditions conducive to microbial proliferation since the soil tended to be P and K deficient (refer to chapter 3).

A massive loss of light fraction C characteristically occurs when a virgin grassland soil is cultivated (Chan, 1997). In our study, the decline in  $C_{org}$  in the Bto compared to grass treatment was 9.5 % whereas that for light fraction C was 61% and the loss of the latter made up 34% of the difference in  $C_{org}$  in the surface 5 cm. Under grassland, large inputs of particulate organic material in the form senescing root tissue, sloughed off root cells and above-ground litter result in high values for light fraction C (Gregorich and Janzen, 1996). Following conversion to sugarcane production, inputs are much reduced due to the wide spacing of the plants (rows were 1.4 m apart) and removal of most of the above-ground biomass at harvest. Thus light fraction dry matter and C were higher under grass than under sugarcane even with trash retention. Even so, increasing returns of above-ground crop residues did cause increases in light fraction dry matter (i.e. Bto < Bt < T).

The lower values for light fraction dry matter and C and C/N ratio for TF than TFo treatment are surprising. Indeed, measurements at the site show mean annual trash inputs for the TF and TFo treatments are 16 and 8.3 Mg kg<sup>-1</sup> respectively. Thus, the results suggest a considerably greater rate of decomposition of particulate organic matter in the fertilized treatments. As already noted, annual fertilizer applications may have stimulated microbial activity and thus promoted decomposition of the light fraction in the 8 month period between trash deposition and soil sampling. Indeed, light fraction is a transient pool and its size and composition will fluctuate seasonally depending upon the timing of residue inputs and their rate of decomposition (Gregorich and Janzen, 1996). That light fraction C was less for the fertilized than unfertilized trashed treatment yet the reverse was the case for mineralizable C demonstrates that although the light fraction undoubtedly contributes to mineralizable C pool, other sources are of considerable importance.

It is also probable that the deposited light fraction under the trashed treatments was more readily decomposable than that in the burnt ones. That is, in the burnt treatments light fraction will have contained some C in the inert form of charcoal. Indeed charcoal can account for a substantial quantity of the light fraction in soil where residues are burnt (Elliott *et al.*, 1991). By contrast, light fraction originating from plant materials under a non-burning regime is enriched with carbohydrates (Skjemstad *et al.*, 1986) which enhances its susceptibility to microbial attack.

#### **4.5 Conclusions**

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 practised 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 to a depth of 30 cm. Thus, soil organic matter quality was greatly altered by the practice.

These changes in organic matter content and quality may greatly effect other soil properties and processes such as aggregation, soil structural condition, N supply to the crop via mineralization and soil microbial and faunal activity and thus improve the sustainability of sugarcane monoculture.

## CHAPTER FIVE

### 5. The long-term effect of trash management and fertilizer additions on soil microbial and enzyme activity.

#### 5.1 Introduction

There is increasing evidence that measures of soil biological activity (i.e. microbial biomass C, basal respiration and soil enzyme activity) hold considerable promise as early indicators of soil degradation or improvement (Dick, 1994; Gregorich *et al.*, 1994; Haynes and Beare, 1996). In particular, these parameters are sensitive to changes in C availability caused by alterations in soil management practice and can change markedly before any changes in total organic matter are detected (Carter, 1986; Powlson *et al.*, 1987; Visser and Parkinson, 1992). Loss of soil organic matter and the attendant loss of soil microbial activity and soil structural condition are major issues when considering the sustainability of current farming practices (Sims, 1990; Doran *et al.*, 1996).

Conservation of crop residues following green cane harvesting provides the best opportunity to maintain soil organic matter content under sugarcane (Meyer *et al.*, 1996). Earlier South African research demonstrated that trash incorporation results in an increase in soil organic matter content and aggregate stability (Thompson, 1966) and an initial immobilization of soil mineral N by the microbial population involved in trash decomposition. Several workers have suggested that the higher productivity under green cane harvesting compared with burning is partly due to higher soil microbial activity, greater soil organic matter turnover and greater storage and release of nutrients (Sutton *et al.*, 1996; Blair *et al.*, 1998).

The long-term (59 years) trash/burning sugarcane field trial situated at Mount Edgecombe showed the positive effect that trash retention and to a lesser extent annual fertilizer additions have on soil organic matter content (refer to chapter 3). Nevertheless, results presented in chapter 3 demonstrated that annual fertilizer

additions, and to a lesser extent trash retention, caused substantial soil acidification. This trial provides an opportunity to investigate how changes in soil organic matter content and quality will interact with soil acidification to influence the metabolic activity of the microbial biomass and its capacity to cycle nutrients (i.e. enzyme activity). The purpose of this chapter is, therefore to investigate the effects of trash retention and fertilizer application on the size and activity of the soil microbial biomass and on the activity of key enzymes involved in C, N, P and S mineralization.

## 5.2 Materials and methods

The experimental site at the South African Sugar Association Experiment Station at Mount Edgecombe was sampled in 1998 to a depth of 30 cm and sectioned into the 0 - 2.5, 2.5 - 10, 10 - 20, and 20 - 30 cm layers (refer to site description in chapter 3). Samples from each plot were bulked. A field moist sub-sample was sieved (< 2mm) and stored at 1°C for not more than 72 h prior to subsequent microbiological and biochemical analysis.

Basal respiration was determined by placing 30 g oven-dry soil equivalent of field-moist soil in 50 ml beakers and incubating the sample in the dark at 25°C in a one L airtight sealed jar along with 10 ml of 0.5M NaOH. The CO<sub>2</sub>-C evolved was determined after 1, 5 and 10 days by titration (Anderson, 1982). The metabolic quotient (qCO<sub>2</sub>) was calculated as basal respiration (μg CO<sub>2</sub>-C day<sup>-1</sup>) g<sup>-1</sup> of C<sub>mic</sub>.

The arginine ammonification rate was measured by the method described by Franzluebbers *et al.* (1995) using an incubation period of 3 h and a temperature of 25°C. The rate of fluorescein diacetate (FDA) hydrolysis was estimated following the method of Schnürer and Rosswall (1982) with the concentration of hydrolysed FDA being determined colorimetrically at 490 nm.

The assays of various enzyme activities were based on the release and quantitative determination of the product in a reaction mixture, the soil samples being incubated

with suitable substrate and suitable buffer solution. Assays were performed to determine the activity of dehydrogenase (EC 3.5.1.5), acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphomonoesterase and arylsulphatase (EC 3.1.6.1) as described by Tabatabai (1994), casein hydrolysing protease (Ladd and Butler, 1972), L-histidine ammonia-lyase (EC 4.3.1.3) (Frankenberger and Johanson, 1982) and invertase (Frankenberger and Johanson, 1983). Enzyme activity was expressed as  $\mu\text{mol}$  product released  $\text{g}^{-1}$  soil  $\text{h}^{-1}$ . It was also calculated as  $\text{mg}^{-1} C_{\text{mic}} \text{h}^{-1}$ .

The effect of soil acidification induced by fertilizer and trash additions on soil enzyme activity were further investigated. In a subsidiary experiment (refer to chapter 3) a 300 g sample from the 0 - 5 cm layer of the TF treatment (pH = 5.0) was treated with  $\text{Ca}(\text{OH})_2$  to give a pH of about 5.8 (i.e. that of unfertilized treatments). Sub-samples of both unfertilized (TFo) and fertilized treatments (TF) with adjusted pH were analyzed for arylsulphatase activity as outlined above.

### 5.3 Results

As discussed in chapter 4, concentrations of  $C_{\text{org}}$  and  $C_{\text{mic}}$  in the surface 5 cm increased with increasing returns of organic residues (Bto < Bt < T) and tended to be higher in fertilized than unfertilized treatments (Figure 4.1). Similarly, basal respiration in the surface 2.5 cm of soil tended to increase in the order Bto < Bt < T although there was no consistent effect of fertilizer (Appendix 5.1; Figure 5.1). By contrast,  $q\text{CO}_2$  decreased in the order Bto > Bt > T and fertilizer additions also tended to depress values (this effect was statistically significant for the Bto and T treatments). Values for  $q\text{CO}_2$  ranged from 37 to 69  $\mu\text{g CO}_2\text{-C}_{\text{mic}}^{-1} \text{day}^{-1}$  for the TF and BtoFo treatments respectively (Appendix 5.1; Figure 5.1).

Measurements of overall microbial activity in the top 2.5 cm soil layer (i.e., FDA hydrolytic activity, arginine ammonification rate and dehydrogenase activity) are shown in Figure 5.2 (Appendix 5.1). The measurements showed a general trend of increasing activities with increasing returns of crop residues (i.e., Bto < Bt < T). FDA

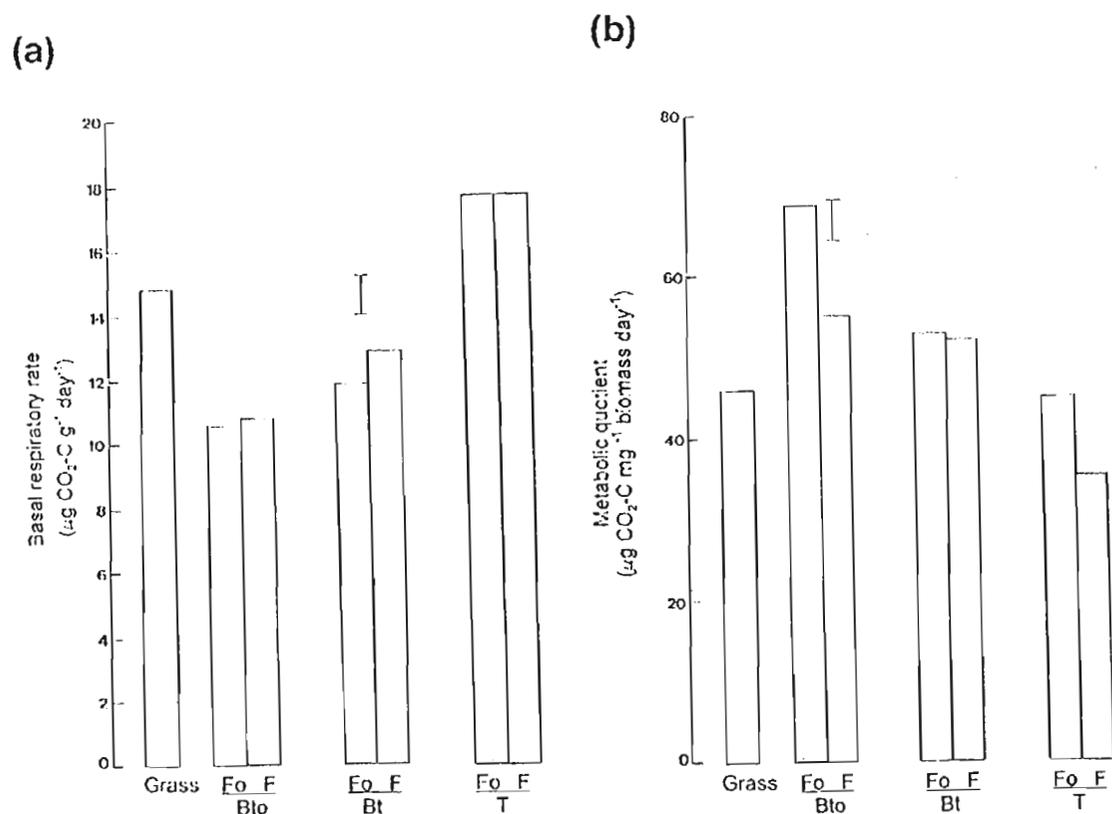
hydrolytic activity was not significantly affected by fertilizer additions but both dehydrogenase activity and the rate of arginine ammonification were decreased.

To demonstrate and explain the effect of trash retention on microbial activity in the soil profile, data from chapter 4 comparing  $C_{org}$  and  $C_{mic}$  in the TF and BtoF treatments in the profile to 30 cm are presented in Figure 5.3 along with data for basal respiration, FDA hydrolytic activity, arginine ammonification rate and dehydrogenase activity. As noted in chapter 4, significant increases in  $C_{org}$  were observed only in the surface 10 cm of soil due to trash retention while significant increases in  $C_{mic}$  were observed to a depth of 30 cm. Similarly, measurements of microbial activity as estimated by basal respiration and overall enzyme activity (FDA hydrolysis rate, arginine ammonification rate and dehydrogenase activity) showed significant increase to a depth of 30 cm in response to trash retention (Appendices 5.1; 5.2; 5.3; 5.4; 5.5; Figure 5.3).

The activities of enzymes involved in transformations of C, N, P and S (i.e. acid- and alkaline phosphatase and arylsulphatase, invertase, L - histidase, protease) are presented in Figures 5.4; 5.5; 5.6 and 5.7. The activities of all enzymes increased in response to increasing amounts of crop residues being returned to the top 2.5 cm soil layer (Appendix 5.6; Figures 5.4 and 5.6; 5.7). Results show that trash retention, compared to burning, resulted in a significant increase in all the soil enzyme activities to a depth of 30 cm (Figure 5.5).

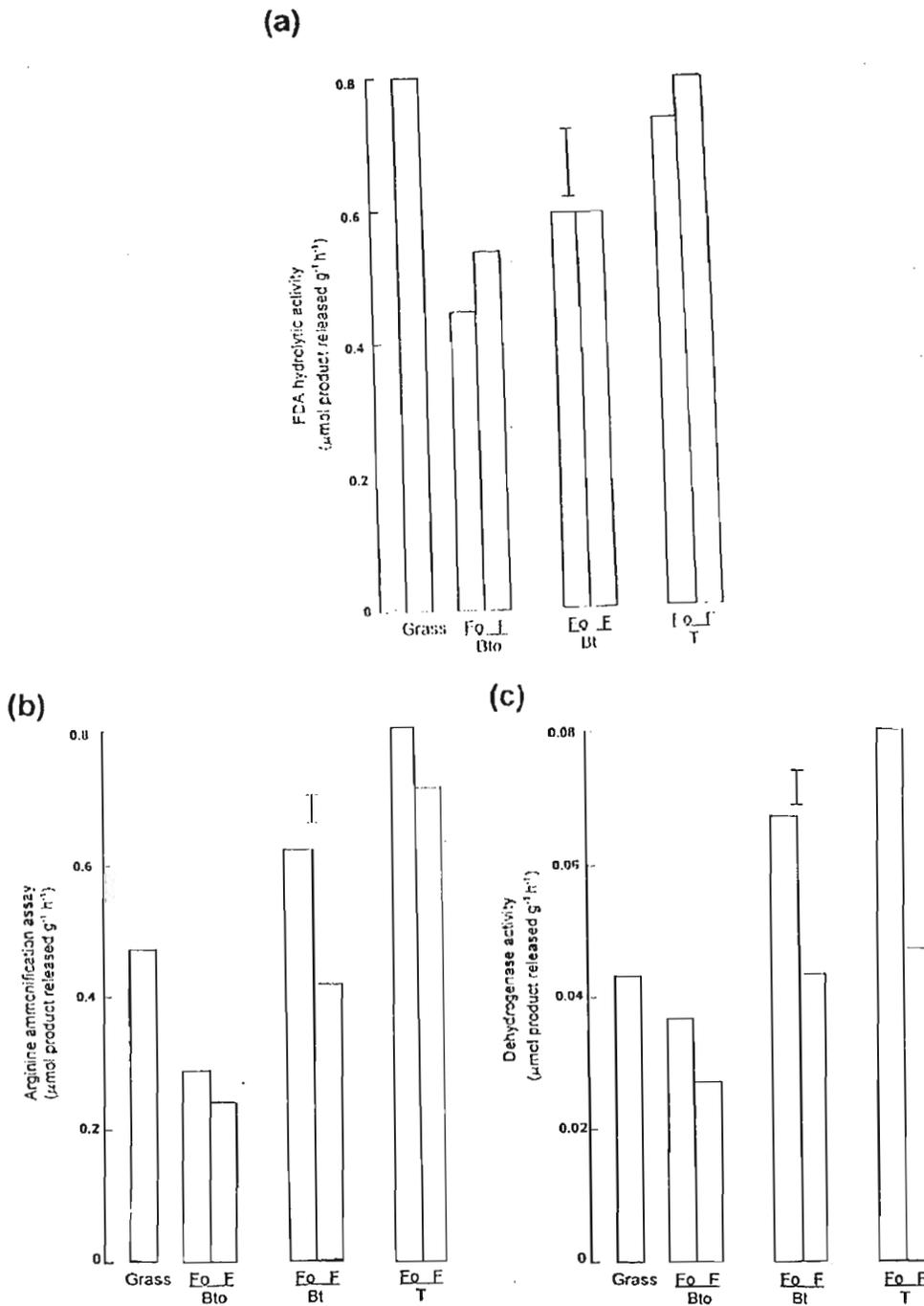
Fertilizer application resulted in varied effects on the activities of soil enzymes (Appendices 5.6; 5.7; 5.8 and 5.9). Fertilizer induced acidification resulted in a significant decrease in L-histidase activity in all crop residue treatments (Figure 5.6) and arylsulphatase activity for Bt and T (Figures 5.4). L-histidase activity was significantly decreased to a depth of 30 cm (Appendix 5.10). There was also a tendency for alkaline phosphatase activity to decrease but the differences were not statistically significant (Figure 5.4). By contrast, fertilizer applications resulted in an increase in invertase activity for all residue treatments (Figure 5.4), acid

phosphatase activity for Bt and T (Figure 5.4) and protease activity for T (Figure 5.7) in the surface 2.5 cm. The increase of protease activity (Figure 5.7) in response to fertilizer application was clearly shown to a depth of 20 cm for treatment T (Appendix 5.9).



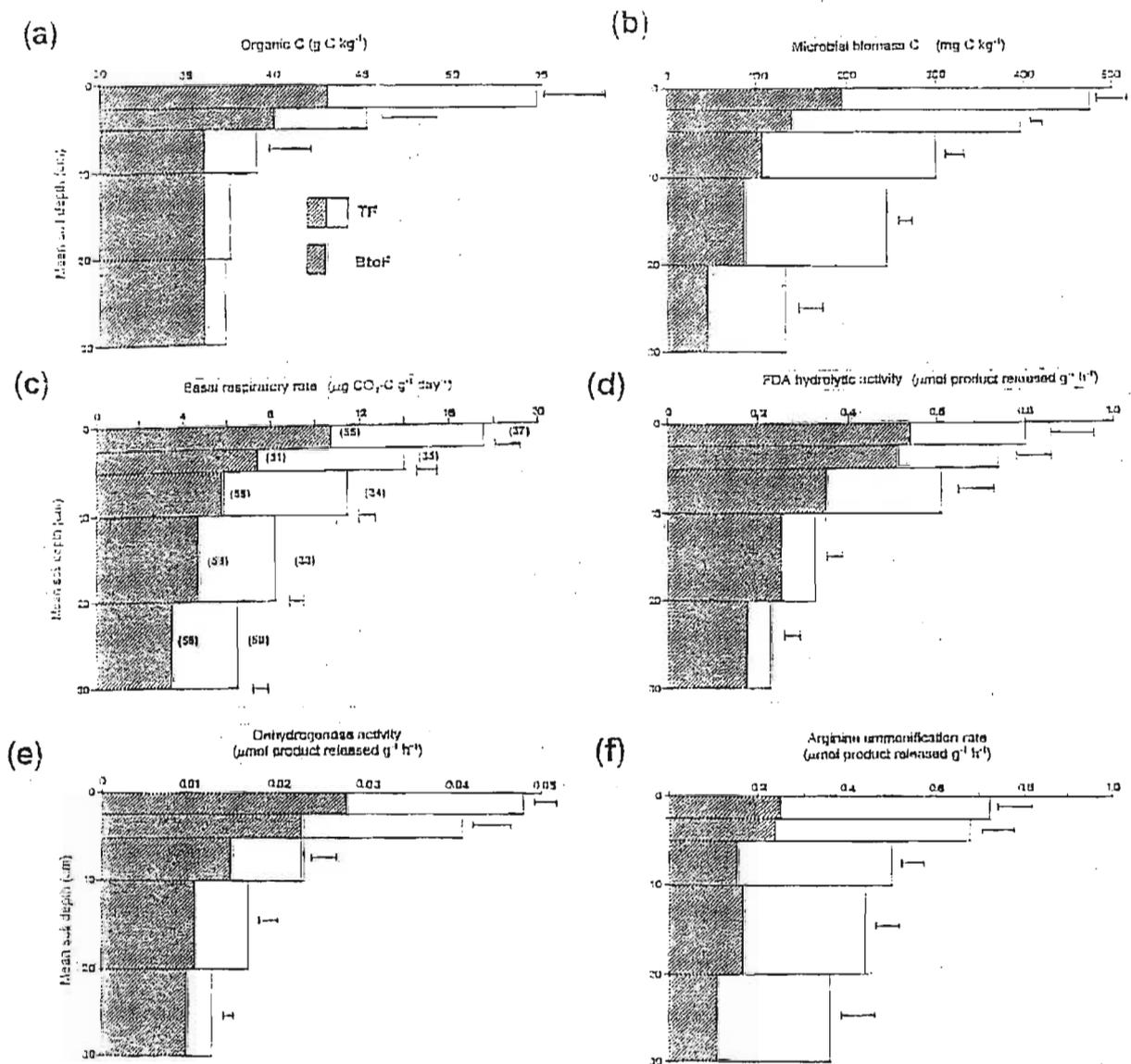
**FIGURE 5.1:**

Effects of long-term residue management practices and fertilizer applications on (a) basal respiration and (b) metabolic quotient in the 0 - 2.5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for comparison between treatments.



**FIGURE 5.2:**

Effects of long-term residue management practices and fertilizer applications on (a) FDA hydrolysis, (b) arginine ammonification rate and (c) dehydrogenase activity in the 0 - 2.5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for comparison between treatments.

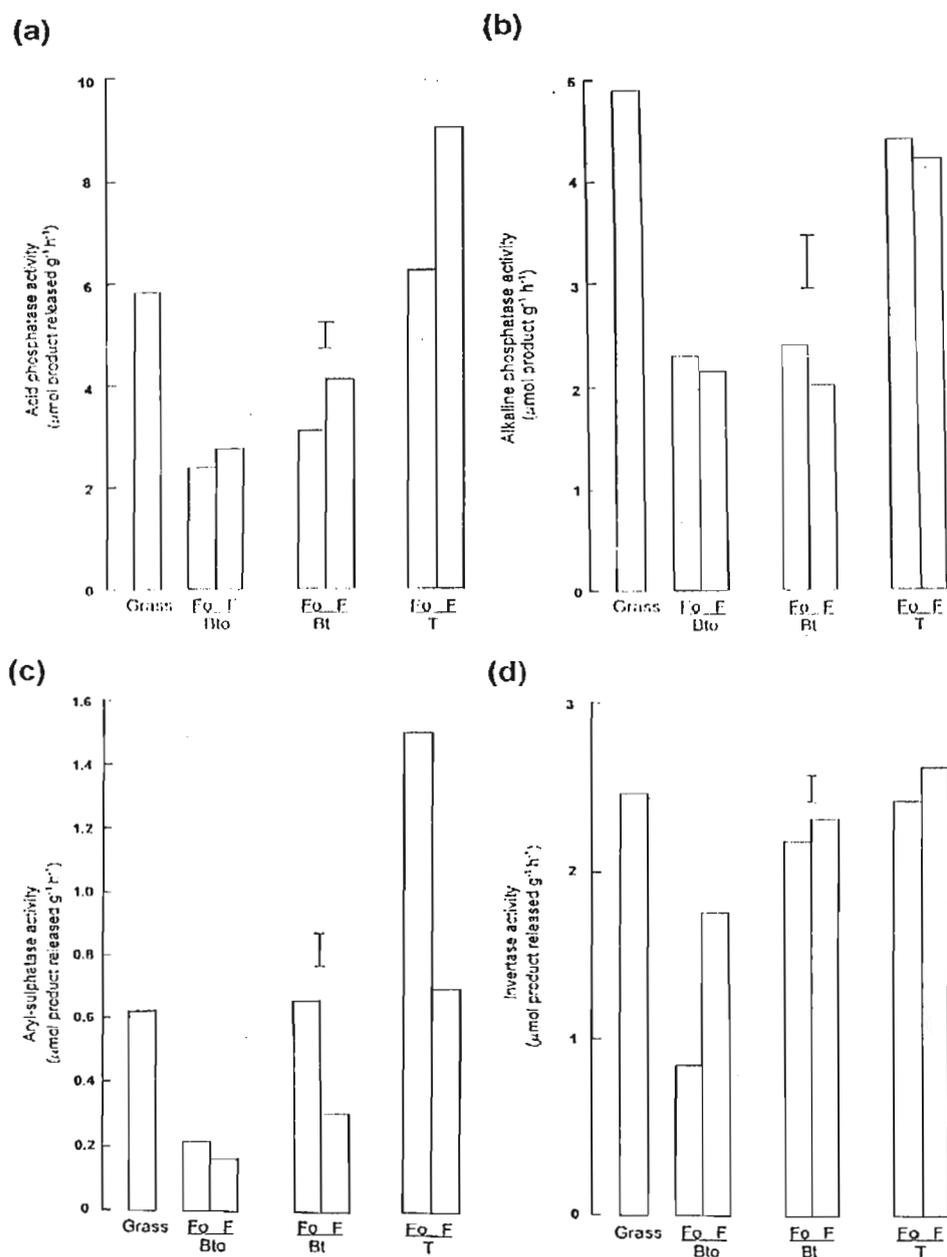


**FIGURE 5.3:** Distribution of (a)  $C_{\text{org}}$ , (b)  $C_{\text{mic}}$ , (c) basal respiration, (d) FDA hydrolysis, (e) dehydrogenase activity and (f) arginine ammonification rate in the soil profile for the BtoF and TF treatments. (Values for the metabolic quotient at each depth are shown in brackets. ( $C_{\text{org}}$  is redrawn from Chapter 3 and  $C_{\text{mic}}$  is redrawn from Chapter 4). T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.

The negative effect of fertilizer-induced acidification is shown in figure 5.8. When  $\text{Ca}(\text{OH})_2$  was added to the TF treatment, the activity of arylsulphatase was increased to almost the same value of the unfertilized treatment.

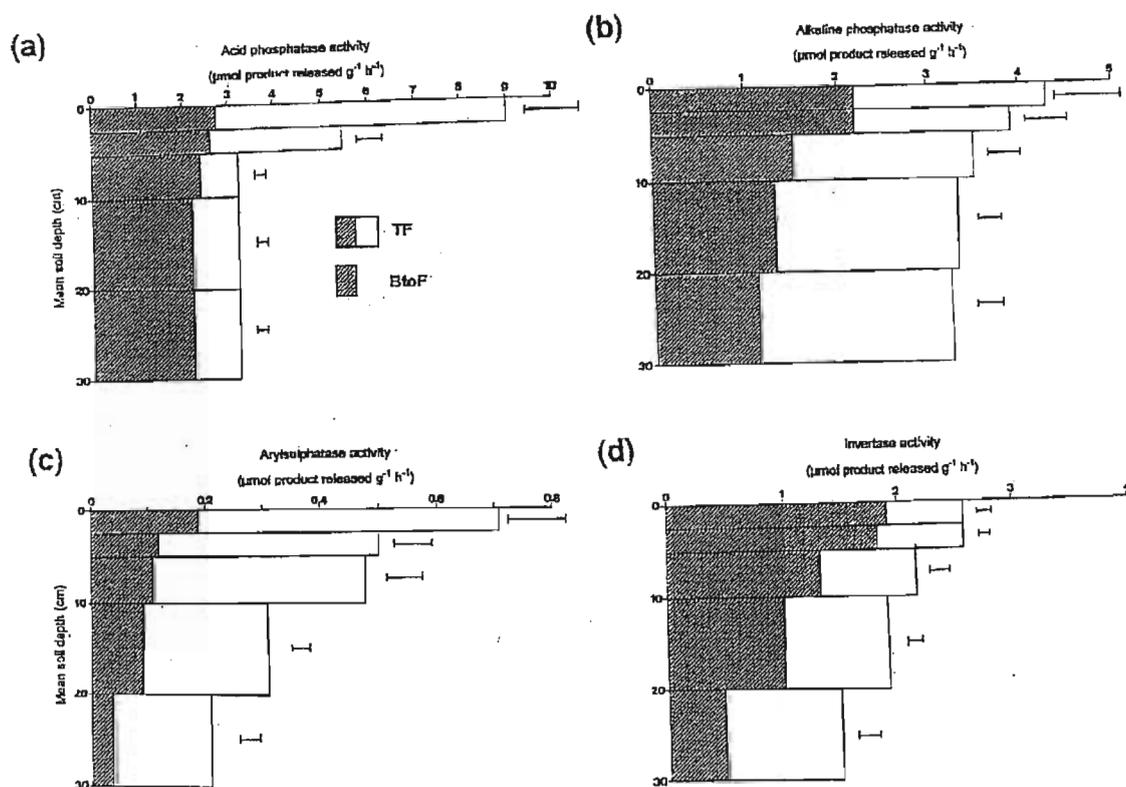
Linear correlation coefficients between the various properties measured in this study are shown in Table 5.1. Both  $C_{\text{org}}$  and  $C_{\text{mic}}$  were closely correlated with other properties, excluding dehydrogenase and L-histidase, which showed poor correlations with  $C_{\text{org}}$ ,  $C_{\text{mic}}$  and other properties. In general, the activities of enzymes involved in C, N, S and P mineralization were significantly correlated with each other, with measures of overall microbial activity and with the size of the microbial biomass.

Enzyme activities were also expressed per unit of  $C_{\text{mic}}$  (Table 5.2). When expressed in this way, increasing crop residue returns increased protease, arylsulphatase and to a lesser extent acid phosphatase activities but had no effect on that of L-histidase. Fertilizer additions decreased L-histidase and arylsulphatase activities but had no consistent effect on acid phosphatase and protease activities. It is also evident (Table 5.2) that the magnitude of the fertilizer-induced decrease in arylsulphatase activity per unit of  $C_{\text{mic}}$  was much reduced after adjustment of soil pH to that of unfertilized treatments.



**FIGURE 5.4:**

Effects of long-term residue management practices and fertilizer applications on (a) acid - and (b) alkaline phosphatase, (c) arylsulphatase and (d) invertase in the 0 - 2.5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for comparison between treatments.

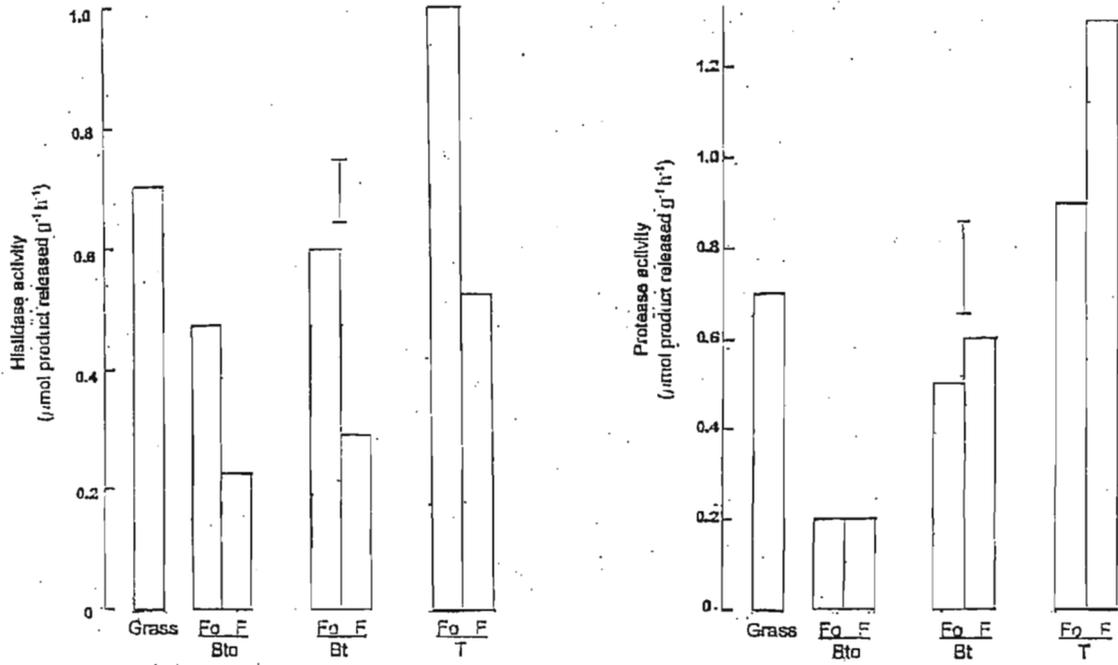


**FIGURE 5.5:** Distribution of (a) acid - and (b) alkaline phosphatase, (c) arylsulphatase activity and (d) invertase in the soil profile for the BtoF and TF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.

**TABLE 5.1:** Linear correlation coefficients (R)<sup>a</sup> between various measures of organic matter and the size and activity of the microbial biomass.

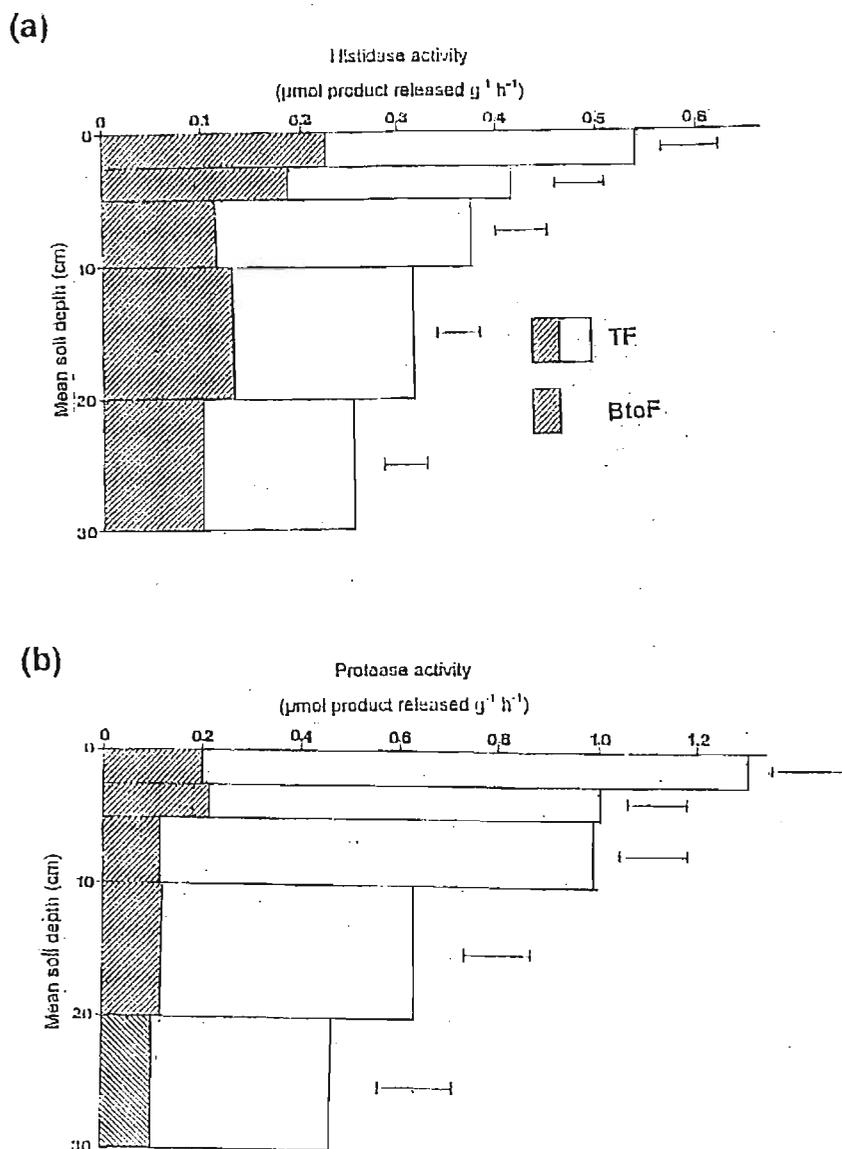
Soil property	Acid Phosphatase	Alkaline Phosphatase	Aryl-sulphatase	Histidase	Protease	Invertase	Dehydrogenase	Arginine ammonification	FDA hydrolysis	Basal respiration	Microbial biomass
Corg	0.911 ***	0.641 **	0.642 **	0.339 ns	0.904 ***	0.718 **	0.316 ns	0.908 ***	0.790 **	0.922 ***	0.923 ***
Cmic	0.975 ***	0.785 **	0.692 **	0.507 *	0.985 ***	0.805 ***	0.504 *	0.857 ***	0.886 ***	0.961 ***	
Basal respiration	0.936 ***	0.837 ***	0.809 ***	0.651 **	0.943 ***	0.766 **	0.617 **	0.853 ***	0.891 ***		
FDA hydrolysis	0.911 ***	0.915 ***	0.587 *	0.600 **	0.868 ***	0.859 ***	0.494 *	0.641 **			
Arginine ammonification	0.817 ***	0.438 *	0.623 **	0.258 ns	0.889 ***	0.721 **	0.345 ns				
Dehydrogenase	0.379 *	0.664 **	0.895 ***	0.938 ***	0.452 *	0.441 *					
Invertase	0.767 **	0.632 **	0.521 *	0.387 *	0.807 ***						
Protease	0.979 ***	0.736 **	0.626 **	0.449 *							
Histidase	0.443 *	0.817 ***	0.843 ***								
Arylsulphatase	0.568 *	0.684 **									
Alkaline phosphatase	0.800 ***										

<sup>a</sup> Statistical significance shown: \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ , \*\*\*  $P \leq 0.001$ , ns = not significant.



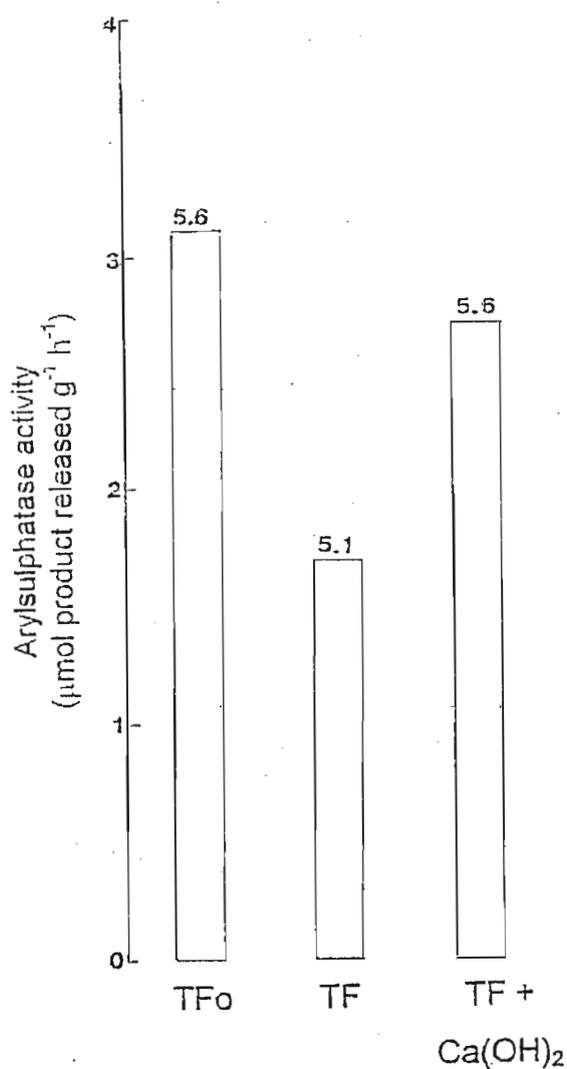
**FIGURE 5.6:**

Effects of long-term residue management practices and fertilizer application on (a) L-histidase and (b) protease in the 0 - 2.5 cm soil layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for comparison between treatments.



**FIGURE 5.7:**

Distribution of (a) L-histidase and (b) protease in the soil profile in the BtoF and TF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.



**FIGURE 5.8:** Arylsulphatase activity of the TFo and TF treatments in the 0 - 5 cm soil layer and arylsulphatase activity following a three week incubation period of the TF treatment with Ca(OH)<sub>2</sub> to raise the pH to that of the TFo treatment. Values in brackets represent the pH. T = green cane harvested with trash retention; F = fertilized annually with N, P and K; Fo = no fertilizer applied. LSD ( $P \leq 0.05$ ) shown for comparison between treatments.

**TABLE 5.2:** Enzyme activity expressed per unit of  $C_{mic}$  in the 0 - 2.5 cm soil layer.

Management Treatment	Histidase activity	Acid phosphatase activity	Alkaline phosphatase activity	Invertase activity	Protease activity	Aryl sulphatase activity	Aryl sulphatase activity (after pH adjustment)
BtoFo	3	15.7	15.1	5.9	1.3	1.3	1.3
BtoF	1.1	13.8	11.2	9.2	1	0.9	1
BtFo	2.7	14.1	10.9	10.9	2.2	2.9	2.9
BtF	1.1	16.8	8.2	9.4	2.4	1.2	2.3
TFo	2.8	16.1	11.4	6.2	2.3	3.9	3.8
TF	1.1	18.9	9.0	5.4	2.7	1.5	2.9

## 5.4 Discussion

Traditionally sugarcane residues are burnt prior to harvest resulting in the volatilization of large amount of C and N and in some accumulation of C in the form of chemically inert char - C (Raison, 1979; Rasmussen and Collins, 1991). Results of the study, demonstrate that 59 years of crop residue retention in the form of a trash blanket caused a significant increase in  $C_{org}$  and  $C_{mic}$  in the surface 10 cm of soil, compared to that of the burnt treatment (refer to chapter 3 and 4). Such an accumulation of  $C_{mic}$  would be expected to result in an increase in soil microbial and enzyme activity in the soil.

In general,  $C_{org}$  changes slowly with changes in soil management practice and changes are often difficult to measure accurately against a large background soil organic matter content already present. By contrast, changes in labile organic matter fractions (i.e.  $K_2SO_4$ -extractable C and light fraction C) (refer to chapter 4) occur more rapidly in response to changes in management (Haynes and Beare, 1996). Similarly, as noted in chapter 4, while  $C_{org}$  was only significantly increased in the surface 10 cm in response to trash retention, there was an increase in labile organic C (e.g.,  $K_2SO_4$  - extractable and light fraction C) and  $C_{mic}$  to a depth of 30 cm. As expected, this also resulted in an increase in basal respiration, microbial activity as estimated by FDA hydrolytic activity, arginine ammonification rate and dehydrogenase activity and in the activity of specific enzymes involved in turnover of C, N, P and S to a depth of 30 cm.

It has been suggested that under a climax ecosystem at equilibrium with its environment, a highly efficient soil microbial community exists that has a low  $qCO_2$  (Sparling, 1997). It was further suggested that  $qCO_2$  increases in response to soil disturbance (Anderson and Domsch, 1989). However, Wardle and Ghani (1995) concluded that  $qCO_2$  fails to distinguish between the effects of disturbance and stress and that it is an index of microbial stress. Thus, the decrease in  $qCO_2$  with increasing returns of crop residues, and to a lesser extent fertilizer additions,

suggests that a shortage of available C and nutrients were limiting microbial activity and causing stress. Thus, the highest  $q\text{CO}_2$  values were encountered for the BtoFo treatment since neither crop residues nor fertilizers were added. By contrast, the TF treatment had large inputs of both organic residues and fertilizer and had the lowest  $q\text{CO}_2$  values. As shown here, where factors limit the size of  $C_{\text{mic}}$  they also tend to increase  $q\text{CO}_2$  C (Sparling, 1997); that is treatments with the lowest  $C_{\text{mic}}$  had the highest  $q\text{CO}_2$  values and vice versa. Other factors could also contribute to an increased  $q\text{CO}_2$ . For example, bacterial communities are less efficient at converting substrate C into cellular C than fungi (Kazumori and Oba, 1994). The composition of the microbial community under the various treatments is considered in chapter 7.

In all treatments, both  $C_{\text{org}}$  and  $C_{\text{mic}}$  decreased substantially with soil depth. Such an accumulation of  $C_{\text{org}}$  near the soil surface is a characteristic of minimum tillage (Haynes and Beare, 1996). There was as expected, similar stratification in the activity of  $C_{\text{mic}}$  and the activity of the enzymes involved in the turnover of N, P and S. Conversely, the  $q\text{CO}_2$  tended to increase with depth. For the past 30 years a minimum tillage system has been used in this trial, thus the input of fresh, easily decomposable plant material (above ground plant material and roots) decreases as a function of depth. The proportion of soil organic matter present as humified, less available, organic matter therefore increases with depth. The metabolic efficiency of the  $C_{\text{mic}}$  is characteristically lower (i.e.,  $q\text{CO}_2$  is higher) when using recalcitrant humic material as a substrate than when the C source is readily decomposable (Kassim *et al.*, 1982). Thus,  $q\text{CO}_2$  tends to increase with depth as a result of the increasing proportion of recalcitrant organic substrate that is present.

The activity of the enzymes involved in C, N, P and S were highly correlated with  $C_{\text{org}}$ . Similarly, in soil under different tillage, rotation and mulching treatments both Dick (1984) and Deng and Tabatabai (1997) observed that the activity of a range of soil enzymes was closely correlated with soil  $C_{\text{org}}$ . Such a relationship was expected since significant correlations were also recorded between the size and activity (i.e. arginine ammonification rate, FDA hydrolysis and basal respiration),  $C_{\text{mic}}$  and  $C_{\text{org}}$ . In

addition, as well as being the substrate for  $C_{mic}$ , soil organic matter plays an important role in protecting soil enzymes since they become immobilized in a three-dimensional network of clay and humus complexes (Tabatabai, 1994). Enzymes originating from microorganisms associated with the decomposition of organic residues contribute significantly to the soil enzyme pool (Johnson, 1957; Balasubramanian *et al.*, 1972; Zantua and Bremner, 1976; Nannipieri *et al.*, 1983).

Several different approaches to measure microbial activity were used in this study. Basal respiration measures the respiratory activity ( $CO_2$  evolution) of the microbial community. The rate of FDA hydrolysis is considered a good index of overall microbial activity because its hydrolysis is carried out by active cells using esterases and fluorescein derivatives and hydrolysed by lipases, esterases and proteases (Schnürer and Rosswall, 1982). Arginine ammonification is used as an index of microbial activity since most heterotrophs possess endocellular ammonifying capacity and its rate has been found to be closely correlated with microbial activity in laboratory studies (Alef and Kleiner, 1987). The activity of the endocellular enzyme dehydrogenase is also often closely correlated with microbial activity but in some cases close correlations are not observed. In this study, dehydrogenase activity was not closely correlated with  $C_{org}$ ,  $C_{mic}$ , FDA hydrolytic activity or arginine ammonification rate. The fact that relatively high rates of fertilizer N are applied to some plots may have been a contributing factor to this. Dehydrogenase activity can be strongly influenced by the presence of nitrate, which serves as an electron acceptor resulting in low measured activities (Casida *et al.*, 1964). This is a reflection of the relative insensitivity of synthetic electron acceptors used in the assay. Goyal *et al.* (1999) found that dehydrogenase activity was not a reliable indicator of microbial activity in N-fertilized soils.

The effects of long-term fertilizer applications on the size and activity of the soil microbial biomass are presumably mainly the result of interactions between the increased soil nutrient status and organic matter content and the decreased soil pH. This interaction resulted in variable effects in that  $C_{mic}$  was increased, basal

respiration and FDA hydrolytic activity were essentially unaffected whilst arginine ammonification rate and dehydrogenase activity were decreased. Thus, long-term fertilizer applications generally caused the accumulation of a larger but less active microbial community. Presumably, whilst the increased nutrient status and accumulation of organic matter allowed the soil to support a larger  $C_{mic}$ , the lowered soil pH limited its activity. Nonetheless, as already noted,  $qCO_2$  was generally decreased suggesting a less stressed microbial community.

The enzymes assayed in this study were chosen because they play central roles in C, N, S and P cycling in soils. Invertase is a key enzyme involved in C mineralization since it catalyzes the breakdown of sucrose to glucose and fructose and consequently provides energy for microbial activity. Both acid and alkaline phosphatase are present in soil and they catalyze the hydrolysis of organic monoester P to inorganic phosphate while soil sulphatases catalyze the hydrolysis of organic sulphate esters to sulphate (Dick, 1997). Protease and L-histidase are important enzymes in relation to N mineralization since proteases catalyze hydrolysis of proteins to polypeptides and oligopeptides to amino acids whilst L-histidase catalyzes deamination of the amino acid L-histidine to urocanate and ammonia (Frankenberger and Johanson, 1982).

Increasing activities of these enzymes with increasing crop residue returns reflect a higher rate of turnover of C, N, P and S. For example, for the two trashed treatments (TFo and TF), the estimated quantities of trash produced annually were 8.3 and 16  $Mg\ ha^{-1}$  respectively (refer to chapter 3). This resulted in an estimated amount of 31 and 61  $kg\ N\ ha^{-1}$  and 3.4 and 6.7  $kg\ P\ ha^{-1}$  recycling through the soil-plant system (refer to chapter 3). Conversely, with burning large amounts of nutrients in organic form are lost via volatilization. In addition, further losses probably occur as a result of ash being blown off burnt plots by strong winds (R van Antwerpen, personal communication, 1999).

The variable effect of fertilizer applications on enzyme activity reflected the variable

effects that were recorded for their effect on the size and overall activity of  $C_{mic}$ . An interaction of factors probably operated. On the one hand, fertilizer applications resulted in a larger  $C_{mic}$ , which would presumably be capable of producing more enzymes. On the other hand, the fertilizer induced soil acidification may have reduced microbial activity and the production of some exocellular enzymes as well as affected the stability and activity of these enzymes in the soil environment.

Soil pH can greatly influence the rate of enzyme catalyzed reactions since change in  $H^+$  ion concentration influences enzymes, substrates and co-factors by altering their ionization and solubility (Tabatabai, 1994). Characteristically, each enzyme has a pH value at which the rate of reaction is optimum. For invertase, for acid phosphatase, alkaline phosphatase, arylsulphatase, protease and histidase these values are about 5.5, 6.5, 11, 5.8, 8.1, and 9.0 respectively (Alef and Nannipieri, 1995). Thus the pH of the unfertilized soils (i.e. 5.8 in the surface 2.5 cm) was already at or well below the optimum pH for activity of the enzymes assayed. Nevertheless, results have shown that the pH stability of soil enzymes is highly dependant on both the enzyme and the soil being considered (Frankenberger and Johnson, 1982).

The interaction of a number of factors apparently resulted in long-term fertilizer applications causing an increase (invertase and acid phosphatase), a decrease (L-histidase and acid phosphatase) or little effect (protease and alkaline phosphatase) on enzyme activity. The decrease in activity of arylsulphatase with acidification confirms the results of others who have recorded a positive correlation between arylsulphatase activity and soil pH (Haynes and Swift, 1988; Dick *et al.*, 1988).

The increase in acid phosphatase activity in the fertilized treatments is of note since there is also a much higher level of extractable inorganic P in the soils (refer to chapter 3). An increase in available soil P may inhibit phosphatase activity since orthophosphate is a competitive inhibitor of acid phosphatase (Tabatabai, 1994) and it may also repress the synthesis of microbial phosphatase (Spier and Ross, 1978).

It is therefore surprising that acid phosphatase activity per unit of  $C_{mic}$  ratio was increased. This increase is possibly explicable in terms of the dominance of the enzyme in acid conditions (Eivazi and Tabatabai, 1977) and its immobilization onto positively charged surfaces (Pant and Warman, 2000). By contrast, alkaline phosphatase expressed per unit of  $C_{mic}$  decreased, demonstrating that there was a lower rate of enzyme production by the microbial biomass, since alkaline phosphatase derives totally from microorganisms (Juma, 1976).

It is interesting to note that readily mineralizable N (as estimated by a 10 - day aerobic incubation) was increased by fertilizer applications (refer to chapter 4). Nonetheless, both arginine ammonification rate and L-histidase activity were decreased and protease activity was only increased in the trashed treatments. The higher total N content in soils from fertilized treatments meant that there was more available substrate for N mineralization and this was presumably the dominant factor that resulted in greater N mineralization. That this occurred despite there being decreases in the assayed activity of some key enzymes systems involved in N mineralization suggests that substrate availability rather than enzyme activity was the main factor limiting N mineralization during the laboratory incubation.

## 5.5. Conclusions

The accumulation of soil organic matter and recycling of C and nutrients held in organic form induced by long-term trash retention, rather than burning, had a substantial positive effect on soil microbial activity. Indeed, all measurements made of the size and activity of the soil  $C_{mic}$  were increased by trash retention. The increased cycling of C, N, S and P induced by trash retention was reflected in the increased activity of enzymes involved in mineralization and cycling of these elements in the soil.

Although trash retention only caused a significant increase in soil organic C and total N to a depth of 10 cm, the increases in labile C and  $C_{mic}$  to a depth of 30 cm resulted

in greater microbial and enzyme activity to that depth. Such results underline the fact that when no changes in total soil organic matter content (organic C and total N) are detected, this does not necessarily mean that changes in soil organic matter quality and soil biological activity have not occurred. The large background levels of total soil organic matter means it is difficult to measure small changes in soil organic matter status that could well alter soil microbial activity substantially. In this case, trash retention only increased total soil organic matter content to a depth of 10 cm yet a larger, more active, microbial community and higher enzyme activity was evident to 30 cm.

The long-term effects of fertilizer applications on microbial activity were somewhat confused. The variable effects were explained in terms of an interaction between fertilizer - induced increases in  $C_{org}$ , soil nutrient status and fertilizer-induced soil acidification. The interaction resulted in a larger less active, more metabolically efficient microbial community with the activity of some enzymes being increased, some decreased and others remaining unaffected. Such results emphasize the fact that measurements of the size and activity of the microbial community and activity of key enzymes are all required in order to understand the effects of various management practices on the function of the soil microbial community.

## CHAPTER SIX

### **6. Size and activity of the soil microbial biomass in the row and inter-row of a sugarcane field under burning and green cane harvesting.**

#### **6.1 Introduction**

Loss of soil organic matter under sugarcane subjected to pre-harvest burning is believed to be a major factor contributing to soil degradation in the South African sugar industry (Meyer *et al.*, 1996; Haynes and Hamilton, 1999). When studies on soil degradation are carried out, generally soil from the inter-row space is sampled (Graham *et al.*, 1999, 2000; Dominy *et al.*, 2001). Under pre-harvest burning, the inter-row space is effectively fallow and inputs of organic material are minimal. When green cane harvesting is employed, the C input to the inter-row area is greatly increased because it is covered with a blanket of trash. Organic matter content and structural stability of soil in the inter-row space is, therefore, greatly increased under green cane harvesting.

Dominy and Haynes (2002) recently suggested that the organic matter content could differ greatly between soil below the row and that in the inter-row space. This is because sugarcane is a perennial crop and can commonly remain in the ground for up to 10 years (i.e. one planted crop and 9 ratoon crops). Under a burning regime the main organic matter input to the soil would occur via rhizodeposition and would be concentrated in the root zone below the sugarcane rows. For this reason, soil in the rows would have a higher organic matter content than that in the inter-row space. In agreement with this suggestion, Hartemink (1998a) observed that soil organic C concentrations tended to be larger in the row than in the inter-row of sugarcane fields in Papua New Guinea. If such gradients were pronounced then measurement of the organic matter content of the inter-row would underestimate the total quantity of organic matter present in a sugarcane field. In addition, such gradients would be expected to result in similar, or even more pronounced, gradients in soil microbial

activity and structural stability. The existence of such gradients has, however, not yet been investigated.

The purpose of this study was to investigate the changes in organic matter status, aggregate stability and soil microbial activity that occur within a sugarcane field at increasing distances out from the centre of the plant row. These changes were investigated under pre-harvest burning and green cane harvesting using the long-term trash management trial at SASEX.

## **6.2 Materials and Methods**

To investigate the variation in organic content across the field, samples were taken from three replicates of the green cane harvested treatment (TF) with all above-ground plant material (tops and leaves) returned to the soil surface (across the row and the inter-row) and the burnt treatment (BtoF) with no above-ground crop residue returned to the soil (inter-row is left fallow).

At the site, sugarcane is planted in rows 1.4 m apart (refer to site description in chapter three). Soils were sampled in (i) the centre of the plant row, (ii) 30 cm out from the row centre (30 cm) and (iii) 60 cm out from the row centre (60 cm) (i.e. the middle of the inter-row area). This procedure was carried out in three randomly chosen areas of each plot; soils were sampled to a depth of 30 cm, sectioned into the 0 - 10, 10 - 20 and 20 - 30 cm layers, and samples from each layer were bulked.

Within 48 hours of collection bulked, field-moist samples were thoroughly mixed and split into three sub-samples. One sub-sample was sieved ( $> 2$  mm) and stored at 2°C prior to biological analysis. Another sub-sample was air-dried, sieved ( $< 2$  mm) and ground ( $< 0.5$  mm) for subsequent analysis of organic C. The third sub-sample was sieved and the 2 - 6 mm diameter aggregates were collected and air-dried for subsequent analysis of aggregate stability.

Soil organic C ( $C_{org}$ ) was measured using a method described by Yeomans and Bremner (1988) (refer to chapter three). Microbial biomass C ( $C_{mic}$ ) was measured using a method described by Vance *et al.*, (1987) (refer to chapter four). The microbial quotient ( $C_{mic}/C_{org}$ ) ratio was calculated by expressing microbial biomass C as a percentage of total organic C. Basal respiration was determined using the method described by Anderson (1982) (refer to chapter four). The metabolic quotient ( $qCO_2$ ) was calculated as basal respiration ( $\mu g CO_2-C day^{-1}$ )  $g^{-1}$  of  $C_{mic}$ . Potassium sulfate - extractable C (extracted from unfumigated soil) was used as a measure of labile soil organic C and light fraction dry matter (Gregorich and Ellert, 1993) was analysed as described in chapter four. Aggregate stability was measured using a wet sieving technique as described by Haynes (1993) (refer to chapter three). The results were expressed as mean weight diameter (mm), which is the sum of the fraction of soil remaining on each sieve after sieving multiplied by the mean diameter of the inter-sieve aperture.

Root samples were collected using the core technique (Fehrenbacher and Alexander, 1955; Kücke *et al.*, 1995). A stainless steel pipe with a 37.5 mm inside diameter was driven into the soil with a motorized hammer. The samples were extracted from the soil after each 10 cm penetration to remove the sample. Samples were sectioned into 0 - 10, 10 - 20 and 20 - 30 cm layers.

Samples were immediately washed free of soil before being stored in a freezer until root length could be determined. Samples were soaked in Calgon solution (20.0 g sodium hexametaphosphate + 8.0 sodium carbonate per liter of water) for at least 24 hours before the roots were separated from the soil. Root length was determined by the line intersect method (Newman, 1966) as modified by Rowse and Phillips (1974). After root length determination, samples were oven-dried and total weight was determined.

In order to calculate the quantities of organic matter in the soil profile to 30 cm, per unit area, concentrations were first converted to a volumetric basis. For this

purpose, bulk density was measured in quadruplet using the core method in the 0 - 10, 10 - 20 and 20 - 30 cm layers. Bulk density samples were taken in the row centre and 30 and 60 cm from the row centre. The quantities of organic matter in the soil profile to 30 cm depth were calculated for each area. In order to calculate the C loads across the entire field, the proportion that each area (i.e. row, 30 cm and 60 cm) contributed to the field was estimated.

The statistical significance of experimental treatments was determined by subjecting the data to Analysis of Variance and Least Significant Differences (LSD) were calculated at the 5 % level.

### 6.3 Results

Both root mass and length density tended to decrease with increasing distance from the plant row (Table 6.1). In the surface 10 cm the burnt treatment had a greater root length density than the trash treatment at each distance from the row centre (Table 6.1). However, although root mass density was greater under burning than trashing below the row, values were similar at 30 cm and, in fact greater under trashing at 60 cm (Table 6.1). Root mass density decreased to a depth of 30 cm (Table 6.1). In the row, significant increases in the root mass density were observed to a depth of 30 cm in response to burning. By contrast, in the inter-row, trash retention resulted in significant increases in root mass density only in the top 10 cm. When root mass was calculated on a per area basis (Table 6.2), it was higher in the burnt than trashed treatment in the row. By contrast, in the inter-row root mass under trashing was approximately twice that under burning. Root mass calculated across the field was similar under TF and BtoF (Table 6.2) so although there were clear differences in root distribution caused by treatment, total root mass remained unchanged.

In the surface 10 cm, concentrations of  $C_{org}$ ,  $C_{mic}$  (Appendix 6.1; Figure 6.1),  $K_2SO_4$ -extractable C and light fraction C (Appendix 6.2; Figure 6.2) decreased, with

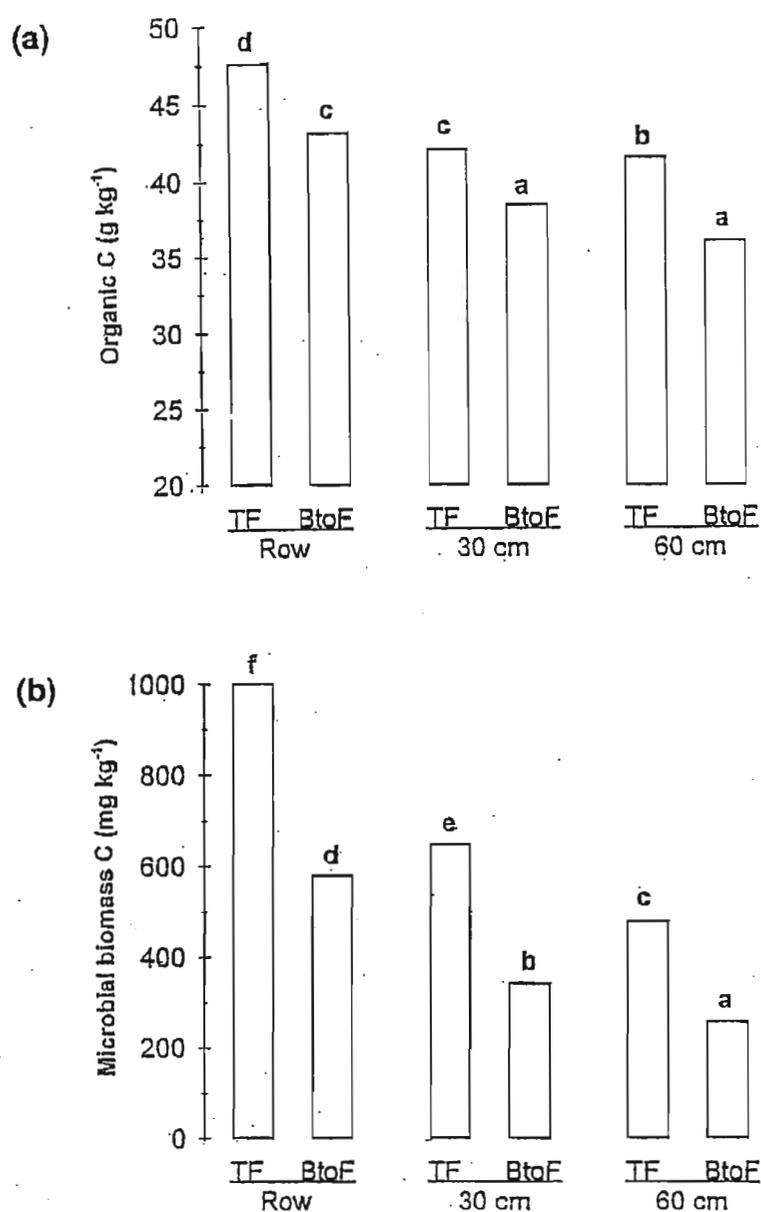
increasing distance from the row centre (i.e. Row > 30 cm > 60 cm) and were higher under trash retention than burning. Since  $C_{mic}$  was considerably more influenced by treatments than  $C_{org}$ , the microbial quotient also decreased with increasing distance for the row centre and was higher under trashing (Figure 6.1). Significant increases in  $C_{org}$  due to trash retention were observed only in the surface 10 cm of soil in the inter-row, but to a depth of 20 cm in the row (Appendices 6.1; 6.2; 6.3; Figure 6.3). By contrast, significant increases in  $C_{mic}$  (Appendices 6.1; 6.2; 6.3; Figure 6.3),  $K_2SO_4$ -extractable C and light fraction C (Appendices 6.1; 6.2; 6.3; Figure 6.5) were observed to a depth of 30 cm in response to trash retention in the row centre as well as in the inter-row. The microbial quotient decreased with increasing soil depth but was higher under trash retention at all depths (Figure 6.3).

Basal respiration in the surface 10 cm followed the same trends as that of  $C_{mic}$  and labile organic matter fractions (Appendix 6.1; Figure 6.4) and as a result the  $qCO_2$  increased with increasing distance from the row and was higher under burning than trashing (Figures 6.4 and 6.5). Basal respiration decreased with increasing soil depth whilst  $qCO_2$  tended to increase (Appendices 6.1; 6.2; 6.3; Figure 6.5).

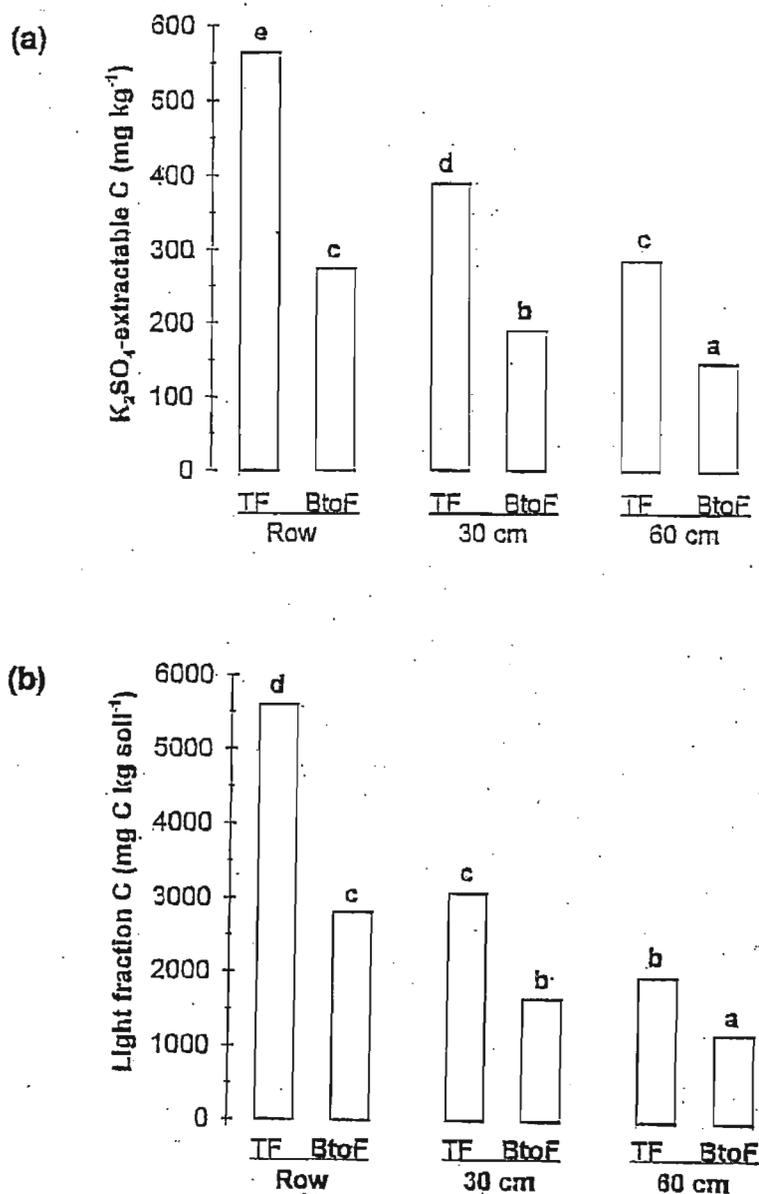
Although concentrations of organic C were lower in the inter-row than the row, the bulk density was considerably higher in the inter-row. As a result, when quantities of  $C_{org}$  under both trashed and burnt treatments were calculated on a per-hectare basis to a depth of 30 cm, there were no significant differences between values for the row, inter-row or on a field basis (Table 6.2). For  $C_{mic}$ ,  $K_2SO_4$  - extractable C and light fraction C values were highest in the row, lowest in the inter-row and intermediate when calculated on a field basis (Table 6.2). Trashing resulted in larger quantities of  $C_{mic}$ ,  $K_2SO_4$  - extractable C, light fraction C and to a lesser extent  $C_{org}$ , on a per hectare basis, in the row, inter-row and per field.

The size distribution of aggregates following wet sieving of samples from the 0 - 10 cm layer is shown in Figure 6.6. The percentage of sample remaining in the 2 - 6 mm class and the mean weight diameter was greater for the trashed treatment,

decreasing with increasing distance from the row centre. Bulk density and aggregate stability in the soil profile is shown in Figure 6.7. Bulk density increased with increasing depth. In the row, bulk density was very similar for both the burnt and trash treatments. By contrast, in the inter-row, burning resulted in significant increases in the bulk density to a depth of 30 cm. In the row area, aggregate stability was significantly increased to a depth of 30 cm in response to trash retention, however this effect was only statistically significant in the top 10 cm of inter-row area.



**FIGURE 6.1:** (a) Organic C and (b) microbial biomass C in the 0 - 10 cm soil layer with increasing distance from the row centre (row, 30 cm and 60 cm) for both TF and BtoF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



**FIGURE 6.2:**

(a)  $K_2SO_4$  extractable C and (b) light fraction C in the 0 - 10 cm soil layer with increasing distance from the row centre (row, 30 cm and 60 cm) for both TF and BtoF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).

**TABLE 6.1:** Root mass - and root length density in the row, 30 cm and 60 cm into the inter-row, at each depth.

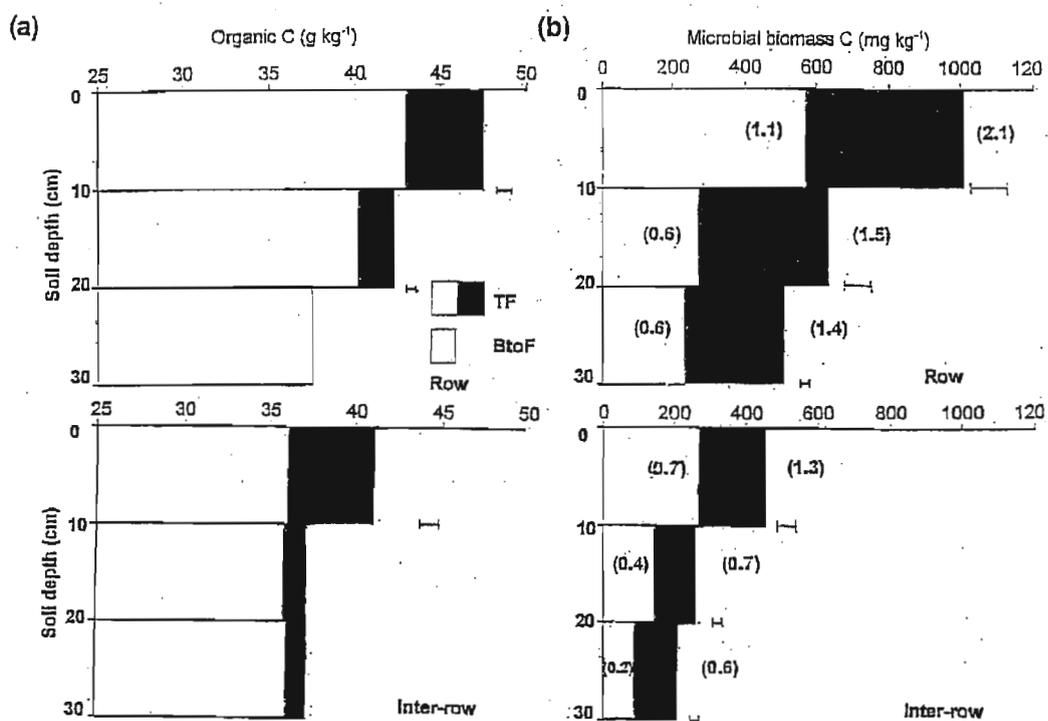
Treatment and depth		Root mass density (x 0.001 g cm <sup>-3</sup> )				Root length density (cm cm <sup>-3</sup> )			
		Row	30 cm	60 cm	LSD	Row	30 cm	60cm	LSD
10 cm	BtoF <sup>1</sup>	0.832	0.366	0.120		1.046	0.608	0.395	
	TF	0.397	0.235	0.242	0.08	0.408	0.286	0.268	0.04
- 20 cm	BtoF	0.398	0.071	0.032		0.392	0.164	0.149	
	TF	0.223	0.065	0.069	0.01	0.216	0.119	0.113	0.03
- 30 cm	BtoF	0.208	0.097	0.026		0.298	0.103	0.100	
	TF	0.067	0.051	0.027	0.01	0.164	0.111	0.093	0.02

<sup>1</sup> = green cane harvesting with a trash blanket left at the soil surface, Bto = burnt with harvest residues removed, F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) for comparison between treatments shown.

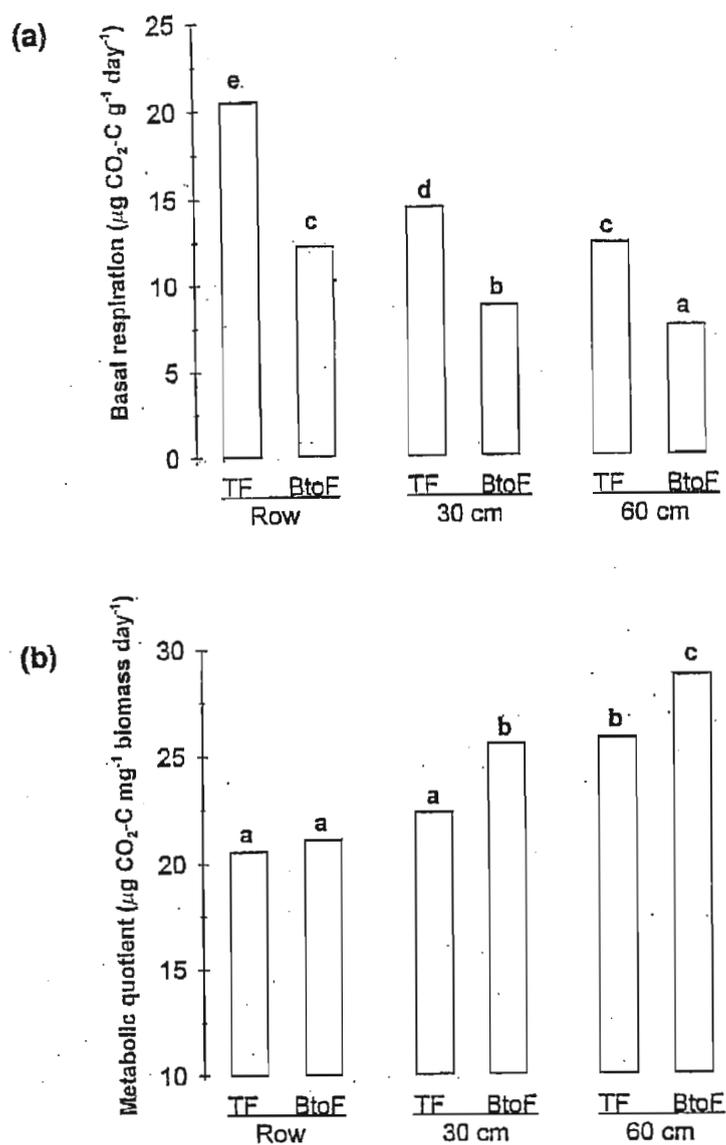
**TABLE 6.2:** Quantities of root biomass C, organic C, microbial biomass C and K<sub>2</sub>SO<sub>4</sub>-extractable C in the surface 30 cm of soil in the row, inter-row (60 cm) and across the field respectively.

Area	Root mass (kg m <sup>-2</sup> )		Root biomass C (Mg ha <sup>-1</sup> )		Organic C (Mg ha <sup>-1</sup> )		Microbial biomass C (kg ha <sup>-1</sup> )		K <sub>2</sub> SO <sub>4</sub> - extractable C (kg ha <sup>-1</sup> )	
	TF <sup>1</sup>	BtoF	TF	BtoF	TF	BtoF	TF	BtoF	TF	BtoF
Row	85.8	124	411	594	136	129	2284	1107	1459	694
Inter-row	39.7	19.4	190	92	134	128	1045	527	685	402
Field	50.8	47.6	245	249	135	129	1459	795	914	503
SD ( $P \leq 0.05$ )	23.6	19.8	113	147	2.87	1.56	136	128	142	74

<sup>1</sup> T = green cane harvesting with a trash blanket left at the soil surface, Bto = burnt with harvest residues removed, F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) for comparison between treatments shown

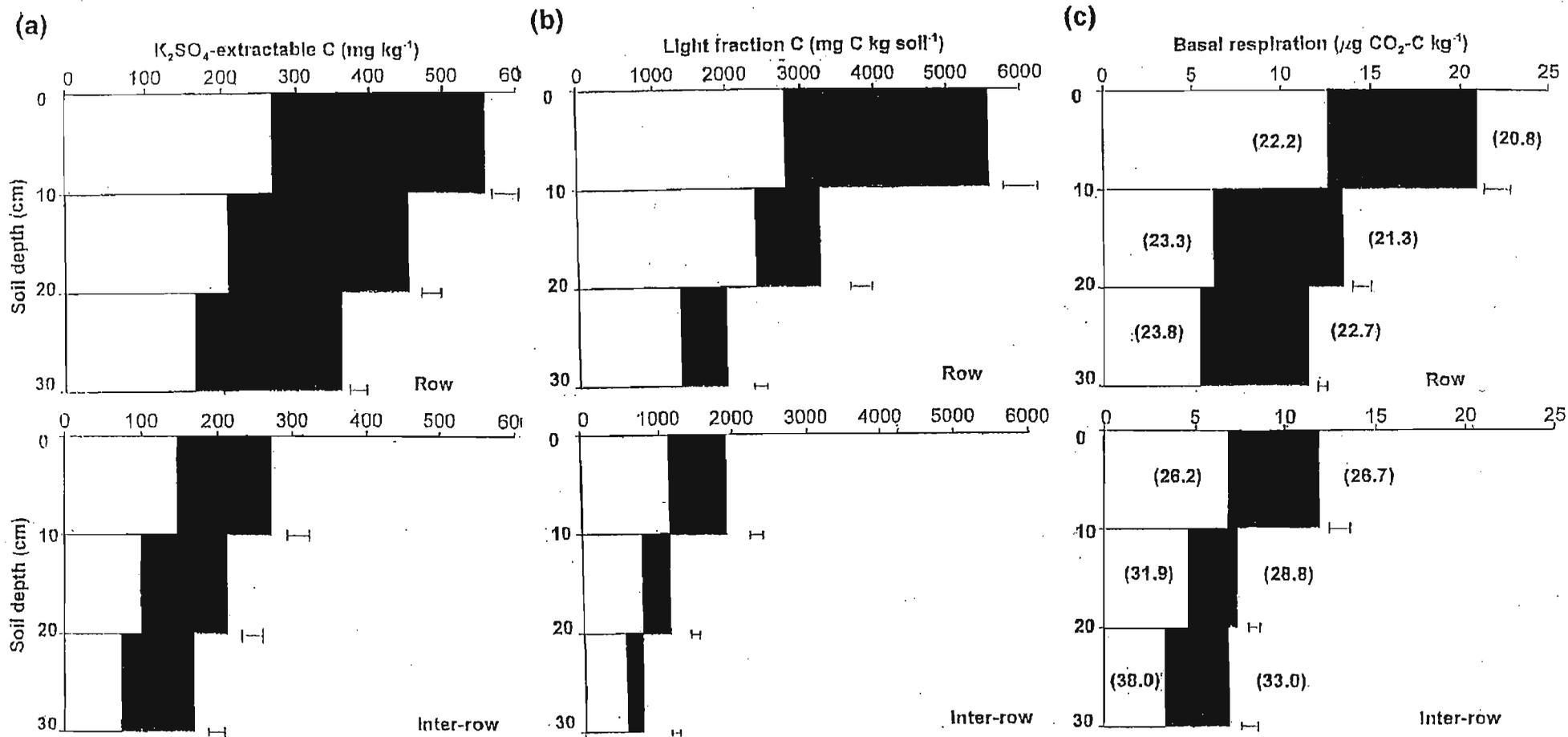


**FIGURE 6.3:** Effects of burning and green cane harvesting on soil (a) organic C and (b) microbial biomass C content in the soil profile in the row and inter-row. Values for the microbial quotient at each depth are shown in brackets. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.



**FIGURE 6.4:**

(a) Basal respiration and (b) metabolic quotient in the 0 - 10 cm soil layer with increasing distance from the row centre (row, 30 cm and 60 cm) for both TF and BtoF treatments. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. Means associated with the same letter are not significantly different (Based on LSD,  $P \leq 0.05$ ).



**FIGURE 6.5:** Effects of burning and green cane harvesting on (a)  $K_2SO_4$ -extractable C, (b) light fraction C and (c) basal respiration in the soil profile in the row and inter-row. Values for the metabolic quotient at each depth are shown in brackets. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) shown for treatment differences for each soil layer.

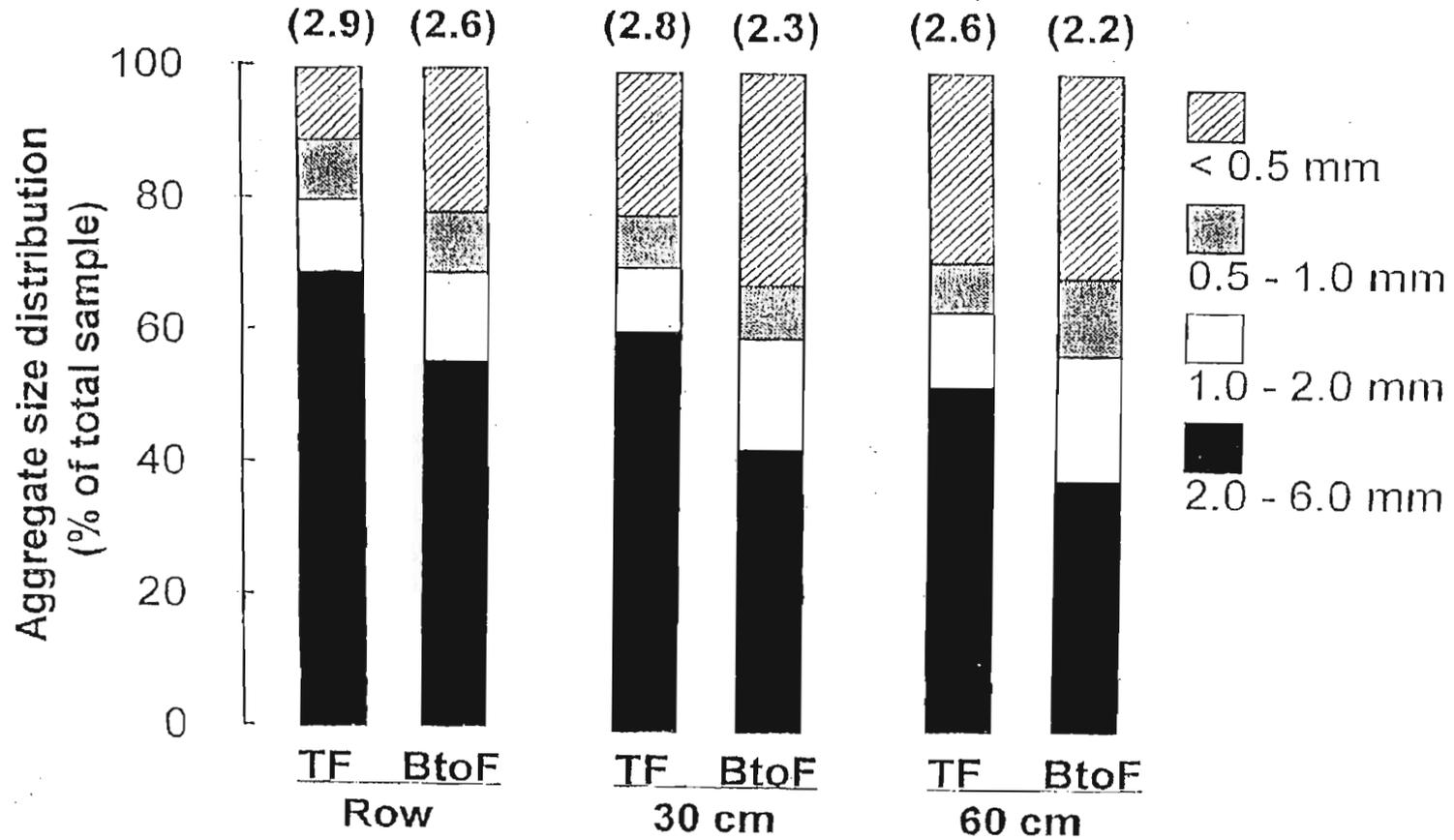
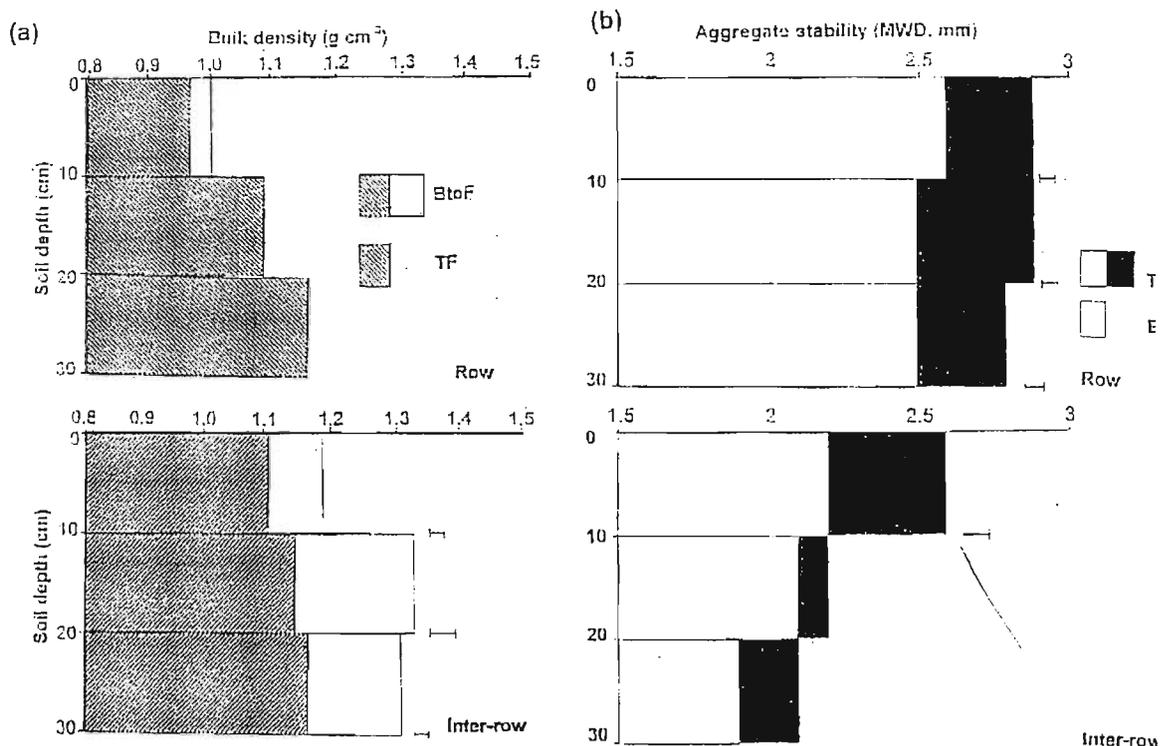


FIGURE 6.6:

Size distribution of aggregates following wet sieving in the 0 - 10 cm soil layer with increasing distance from the row centre (row, 30 cm, 60 cm) for both TF and BtoF treatments. Values for the MWD (mm) are shown in brackets. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K.



**FIGURE 6.7:** (a) Bulk density and (b) aggregate stability (MWD, mm) in the soil profile in the row and inter-row. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K. LSD ( $P \leq 0.05$ ) for comparison between treatments.

## 6.4 Discussion

Under pre-harvest burning, the main C inputs to the soil are in the form of turnover of sugarcane roots. It is clear that under burning sugarcane roots are concentrated in the row below the stem clump. This has also been observed by other workers (Ball-Coulho *et al.*, 1992; Hartemink, 1998b) and, as shown here, this is translated into a concentration of total and labile organic matter and microbial activity in the soil below the plant rows.

Root biomass contributes large amounts of organic matter to the soil. Rhizodeposition of organic material occurs in several ways including exudation of mucilaginous material which is dominantly polysaccharides (Morel *et al.*, 1991). Input of root tissue also occurs from sloughing of root tissues during root senescence, conversion of very fine roots to those with secondary thickening (autolysis of cortical tissues) releasing epidermal and cortical tissue, senescence of woody or fine roots, and root tissue loss or damage due to faunal grazing or microbial root diseases (Haynes and Beare, 1996). However, it is root mortality that generally contributes most to the formation of soil organic matter (Clarke *et al.*, 1967). This C supply from the roots encourages microbial proliferation in the rhizosphere and as a result, microbial activity was significantly increased in the row area. Indeed, the microbial quotient was considerably higher in the row (mean 1.22) than the inter-row (mean 0.65) (Figure 6.1). As noted previously (refer to chapter four), the microbial quotient is notably low in the inter-row since values normally range from 1 - 2 % (Sparling, 1997).

Factors such as soil compaction within the inter-row also contribute to poor root proliferation in the inter-row area. For example, wheeled traffic during harvesting and other field operations cause considerable compaction especially during wet harvesting seasons (Haynes and Hamilton, 1999). As a result, the bulk density and penetration resistance increases and pore space decreases causing poor root

proliferation in the inter-row (Hartemink, 1998a).

The much larger root length and mass in the row under burning than trashing is probably related to the lack of a surface mulch and thus a shortage of soil water in the burnt treatments. Water stress often stimulates root growth resulting in longer, finer roots which are able to explore the soil volume for water more effectively (Sharp and Davies, 1979). Indeed, at all three sampling positions, the root length density was greater under burning.

Despite the smaller root mass,  $C_{org}$  was significantly increased in the row to 20 cm in response to trash retention. Large amounts of leaves and tops are returned annually to the soil with green cane harvesting, covering the soil surface in both the row and the inter-row. This explains the accumulation of organic matter in the row. Earthworm communities tend to be concentrated in the row area of sugarcane fields (Spain *et al.*, 1990) and they could contribute to downward movement of organic material to 20 cm. Earthworms are actively involved in the redistribution of particulate organic matter fractions within the profile (see chapter four). They can ingest / collect the decaying plant material from the soil surface and redistribute it within their burrows. Earthworm casts frequently have a higher organic matter content than bulk soil (Haynes and Beare, 1996).

Return of trash to the soil surface has been shown to have a positive effect on various soil chemical (see chapter three), biological (see chapter four and five) and physical (see chapter four) properties. Surface mulches also result in water conservation by reducing runoff, increasing infiltration and reducing losses by evaporation. The moist soil conditions below the mulch stimulated root proliferation in the 0 - 10 cm layer of the inter-row under trashing. The redistribution of roots into the inter-row is also favoured by the large amounts of nutrients leaching from the trash blanket (Chapter three). Similarly Ball-Coelho *et al.* (1992) reported a concentration of roots at the soil surface and in the litter layer of mulch treatments. Although the root biomass was greater in the inter-row under trashing, there was no

significant difference in total root biomass between the two treatments. Nevertheless, the redistribution of roots towards the inter-row under trashing will result in more rhizodeposition of C in the inter-row. This will contribute to the greater organic matter, higher biological activity and greater aggregate stability in the inter-row under trashing.

When sugarcane is burnt very little above ground plant material is returned to the soil and the inter-rows are left fallow. The metabolic quotient was considerably higher in the inter-row of burnt than trashed treatments. An increase in the metabolic quotient has been interpreted as a response by soil microflora to adverse environmental conditions (either environmental stress or disturbance) (Wardle and Ghani, 1995). In the inter-row of the burnt treatment the main stresses are likely to have been a scarcity of labile C, water stress and possibly, also compaction.

The relative decrease in  $C_{mic}$  in response to both burning and increasing distance away from the row was much greater than that for  $C_{org}$  (Figure 6.3). As a result, the microbial quotient was higher in the row than inter-row and higher under trashing than burning. A similar trend was also evident for  $K_2SO_4$  - extractable C, light fraction C and basal respiration (Figure 6.5 and 6.6). Thus, as noted by a number of other workers (Gregorich *et al.*, 1994) the effects of agricultural practice on organic matter status are more obvious, and noticed first, when measuring labile C fractions or the size and activity of  $C_{mic}$  rather than simply measuring  $C_{org}$ .

Changes in  $C_{org}$  and labile organic matter fractions play a major role in soil aggregation (Haynes and Beare, 1996). The major organic binding agents in soil are humic molecules and polysaccharides. These molecules can strongly bind to the mineral components of soils, and to each other, and are therefore of central importance in relation to binding aggregates together. The microbial biomass is also important due to its production of polysaccharide binding agents (mucilages) and the binding ability of fungal hyphae. Thus, the smaller  $C_{org}$  and  $C_{mic}$  concentrations in the surface soil under burning resulted in a substantially lower aggregate stability

than under trashing. This means that soil in the inter-row of the burnt treatment is predisposed to structural breakdown and compaction. Such compaction occurs commonly in sugarcane inter-rows, and along with poor aggregation, tends to decrease infiltration and increase surface runoff (Wood, 1985; Prove *et al.*, 1995). The higher aggregate stability in the row than inter-row is likely to be not only because of the larger  $C_{org}$  and  $C_{mic}$  but also due to the direct and indirect effects of the presence of crop roots. Roots exert a stabilizing influence on soil structure partially through mucilage produced by the microbial population of the rhizosphere and by exudation of mucigel from the root (Russell, 1971). Roots and root hairs of plants as well as associated mycorrhizal hyphae, can act as aggregating and temporary binding agents. They have been shown to enmesh fine particles of soil into aggregates (Clarke *et al.*, 1967; Coughlan *et al.*, 1973; Haynes and Beare, 1996).

Although  $C_{org}$  concentrations were less in the inter-row than row, bulk density was greater in the inter-row. As a result, when calculated on a per hectare basis,  $C_{org}$  values based on samples taken from the inter-row did not underestimate the C reserves in the field. Nonetheless, the equivalent values for  $K_2SO_4$  - extractable C, light fraction C and  $C_{mic}$  were clear underestimates (Table 6.2). Where C reserves are being compared under various land uses, sampling randomly over the entire fields rather than between the row is obviously an important consideration.

However, from an agronomic viewpoint, the soil organic matter status and soil physical conditions in the inter-row are of great importance. The inter-row space represents about 60 % of the surface area of a sugarcane field and it is the main area where surface runoff, erosion and compaction occurs. Indeed, on the rolling land of the South African sugar belt, losses of soil through water erosion are common. An increase in organic matter, microbial activity and aggregate stability in the inter-row space induced by green cane harvesting is therefore of considerable significance.

## 6.5 Conclusions

It is clear that under burning, the roots of sugarcane are concentrated below the stem clump down the plant rows. This results in a large gradient in organic matter content, soil microbial activity and aggregate stability across the fields with values being greatest below the rows and least in the middle of the inter-rows. Conversion to green cane harvesting results in redistribution of root mass and a concentration of roots in the surface soil in the inter-row space below the trash mulch. As a result of the large organic matter inputs via the trash itself, and in the form of root turnover, the organic matter status, size and activity of the microbial community and aggregate stability are all appreciably increased in the inter-row space under green cane harvesting. The likely results of these changes include stabilization of the surface soil, increased infiltration, a greater rate of nutrient turnover and reduced runoff and erosion and thus conservation of the soil resource in the sugar belt.

## CHAPTER SEVEN

### 7. Effect of agricultural land-use on soil microbial biodiversity.

#### 7.1 Introduction

In recent years, considerable interest has been generated in assessment of the quality of agricultural soils (Carter *et al.*, 1997). Organic matter content and quality are considered as key attributes of soil quality since they are important in determining the chemical, physical and biological properties of soils (Gregorich *et al.*, 1994). Although the soil microbial biomass constitutes a small proportion (typically 1 - 5 %) of soil organic matter (Sparling, 1997) the composition and activity of soil microbial communities largely determine biogeochemical cycles, the turnover processes of organic matter, and the fertility of soil (Zelles, 1999).

There are believed to be several million species of microorganisms present in soils and most cannot be characterized by conventional culture techniques and generally, plate counts are not considered reliable measures of soil microbial diversity (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 (PLFA) (Zelles, 1999). PLFA analysis is a valuable approach, because it is a biochemical method that provides direct information about the structure of the viable microbial community, free of the limitations inherent in culturing microorganisms. This technique has been used to elucidate different strategies employed by microorganisms to adapt to changed environmental conditions under wide ranges of soil types (Frostegård *et al.*, 1993), management practices (Zelles *et al.*, 1995; Lundquist *et al.*, 1999), climatic origins (Steinberger *et al.*, 1999) and different perturbations (Kelly *et al.*, 1999).

Soil microbial biodiversity has received little attention in South Africa. The long-term

sugarcane residue management trial provides an ideal opportunity to investigate the effect of burning and trashing on soil microbial diversity by analyzing phospholipid fatty acids. Diversity under sugarcane is also compared to that under other agricultural land uses in the midlands area of the province of KwaZulu-Natal. The principal objective was to study how residue management under long-term sugarcane production and other common agricultural land uses changes the composition of soil microbial communities.

## **7.2 Materials and Methods**

To study the effect of residue management on the soil microbial community structure, samples were taken from the long-term residue management trial, BT1, at Mount Edgecombe (refer to chapter 3). The largest differences in chemical, physical and biological properties were found between the burnt treatment with all the above ground residues removed and the green cane harvested treatment with a trash blanket on the soil surface. For this reason the BtoFo, BtoF, Tfo and TF treatments were sampled at a depth of 0 - 5 cm. The grass rows between the replicates were also sampled as a control for comparison (refer to site description in chapter 3). Samples were also taken from the 0 - 5 cm layer from fields under various agricultural land uses at a site in the KwaZulu-Natal midlands. The fields were on "Baynesfield Estate" (27°22'S and 30°45'E) and known cropping histories of fields were > 50 yr Kikuyu grass pasture (KIK), > 50 yr annual rye grass pasture (RYE), > 30 yr continuous burnt sugarcane (SUG), > 30 yr continuous maize (MAI) and undisturbed grassveld (VEL). The soil at the site was a Hutton form (Farmingham series) (Soil Classification Working Group, 1991) or a Rhodic Ferralsol (FAO) with a clay content of about 62 %. Its mineralogy is dominated by kaloinite plus halloysite and there are also appreciable amounts of crystalline sesquioxides, gibbsite and inter-layered chlorite.

The modified one-phase extraction procedure was used for extraction of phospholipids and separation of fatty acids (Zelles, 1996). The phospholipid fatty

acids were extracted from the field-moist soil samples using a mixture of buffer solution, methanol and chloroform and this was followed by a mild alkaline hydrolysis step. Fatty acids and their derivatives were then fractionated into various chemically relevant groups and determined by gas chromatography (Zelles, 1999).

The total phospholipid fatty acids are designated throughout this chapter as PLFAs. The total amount of PLFA generally represents the total microbial biomass (Petersen *et al.*, 1991; Bååth *et al.*, 1992; Balkwill *et al.*, 1988; Zelles *et al.*, 1994; 1997). They are composed of the ester-linked phospholipid fatty acids (EL-PLFA) and non-ester-linked phospholipid fatty acids (NEL-PLFA). The main EL-PLFA's are subdivided into saturated (EL-SATFA), monounsaturated (EL-MUFA) and polyunsaturated (EL-PUFA) fatty acids (Zelles, 1999). EL-PLFA are the signature fatty acids for aerobic microorganisms and NEL-PLFA are the indicator fatty acids for anaerobic microorganisms. The EL-SATFA are generally regarded as signature fatty acids for the Gram - positive bacteria and EL-MUFA as signature fatty acids for Gram - negative bacteria. The EL-PUFA are generally regarded as signature fatty acids for fungi. However, linoleic acid (18:2 $\omega$ 6) has been considered as a more accurate indicator fatty acid for fungi (Federle, 1986; Frostegård and Bååth, 1996). The fungal / bacterial ratio is calculated as the ratio of 18:2 $\omega$ 6 PLFA to total branched SATFA.

Individual PLFAs (expressed as log<sub>10</sub> mol%) were subjected to principal component analysis (PCA) to elucidate major variation and covariation patterns using CANOCO software (Microcomputer Power, Ithica, N.Y.). Using this software, variation in PLFAs was correlated with environmental variables by redundancy analysis (RDA). RDA allows direct assessment of the relationship between environmental variables and variation in the multivariate data. Thus the degrees of similarity among communities or environmental effects on a community can be quantified and tested for significance. Environmental variables tested included organic C, pH, exchangeable K, Na, Mg and extractable P. The Monte Carlo permutation test was used to test the statistical significance of the relationship between environmental

variables and variation in PLFA profiles.

Shannon's diversity index (H), PLFA richness (S) and PLFA evenness (E) were used to evaluate microbial diversity as affected by the treatments. PLFA richness is the number of different PLFA's that were isolated. PLFA evenness measures the distribution of individual PLFAs among the total number present; this indicates whether there is dominant PLFA's present (as indicated by low values for evenness). The Shannon index integrates richness and evenness into a single diversity value.

### 7.3 Results

A summary of the chemical properties as affected by agricultural land uses is given in Table 7.1. Soil organic C increased in the order  $SUG = MAI < RYE \leq VEL < KIK$ . Soil pH increased in the order  $VEL < MAI = SUG \leq RYE < KIK$ . Extractable P and exchangeable K were considerably higher in agricultural fields than under undisturbed grassveld. Bulk density was least under kikuyu pasture and undisturbed grassveld and greatest under pre-harvest burnt sugarcane. Values were intermediate under maize and annual ryegrass. Chemical properties of the BtoFo, BtoF, Tfo, TF and grass treatments are given in chapter 3.

The fatty acid data was analyzed in two steps: First, the PCA was carried out for the total PLFA's. Secondly, some selected indicator fatty acids were used to indicate which microbial communities predominate in the soils under different management practices.

PCAs of the PLFA profiles for the soils from the different sugarcane management practices and different agricultural land management practices are shown in Figure 7.1. The component weights are plotted for the first two variables (Figure 7.1). Under sugarcane residue management the first variable (PC 1) accounted for 85.3 % and the second variable (PC2) only accounted for 8.1 % of the total variance in the PLFA data. Under agricultural land management practices the first variable (PC1)

accounted for 74.2 % and the second variable (PC2) accounted for 23.4 % of the total variance in the PLFA data. This analysis indicated that there were clear differences in PLFA compositions (microbial communities) under different sugarcane residue management and under different land management practices.

RDA results are displayed on the biplots which depict the relationship between the soil chemical properties and individual PLFAs (Figure 7.1). The closer the vector for an individual variable aligns with a principal component axis the more that particular chemical variable can be used to explain the variation in the data along that axis. The general alignment of organic C with the PC1 axis is evident in both biplots.

Under sugarcane residue management organic C was found to be significantly correlated ( $P < 0.005$ ) with PC 1 and accounted for 67.1 of the explained variance in the PLFA profile (Table 7.2). Exchangeable potassium was also significantly correlated ( $P < 0.005$ ) with PC 1 and accounted for 10.2 % of the explained variance in the PLFA profile. Since PC 2 only accounted for 8.1% of the total variance in the PLFA data, there was no significant correlation between PC2 and any of the environmental variables. However, there was a tendency for the variance along PC2 to be related to changes in soil pH.

Under agricultural land management practices organic C was also found to be significantly correlated ( $P < 0.005$ ) with PC1 and accounted for 66.4 % of the explained variance in the PLFA profile (Table 7.3). The vertical distribution of treatments (PC2) was significantly correlated ( $P < 0.005$ ) with soil pH and accounted for 24 % of the explained variance in the PLFA profile.

Under sugarcane residue management a good correlation was found between PLFA's and  $C_{mic}$  as determined by fumigation extraction. ( $r = 0.90$ ,  $P \leq 0.001$ ). The concentration of both  $C_{mic}$  and PLFA's followed the order: BtoFo = BtFo < grass ≤ TFo < TF (Figure 7.2). Values for EL-PLFA, EL-SATFA and EL-MUFA followed the order BtoF ≤ BtoFo < grass = TFo < TF but those for NEL-PLFA were greater under BtoF than BtoFo (Figures 7.3 and 7.4). The proportion of PLFA's present as EL-

PLFAs showed a similar trend to that for EL-PLFA values while the proportion of PLFAs present as NEL-PLFAs showed the opposite trend (Figure 7.3). The proportion of EL-PLFA present as EL-SATFA followed the order: grass < TFo = TF BtoF ≤ BtoFo. By contrast the proportion of EL-PLFA's present as EL-MUFA followed the order; BtoF ≤ BtoFo < TF ≤ Grass = TFo. The ratio of MUFA / SATFA were 0.12, 0.13, 0.2, 0.22, and 0.22 for the BtoFo, BtoF, TFo TF and Grass treatments respectively ( data not shown). The 18:2w6 - PLFA followed the order of BtoFo ≤ BtoF < TFo < TF < Grass (Figure 7.5). The fungal / bacterial ratio followed the order: BtoFo < BtoF = TF ≤ TFo < Grass (Figure 7.5).

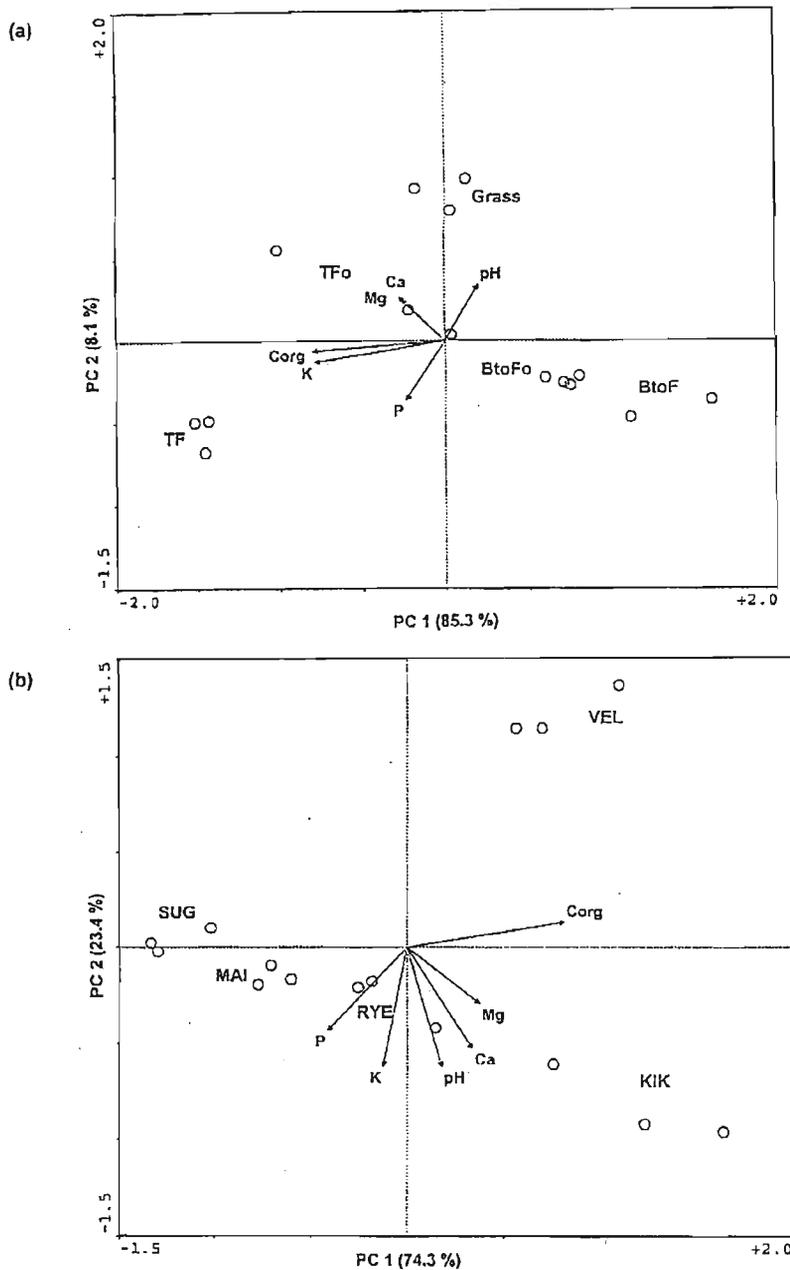
Phospholipid fatty acid (PLFA) richness, PLFA evenness and Shannon's diversity index values are shown in figure 7.6. PLFA richness (i.e. the number of different PLFA's) increased in the order: BtoFo ≤ BtoF < TFo = Grass < TF. PLFA evenness followed the order: TF < TFo < Grass = BtoFo ≤ BtoF (i.e. reduced evenness indicating increased dominance of a few PLFA's). The PLFA diversity index increased with increasing organic matter returned (i.e. BtoFo < BtoF < TFo < TF < Grass) (Figure 7.6).

Under different agricultural land management practices at Baynesfield Estate the concentrations of  $C_{mic}$  and PLFA's followed the order SUG ≤ MAI ≤ RYE ≤ VEL ≤ KIK and the two measurements were closely correlated ( $r \leq 0.71$ ,  $P \leq 0.001$ ) (Figure 7.2). Values for EL-PLFA, NEL-PLFA, EL-SATFA and EL-MUFA followed a similar trend (Figures 7.3 and 7.4). The proportion of PLFA present as EL-PLFA followed the order: SUG < MAI < RYE ≤ VEL < KIK while that for NEL-PLFA followed the opposite trend. Even though the absolute values of EL-SATFA and EL-MUFA followed the same trend as PLFA the proportion of EL-PLFA present as EL-SATFA followed the order VEL ≤ KIK ≤ RYE < MAI ≤ SUG. By contrast the proportion of EL-PLFA present as EL-MUFA followed the inverse trend. Indeed, in this study the ratio of MUFA / SATFA was 0.11, 0.2, 0.31, 0.37, 0.44 for the SUG, MAI, RYE, KIK and VEL treatments respectively. The 18:2w6-PLFA followed the order: SUG < MAI = RYE < KIK < VEL (Figure 7.5). The fungal / bacterial ratio followed the order: KIK < RYE < SUG ≤ MAI ≤ VEL (Figure 7.5).

**TABLE 7.1:** Soil properties under different agricultural land uses (0 - 5 cm) from Baynesfield Estate in the KwaZulu-Natal midlands.

	Organic C (g kg <sup>-1</sup> )	Bulk density (g cm <sup>-3</sup> )	pH <sub>(water)</sub>	Extractable P (mg kg <sup>-1</sup> )	Ca	Exchangeable Mg (mmol <sub>c</sub> kg <sup>-1</sup> )	K
Undisturbed grassveld (VEL)	44	1.11	5.0	8	30	7.2	2.3
Conventionally tilled maize (MAI)	28	1.25	5.3	49	34	6.4	3.8
Pre-harvest burnt sugarcane (SUG)	27	1.37	5.3	26	33	6.6	4.1
Annual rye grass (RYE)	40	1.28	5.4	35	36	10.5	4.6
Kikuyu grass pasture (KIK)	67	1.05	5.6	29	42	9.9	4.7

Values for PLFA richness, evenness and diversity are shown in Figure 7.6. It is evident that trashed treatments had a greater PLFA richness and lower evenness (i.e. there was a dominance of some PLFAs) than Bto treatments. Overall PLFA diversity was greater under trashing than burning and greatest under grass. For agricultural management practices, richness was least and evenness greatest under sugarcane. That is, there were a relatively small number of PLFAs present but they were all present in approximately equal amounts. PLFA diversity was least under sugarcane and greatest under maize and ryegrass.



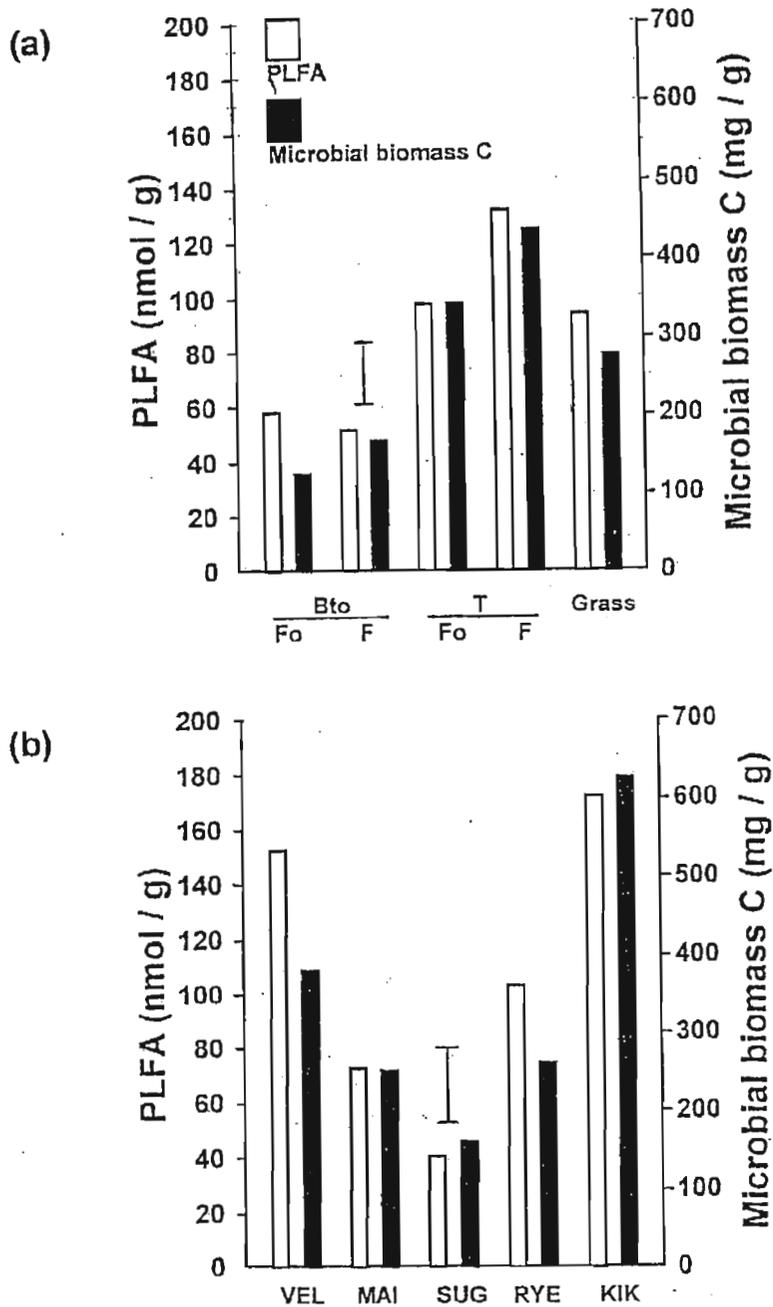
**FIGURE 7.1:** Redundancy analysis ordination bi-plots of PLFA profiles for (a) sugarcane residue management and (b) agricultural land management practices. The percent of the variation explained by the two ordination axes are included on the bi-plot. Six site variables showing correlations with variation along PC1 and PC2 axes are shown. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. VEL = grassveld; MAI = maize; SUG = burnt sugarcane; RYE = rye grass; KIK = kikuyu pasture.

TABLE 7.1: Redundancy analysis (RDA) showing the effects of sugarcane residue management practices on PLFA composition.

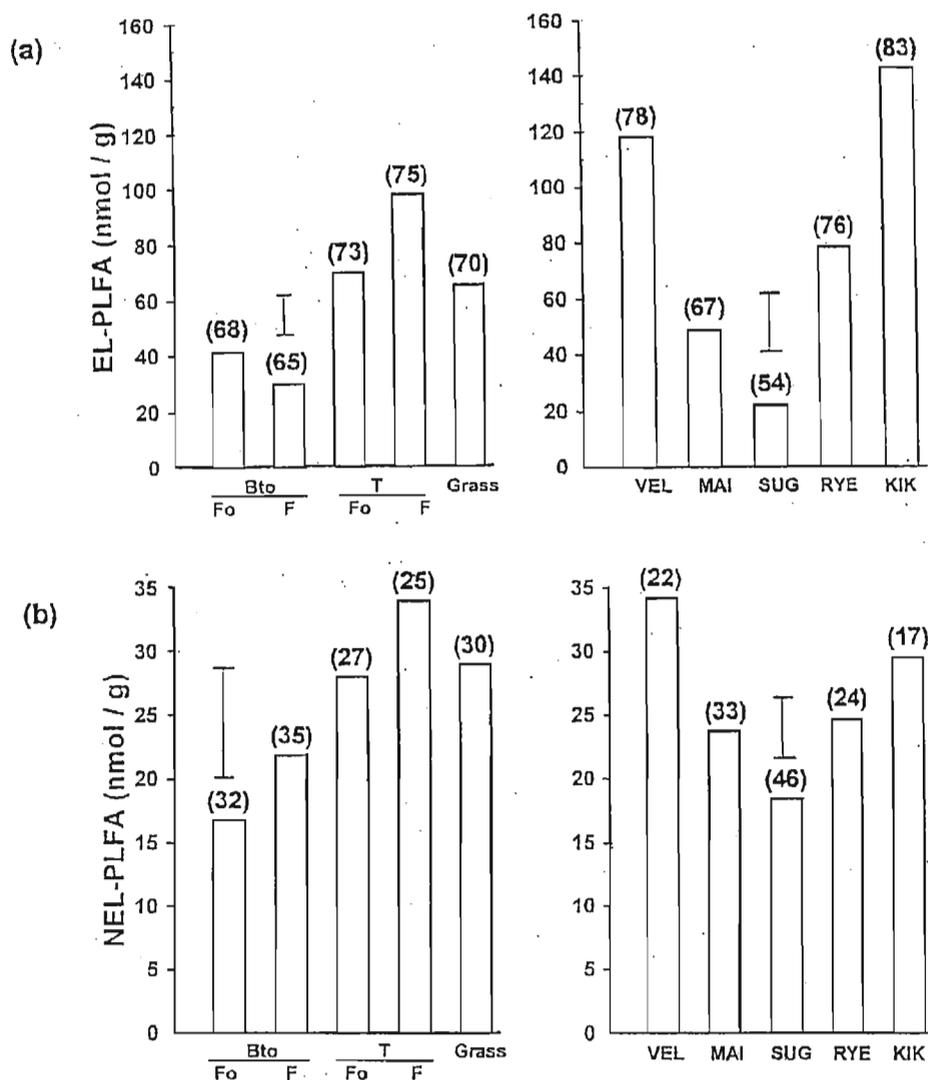
Variable	Eigenvalue	% of explained variance	Montecarlo test		Environment	Correlations	
			F	P		RDA Ax1	RDA Ax2
Organic C	0.59	67.1	18.87	0.005**		-0.85	-0.12
Mg	0.11	12.5	4.44	0.025ns		-0.29	0.49
K	0.09	10.2	4.66	0.005**		-0.83	-0.24
P	0.05	5.6	2.79	0.055ns		-0.25	-0.61
Ca	0.02	2.2	1.73	0.185ns		-0.27	0.46
PH	0.02	2.2	1.1	0.34ns		0.21	0.67
Sum of all canonical eigenvalues	0.879						
Total inertia	3.937						
RDA Ax1	0.812	85.3	3.849	0.005**	0.84		
RDA Ax2	0.073	8.1			0.74		
RDA Ax3	0.036	4.3			0.78		
RDA Ax4	0.011	1.3			0.77		

TABLE 7.2: Redundancy analysis (RDA) showing the effects of agricultural land management practices on PLFA composition.

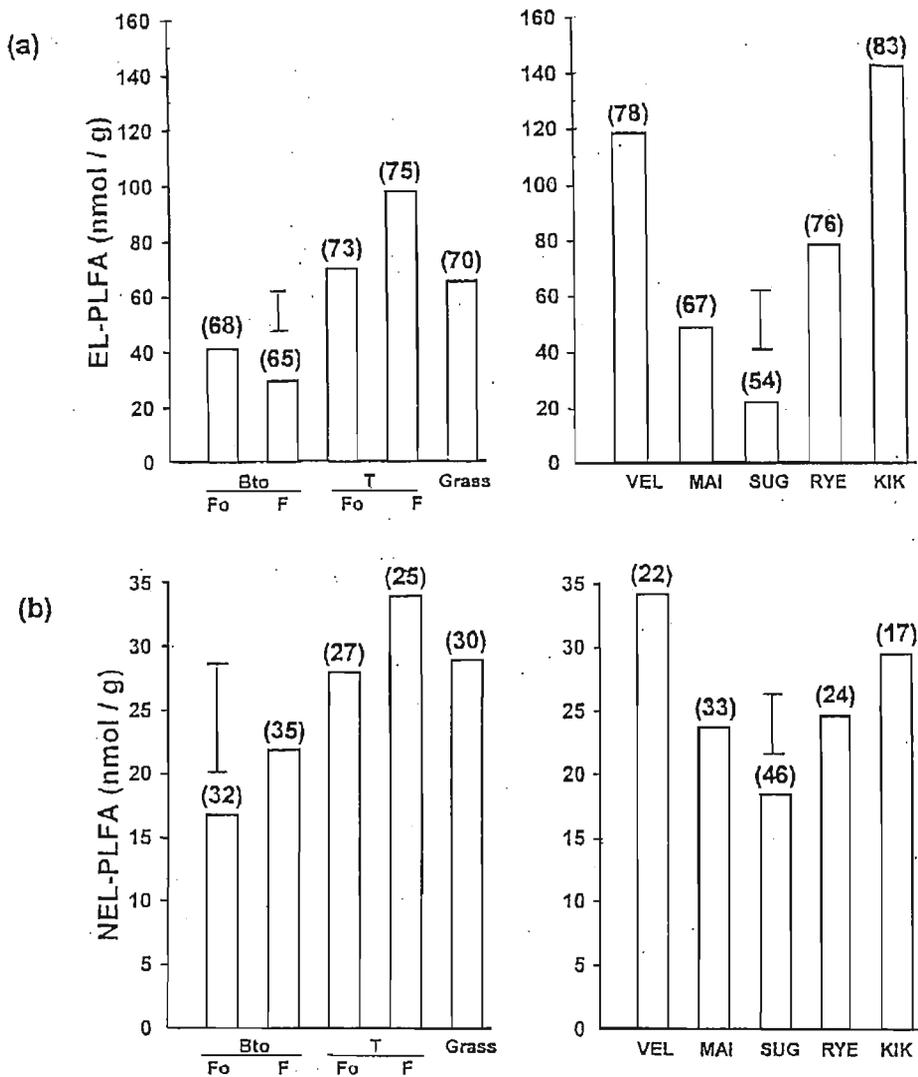
Variable	Eigenvalue	% of explained variance	Montecarlo test		Environment	Correlations	
			F	P		RDA Ax1	RDA Ax2
Organic C	0.58	66.4	18.27	0.005**		0.88	0.19
Mg	0.04	4.5	2.55	0.09ns		0.40	-0.42
K	0.02	2.2	1.11	0.345ns		-0.13	-0.91
P	0.0	0.0	0.36	0.735ns		-0.44	-0.63
Ca	0.02	2.2	1.67	0.195ns		0.36	-0.77
PH	0.21	24	11.48	0.005**		0.19	-0.91
Sum of all canonical eigenvalues	0.874						
Total inertia	4.735						
RDA Ax1	0.65	74.3	2.365	0.004**	0.82		
RDA Ax2	0.204	23.4			0.83		
RDA Ax3	0.011	1.2			0.79		
RDA Ax4	0.006	0.7			0.82		



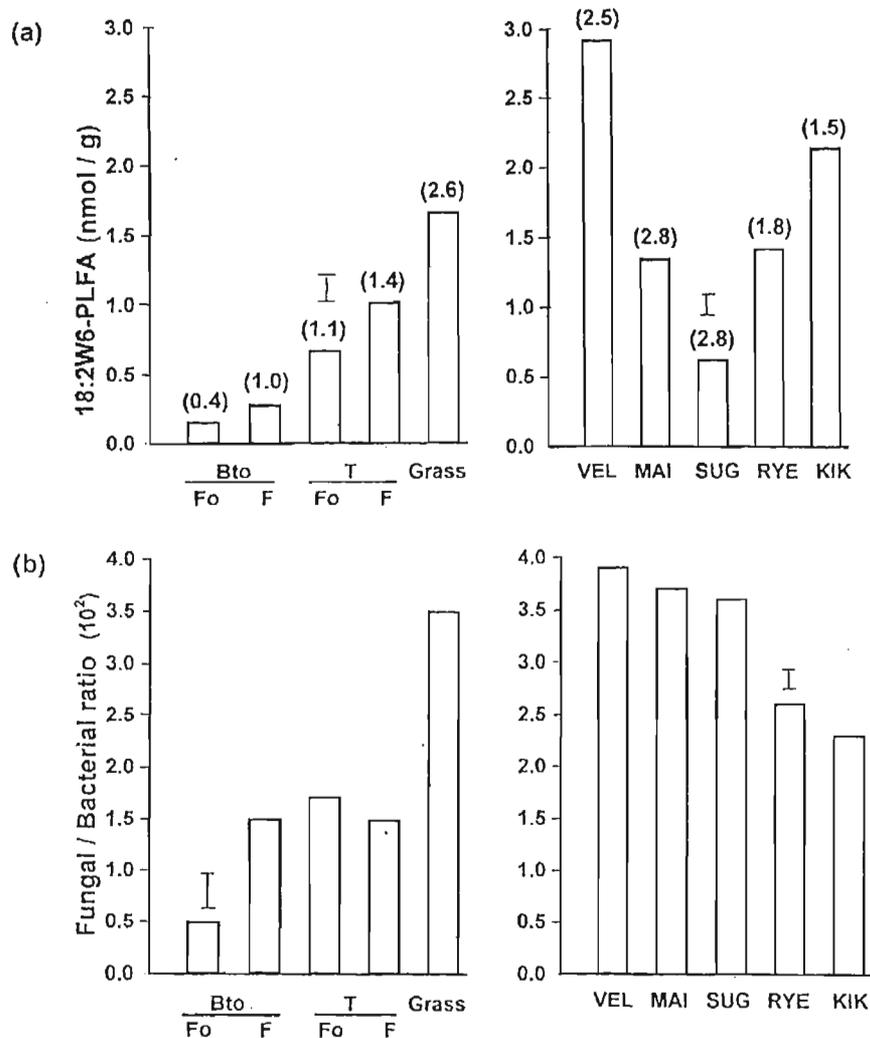
**FIGURE 7.2:** The effects of (a) sugarcane residue management and (b) agricultural land management practices on the amounts of total phospholipid fatty acids (PLFA's) and microbial biomass C as determined by fumigation extraction procedure in the surface 0 - 5 cm layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. VEL = grassveld; MAI = maize; SUG = burnt sugarcane; RYE = rye grass; KIK = kikuyu pasture. LSD ( $P \leq 0.05$ ) for comparison between treatments.



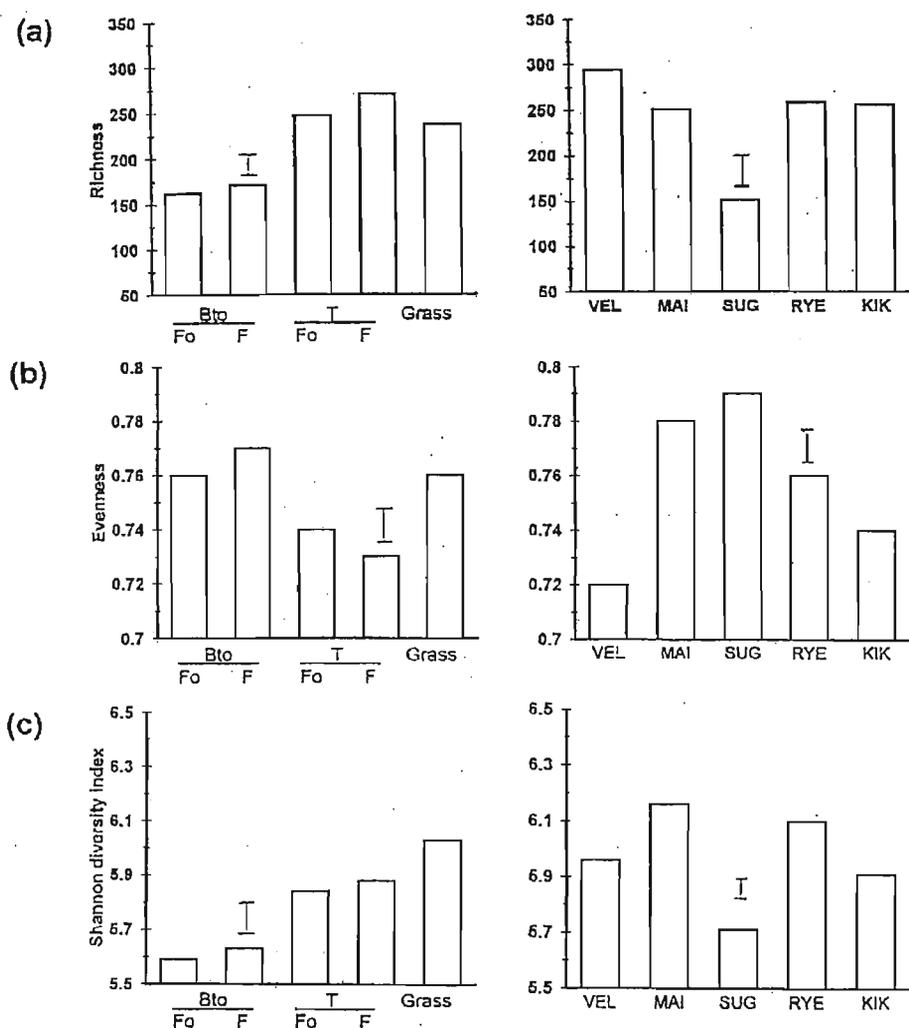
**FIGURE 7.3:** The effects of sugarcane residue management and agricultural land management on the (a) ester-linked PLFA (EL-PLFA) (The values in brackets represent the proportion of PLFA present as EL-PLFA) and (b) non-ester-linked PLFA (NEL-PLFA) (The values in brackets represent the proportion of PLFA present as NEL-PLFA) in the surface 0 - 5 cm layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. VEL = grassveld; MAI = maize; SUG = burnt sugarcane; RYE = rye grass; KIK = kikuyu pasture. LSD ( $P \leq 0.05$ ) for comparison between treatments.



**FIGURE 7.4:** The effects of sugarcane residue management and agricultural land management on the amounts of (a) saturated fatty acids (EL-SATFA) (The values present in brackets present the proportion of EL-PLFA present as EL-SATFA) and (b) monounsaturated fatty acids (EL-MUFA) (The values in brackets represent the proportion of EL-PLFA present as EL-MUFA) in the surface 0 - 5 cm layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. VEL = grassveld; MAI = maize; SUG = burnt sugarcane; RYE = rye grass; KIK = kikuyu pasture. LSD ( $P \leq 0.05$ ) for comparison between treatments.



**FIGURE 7.5:** The effects of sugarcane residue - and agricultural land management practices on (a) fungi (18:2ω6 - PLFA) (The values present in brackets present the proportion of EL-PLFA present as 18:2ω6-PLFA) and (b) fungal / bacterial ratio (18:2ω6 - PLFA / total branched SATFA) in the surface 0 - 5 cm layer. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. VEL = grassveld; MAI = maize; SUG = burnt sugarcane; RYE = rye grass; KIK = kikuyu pasture. LSD ( $P \leq 0.05$ ) for comparison between treatments.



**FIGURE 7.6:** The effect of sugarcane residue – and agricultural and management practices on (a) PLFA richness, (b) PLFA evenness and (c) Shannon diversity index. Grass = unfertilized grass; T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied. VEL = grassveld; MAI = maize; SUG = burnt sugarcane; RYE = rye grass; KIK = kikuyu pasture. LSD ( $P \leq 0.05$ ) for comparison between treatments

### 7.3 Discussion

Since phospholipids are essential membrane components of all living cells and are rapidly metabolized when a cell dies in soil, they have been proposed as an index of the viable microbial biomass (White *et al.*, 1996, Zelles, 1999). Similarly, in this study a good correlation was found between the total amount of PLFA's and the microbial biomass C determined by the fumigation extraction method, indicating that phospholipids do provide an accurate measurement of living biomass. Other workers have recorded similar results. For example, the *r* value for the correlation of the total amount of PLFA's to biomass, measured by the substrate-induced respiration (SIR) technique, varied between 0.9 and 0.98 (Zelles *et al.*, 1992; 1994; 1995). Signature fatty acids for aerobic microorganisms (i.e. EL-PLFA's) showed very similar trends to those of total PLFA's. They have also been used as indicators of microbial biomass in environmental samples by other workers (Balkwill *et al.*, 1988; Zelles *et al.*, 1994; 1995), since they contribute 50 – 90 % of the total PLFA's (Figure 7.3).

Organic matter is frequently the major limiting factor for growth of heterotrophic microorganisms and the size of the microbial biomass is generally stimulated by organic amendments (Knapp *et al.*, 1983; Schnürer *et al.*, 1985). The large total PLFA content that was associated with kikuyu pasture and natural grassveld was a direct result of the large organic matter input arising from senescing plant tops and particularly from turnover of the large root biomass (Haynes and Beare, 1996). Annual ryegrass pastures are ploughed in each spring and resown. Conventional tillage increases aeration and breaks up aggregates exposing organic matter to microbial attack that was previously physically protected (Haynes and Beare, 1996). The effects of a greater organic matter input (with pasture growth) and the degradative effects of conventional tillage apparently balanced out and as a result annual pasture and native grassland had similar organic matter contents. Nevertheless, the microbial biomass C and the PLFA's were notably greater under native grassland. This is possibly the result of larger fungal biomass under natural grassveld than under annually cultivated land (see below).

The lower PLFA's under maize than annual ryegrass reflect the lower organic matter inputs for the maize crop. This occurs because of the wide spacing of maize plants, their sparse root system and the removal of substantial amounts of dry matter at harvest (Haynes and Francis, 1993). Whilst maize fields are tilled each year, for sugarcane, tillage occurs at replanting about every 8 years (a planted crop plus seven ratoon crops). However, the inter-row area is effectively fallow since it receives negligible inputs of organic matter (refer to chapter six). That is, the crop is pre-harvest burnt to remove dead leaves, the cane is removed, and harvest residues are also burnt. As a result, the inter-row area under burnt sugarcane supports a lower microbial population compared to maize. When the crop is green cane harvested the inter-row area is covered in decaying trash and the microbial community is stimulated. This stimulation arises from larger organic matter inputs, more moist soil conditions below the mulch and greater crop root growth in the surface soil below the mulch (see chapter 6).

Recent research has shown that PLFA data can be used to separate certain groups of organisms (Zelles, 1999). Multivariate statistical analysis such as PCA has been a useful tool to detect similarities in PLFA patterns (Zelles, *et al.*, 1997). It separates different groups of PLFA's that are extracted, indicating that these groups have a different microbial community structure. The ordination of PLFA data showed distinct differences in community composition under both sugarcane residue and agricultural land management practices.

The advantage of PLFA analysis, in comparison to other techniques for determining the microbial biomass, is that the sub-fractions of PLFA's can be used as signature fatty acids for specific groups of microorganisms (Tunlid and White, 1992). It has been shown that certain fatty acids are found predominantly in certain groups of microorganisms (Zelles, 1999). It is these sub-fractions that are responsible for the shift in microbial communities as expressed by RDA under different management

practices.

Soil organic matter content was included in the statistical analysis as the main environmental variable that influences the soil microbial community structure. The availability of carbon substrates to microorganisms was linked to changes in relative abundances of specific fatty acids in soils since organic C content contributed the greatest variance in the data along the first axis (PC1) under both residue - and land management practices.

Thus, increasing soil organic matter not only increased the size of the microbial biomass, but also affected the composition of the microbial community. The increase in labile organic matter pools with the addition of organic matter (refer to chapter 3; Haynes *et al.*, 2002) had a direct effect on the size and the proportion of MUFA's. MUFA's can occur in both Gram-negative and Gram-positive bacteria, although their relative contribution to total PLFA content in Gram-positive bacteria is typically very small (e.g. < 20 %). Thus MUFA's can be used as general biomarkers for Gram-negative bacteria (Ratledge and Wilkinson, 1988). Gram negative bacteria are nutritionally a very diverse group of microorganisms (utilize many carbon sources), fast growing and they adapt quickly to a variety of environments. These characteristics result in their proportion increasing with increasing organic matter returns. Soils under kikuyu grass and undisturbed grassveld contain a large senescing root mass which selectively stimulates gram negative bacteria (Rovira and Campbell, 1974). A relationship between high MUFA's and high substrate availability was also identified by Zelles *et al* (1992) in agricultural soils. Thus, the MUFA's seem to be a sensitive and general indicator of higher substrate availability (Bossio *et al.*, 1998). The ratio of MUFA/SATFA has also been tied to nutrient availability. In this study the ratio of MUFA/SATFA PLFA's increased with trash retention and was higher in soils under land managements which had higher soil organic matter contents. Similar results have been found with increasing straw inputs in a rice farming system (Bossio and Scow, 1998), and the ratio was higher in

grassland or wheat soils than under potato cultivation or fallow (Zelles *et al.*, 1992).

Prokaryotic organisms almost exclusively produce saturated and monounsaturated fatty acids, and hence the fatty acids in the EL-PUFA fraction are only found in eukaryotes. Unfortunately, since the composition of EL-PUFA's in fungi and plants is similar, no discrimination can be made between the proportions derived from fungi and from plants. The fatty acid, 18:2 $\omega$ 6, has, however, been proposed as a fungal biomarker (Vestal and White, 1989). The surface application of residues characteristically stimulates fungal growth (Hendrix *et al.*, 1986). A reason for this is that fungal hyphae link surface residue to the soil below more readily than do bacteria (Holland and Coleman, 1987). Thus, for Fo, trash retention greatly increased the fungal to bacterial ratio. However, in fertilized plots no such trend was observed. It is probable that acidification in fertilized plots (see chapter 3) favoured fungal growth regardless of whether burning or trashing was practised. Indeed, soil pH can have a significant effect on microbial community structure because each microbial strain has an intrinsic range of pH within which it can function. Fungi are generally more tolerant of acidity than bacteria (Doran, 1980) and acidification generally favours fungal dominance.

Increases in the soil fungal/bacterial ratio along natural successional gradients are thought to reflect enhanced ecosystem efficiency and food web complexity (Sakamoto and Oba, 1994; Wardle *et al.*, 1995). The findings in this study are in accordance with this in that at both sites undisturbed grassy vegetation had the highest fungal/bacterial ratios. The fungal dominance in undisturbed grasslands noticed here is common (Bargett, 1996) and the low pH under undisturbed grassveld may well have played a role in this. Surprisingly, for the comparison of agricultural land management, the fungal/ bacterial ratio was only slightly lower under maize and sugarcane than veld. However, the ratio was substantially lower under ryegrass and particularly kikuyu pasture. Under these intensively grazed pastoral systems large amounts of organic matter and nutrients are returned to the soil in the form of dung and urine. The increases in soluble organic matter particularly in urine patch areas

of pasture soil may well stimulate bacterial rather than fungal growth, thus lowering the fungal/bacterial ratio.

The shift in the microbial communities from low organic matter input management practices to that of high organic matter inputs could be partially related to changes in soil physical conditions (Haynes and Beare, 1996). The addition of organic matter to the soil is known to improve soil physical conditions by increasing soil aggregation, soil porosity and decreasing soil bulk density (Haynes and Beare, 1996). This increase in oxygen supply stimulates the growth of aerobic microorganisms. The increase in EL-PLFA's was clearly observed under management practices with high organic matter return (i.e. kikuyu pasture and natural grassveld). Even though some compaction occurred under annual ryegrass the proportion of aerobic bacteria was significantly increased compared to arable crops. High pore continuity due to cracking and perhaps earthworm activity, may have resulted in high air permeability and good aeration under this land use. Under burnt sugarcane fields, the inter-row space is effectively fallow and predisposed to structural breakdown and compaction as evidenced by the high bulk density. By contrast under green cane harvesting large amounts of organic matter are returned to the row space improving aggregation and lowering bulk density. The microbial communities that proliferate under lower oxygen levels (i.e. under burnt sugarcane) shift their community structure towards anaerobic microorganisms (NEL-PLFA's). It has been previously shown that NEL-PLFA's increased with lower oxygen availability under rice paddies and under increased compaction (Bai, *et al.*, 2000).

The potassium removals by sugarcane are characteristically large and exchangeable K was significantly correlated with the shift in the microbial community with change in sugarcane residue management practice (PC1). As shown in chapter 3, concentrations of exchangeable K in the surface soil are markedly higher under the trashed than Bto treatments and levels under the Bto treatments are considered low. Thus, and inadequate supply of available C and K may have both been limiting microbial growth and diversity in the Bto treatment.

Management practices such as burning are known to change soil physical and biochemical properties and the quantity and quality of C input to the soil (Raison, 1979; Rasmussen and Collins, 1991). Under adverse conditions, microbial cells may enter a reduced metabolic state that allows them to survive (i.e. by forming resistant structures e.g. endospores). The importance of endospores is largely due to their heat resistance. In general, all endospore forming bacteria are gram positive. Saturated fatty acids (SATFA) mainly indicate gram positive bacteria (Zelles, 1999). Therefore it was not surprising to find that the burnt sugarcane fields had a larger proportion of gram positive bacteria than the green cane harvested treatments or any of the other land uses investigated. Endospore formation can also be associated with depletion of energy sources (Schlegel, 1992) which will occur in the effectively fallow inter-row of burnt sugarcane and also under continuous maize.

It is important to note here that individual fatty acids in a soil PLFA profile cannot be used to represent specific species, both because an individual microbial species can have numerous fatty acids, and because the same fatty acids occur in many different organisms (Bossio *et al.*, 1997). Therefore using PLFA as an index of diversity does not directly indicate the diversity of soil microbial communities, but diversity in the different PLFA's. The generally negative relationship between PLFA evenness and richness observed (Figure 7.6) indicates that where there are a small number of PLFAs present they accumulate in relatively similar amount whereas when there are a large number of PLFAs a small number predominate. A possible explanation for this that a lack of microbial diversity leads to a small community consisting of a relatively small number of resilient groups and populations. A diverse community, however, still tends to be dominated by a relatively small group of populations and associations.

The notably lower Shannon diversity index under preharvest burnt sugarcane compared to the other land uses underlies the lack of diversity in the microbial community under burnt sugarcane. Similarly, the greater PLFA diversity under

trashed than Bto treatments suggests a more taxonomically diverse community had developed under trash retention. Thus, trash retention with large inputs of organic material favours a diverse microbial community.

## 7.5 Conclusions

Management practices that resulted in soil organic matter accumulation not only increased the size of the microbial biomass, but also changed the composition of the microbial community. This was thought to be a result of increased C supply and possibly a change in soil physical conditions induced by a higher organic matter content.

The soil community structure is also dependent on the type of organic matter that is returned to the soil. Under the fertilized pastures the extensive rhizosphere and the addition of animal urine and dung were thought to increase labile C pools and selectively stimulate bacterial communities. However surface applied crop residues (i.e. trash management) stimulated fungal growth.

The number and diversity of PLFA's under burnt sugarcane were significantly reduced compared to trash retention and other land managements studied, reflecting the negative effect of burning in supporting a diverse microbial community. Conversion from burning to trash management increases the soil organic matter content and the size and diversity of the microbial biomass.

## CHAPTER EIGHT

### 8 Effect of agricultural land use on soil functional diversity

#### 8.1 Introduction

Depletion of soil organic matter can result in a reduction in the size and activity of the microbial biomass as well as losses in water-holding and nutrient retention capacity (Gregorich *et al.*, 1994). An understanding of whether these changes affect the diversity of microbial communities or, more important, their functioning, is necessary to evaluate long-term effects of land uses on soil quality. Soil microbial communities are intimately involved in functions such as decomposition, nutrient transformations, aggregate formation and stabilization and plant growth promotion and suppression (Giller *et al.*, 1977). Indeed, the diversity of decomposition functions performed by heterotrophic microorganisms represents one important component of microbial functional diversity. A simple approach to measuring this is to examine the number of different C substrates used by the microbial community. The two most commonly used methods of measuring substrate utilization patterns are the: (a) the Biolog plate and (b) substrate induced respiration techniques.

Biolog plates contain 95 different C substrates to which a growth medium and tetrazolium violet is added. Upon inoculation the C source is oxidized and the tetrazolium salts are reduced to a coloured, insoluble formazan that is indicative of C substrate utilization (Garland and Mills, 1991; Zak *et al.*, 1994). Degens and Harris (1997) developed an alternative method based on substrate induced respiration where, various substrates are added to the soil and the CO<sub>2</sub> levels are measured over a short-term incubation period. This method has the advantage of assessing the catabolic diversity of microbial communities without extracting or culturing organisms from the soil.

The objective of this study was to investigate how residue management under long-term sugarcane production has influenced the metabolic diversity of soil microbial communities. In addition the long-term effects of burnt sugarcane are compared with that of other common agricultural land uses in the locality. Both the Biolog system and the substrate induced respiration methods were used as a comparative evaluation of techniques in assessing microbial functional diversity.

## 8.2 Materials and Methods

### Experimental sites

To study the effect of sugarcane residue management on the soil functional diversity, samples were taken from the long-term residue management trial, BT1, at Mount Edgecombe (refer to chapter 3). The largest differences in chemical, physical and biological properties were found between the burnt treatment with all the above ground residues removed and the green cane harvested treatment with a trash blanket on the soil surface. For this reason we only sampled BtoFo, BtoF, Tfo and TF at a depth of 0 - 5 cm for this study. The grass rows between the replicates were also sampled as a control for comparison (refer to site description in chapter 3). Samples were also taken from the 0 - 5 cm layer from fields under various agricultural land uses at a site in the KwaZulu-Natal midlands. The fields were on "Baynesfield Estate" (refer to site description in chapter 7) and known cropping histories of fields were > 50 yr Kikuyu grass pasture (KIK), > 50 yr annual rye grass pasture (RYE), > 30 yr continuous burnt sugarcane (SUG), > 30 yr continuous maize (MAI) and undisturbed grassveld (VEL).

### Experimental measurements

The Biolog microplates used in this research rely on the redox dye tetrazolium violet to detect utilization of sole carbon sources. The 96-well Biolog microplate comprises 95 substrate-containing wells and a control well without a carbon source. Substrate, dye, and nutrients are supplied in each well in a dried-film form which is reconstituted

upon addition of sample. Ten grams of field-moist sieved soil was shaken in 90 mL of autoclaved physiological saline solution (0.85% NaCl w / v) for 60 min and brought to a final dilution of  $10^{-3}$ . The diluted soil suspension (50 ml) was then placed in a sterile Petri dish. An aliquot of 150  $\mu$ l of the diluted suspension was inoculated into Biolog plates. Three replicate Biolog plates were inoculated from each extraction. The plates were incubated at 30°C. The redox dye turns purple in the presence of electron transfer, indicating the substrate has been utilized by the inoculated microbes. Absorbance readings (590 nm) were obtained after 24, 48, 72, 96 and 120 h of incubation using an ELISA plate reader.

The substrate induced respiration method is based on the measurement of respiration responses ( $\text{CO}_2$ ) to 36 substrates (Degens and Harris, 1997). Various substrates were added as 2 mL solutions to 1 g equivalent dry weight of soil in McCartney bottles sealed with Suba-seals. De-ionised water was added to additional soil samples to determine respiration non-amended soil and to confirm that the responses to substrates were greater than the background respiration. The soil with the added substrate was incubated for 4 h at 25°C in darkness. During the incubation, all bottles were vigorously mixed using a vortex mixer at 1 to 1.5 h after substrate addition and 10 min before measuring the  $\text{CO}_2$  -flux, using an infrared gas analyzer.

The substrates consisted of three amines (D-glucosamine, L-glutamine, N-methyl-D-glucamine), one amide (Succinamide), ten amino acids (L-arginine, L-asparagine, L-cystein, L-glutamic acid, L-histidine, L-leucine, L-lysine, L-serine, L-phenylalanine, DL- tyrosine), two carbohydrates (D-glucose, D-mannose), 17 carboxylic acids (L-ascorbic acid, citric acid, Na-formate, fumaric acid, D-gluconic acid,  $\alpha$ -ketobutyric acid,  $\alpha$ - ketovaleric acid,  $\alpha$ - ketoglutaric acid, DL-  $\alpha$ -hydroxybutyric acid, DL-malic acid, malonic acid, oxalic acid, pantothenic acid, quinic acid succinic acid, L-tartaric acid, uric acid), one aromatic chemical (urocanic acid), and two polymers (cyclodextrin, tween 80). The amines, amides, aromatic chemicals and amino acids were added at 15 mM whereas the carbohydrates were added at 75 mM, the carboxylic acids at 190 mM and the polymers at 30 mM (Degens and Harris, 1997).

All solutions were adjusted to pH 5.5 - 6.0 using HCl or NaOH before addition to the soils.

### Data analysis

For the Biolog data, average well colour development (AWCD) was calculated as described by Garland and Mills (1991). The absorbance value of each well at 120 h was then divided by the AWCD in order to minimize the influence of inoculum density between plates. Normalized absorbance readings were analyzed according to Zak *et al.* (1994) to give substrate richness (the number of substrates used), substrate evenness (the distribution of colour development between substrates) and Shannon's diversity index (a composite measure of richness and evenness).

Normalized absorbances were analyzed by principal component analysis (PCA) using CANOCO software (Microcomputer Power Ithaca, N, Y). Using this software, variation in substrate utilization was correlated with environmental variables using redundancy analysis (RDA) and testing for significant relationships was achieved using the Monte Carlo permutation test. Environmental (soil) variables included were exchangeable Ca and Ma, extractable P, organic C and pH.

The SIR data was also analysed as described above for substrate richness, evenness, Shannon's diversity index, PCA and RDA.

## **8.3 Results**

Color development in Biolog plates (expressed as AWCD) followed a sigmoidal curve over the incubation time of 120 h and followed the order:  $B_{toF_0} \leq B_{toF} < T_{F_0} = TF < \text{Grass}$  under sugarcane residue management practices and  $SUG \leq MAI \leq RYE < VEL < KIK$  under agricultural land management practices (Figure 8.1). Under sugarcane residue management treatments as well as rye and maize, the AWCD

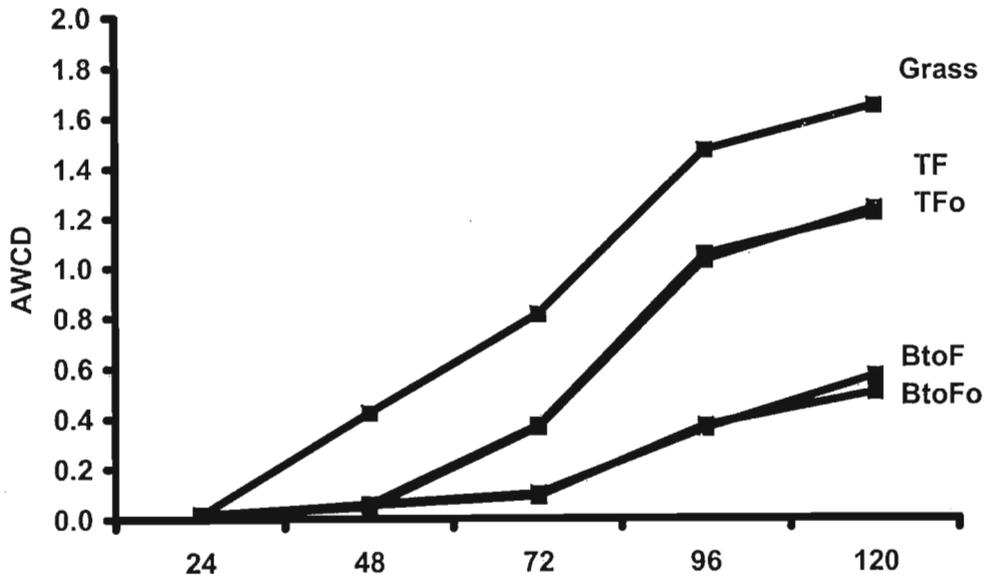
values for the first 48 h showed little, if any change (a lag phase) after which color development proceeded to various levels. By contrast, AWCD for the grass rows, kikuyu pasture and undisturbed grassveld showed an increase in color development after 24 h (Figure 8.1).

Under sugarcane residue management practices, the first variable (PC1) accounted for 44% and 78% and the second variable (PC2) accounted for 8.4% and 8.6% of the total variance in Biolog and SIR data respectively (Figure 8.2). Under different agricultural land management practices, the first variable (PC1) accounted for 57% and 74.7 % and the second variable (PC2) accounted for 28.1% and 10.6 % of the total variance in Biolog and SIR data respectively (Figure 8.3). Confirmation of differences in functional abilities of the soil microbial populations inhabiting the different treatments was demonstrated by their different positions in the plane of the first and second principal components.

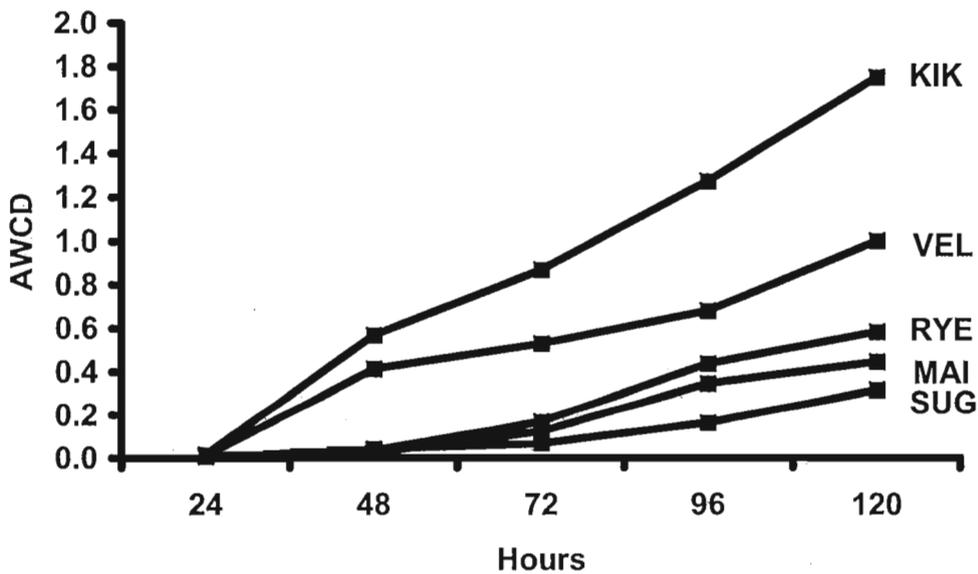
For the sugarcane residue management practices, the Biolog method separated three groups (i) grass, (ii) TF and TFo and (iii) BtoF and BtoFo (Figure 8.2). For the land management practices, the Biolog method also separated three groups (i) sugarcane, (ii) ryegrass and maize and (iii) kikuyu and veld (Figure 8.3). The SIR method separated all of the land uses from one another and, in particular, kikuyu and veld were greatly separated on the PC2 axis.

Substrates most utilized by microorganisms under different treatments are those found in the same position in the zones of the principal component spaces. For the SIR method, this data is shown in Figure 8.4 but the large number of substrates used in the Biolog method made such a presentation impracticable. For residue management practices, a comparison of Figure 8.2b and 8.4b suggests that microorganisms under grass used mannose and ketobutyric, malic, succinic, uracanic and quinic acids most effectively. The community under trash retention used tyrosine, phenylalanine, succinimide and tartaric, uric and glutamic acids most effectively whilst that under preharvest burning used the substrates on the right hand side of the bi-plot most effectively.

(a)



(b)



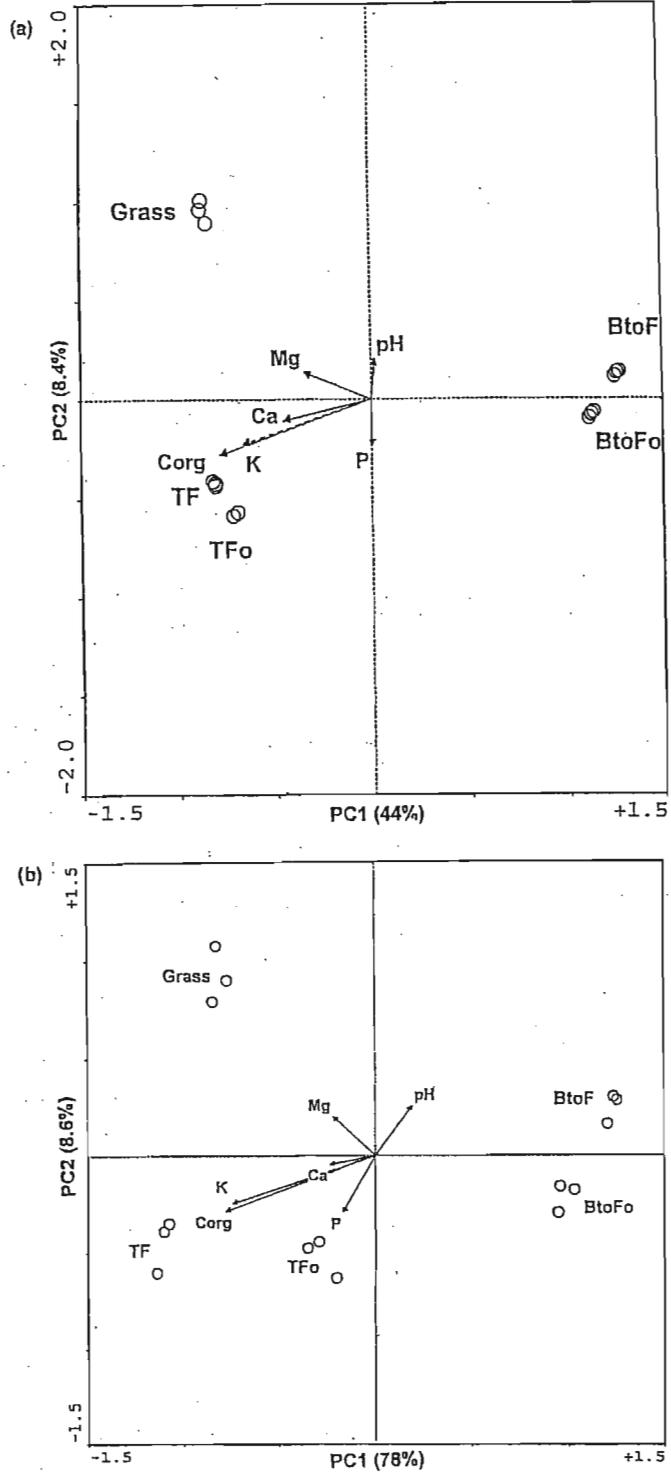
**Figure 8.1:** Variation in average well color development (AWCD) over time under (a) sugarcane residue management and (b) under agricultural land management practices. Grass = unfertilized grass; T = green cane harvesting with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied; KIK = kikuyu pasture; VEL = undisturbed grassveld; RYE = rye grass; MAI = maize; SUG = burnt sugarcane.

For the land management practices the community under sugarcane and maize used tyrosine, phenylalanine, succinimide and uric and tartaric acids most effectively and under veld it was glutamine. Under kikuyu grass, glucosamine, glucamine, arginine, leucine, cysteine and oxalic and ascorbic acids were used most effectively.

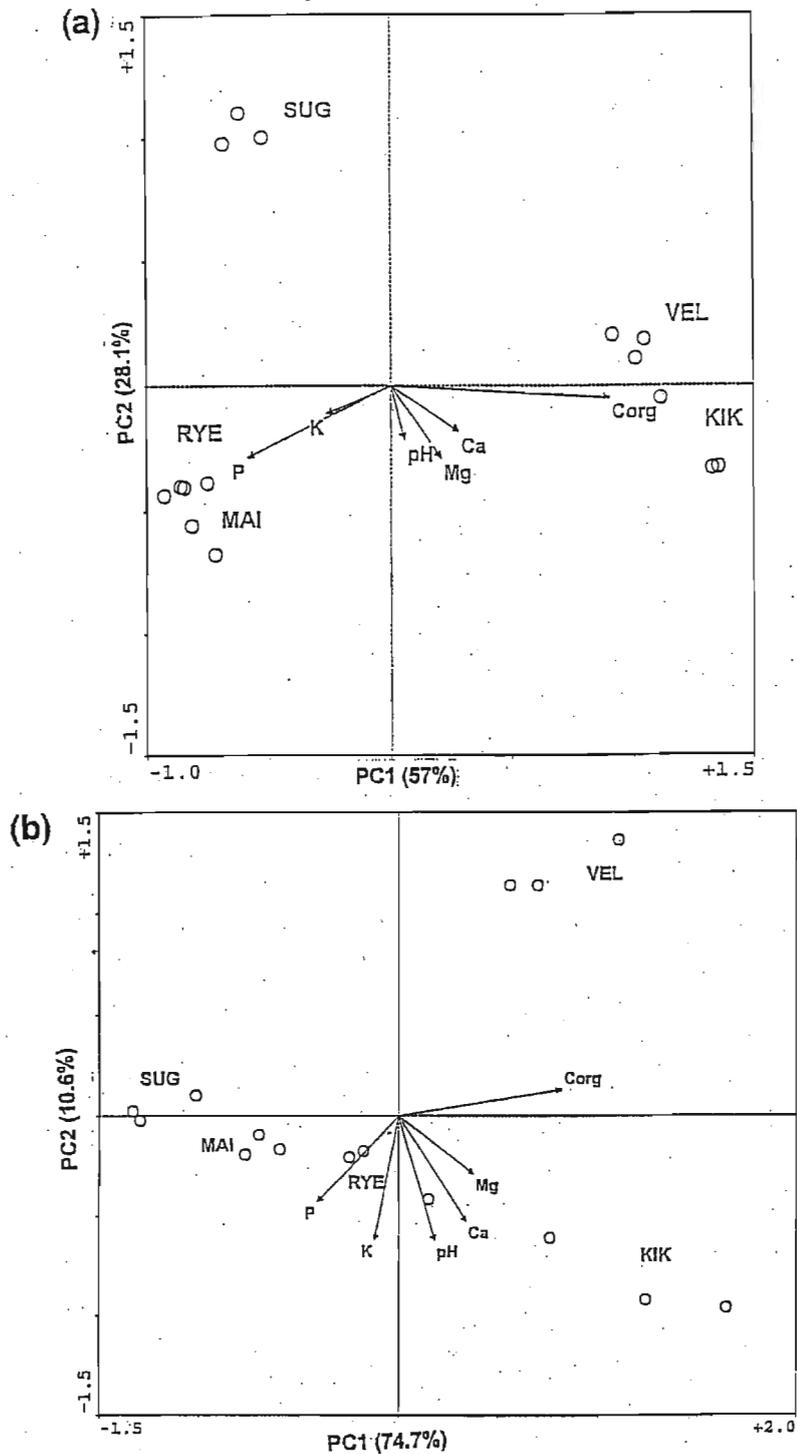
RDA results are displayed on the biplots which depict the relationship between the soil chemical properties and substrate utilization patterns (Figures 8.2 and 8.3). The closer the vector for an individual variable aligns with a principal component axis the more that particular chemical variable can be used to explain the variation in the data along that axis. The general alignment of organic C with the PC 1 axis is evident in all the bi-plots.

Under sugarcane residue management practices, organic C was found to be significantly correlated ( $P < 0.005$ ) with PC1 and accounted for 63.8% and 62.6% of the explained variance in the substrate utilization pattern for Biolog and SIR respectively (Tables 8.1 and 8.2). Phosphorus was also significantly correlated ( $P \leq 0.005$ ) with PC1 and accounted for 17.6% of the explained variance in the SIR data. Since PC2 only accounted for 8.4% and 8.6% of the total variance of the substrate utilization pattern for Biolog and SIR respectively, there was no significant correlation between PC2 and any of the environmental variables.

Under agricultural land management practices, organic C was also found to be significantly correlated ( $P \leq 0.005$ ) with PC1 and accounted for 52% and 63.8% of the explained variance in the substrate utilization pattern for Biolog and SIR respectively (Tables 8.3 and 8.4). Phosphorus was also significantly correlated ( $P \leq 0.005$ ) with PC1 and accounted for 14.3% of the explained variance in the SIR data. PC2 was not significantly correlated with any of the environmental variables.

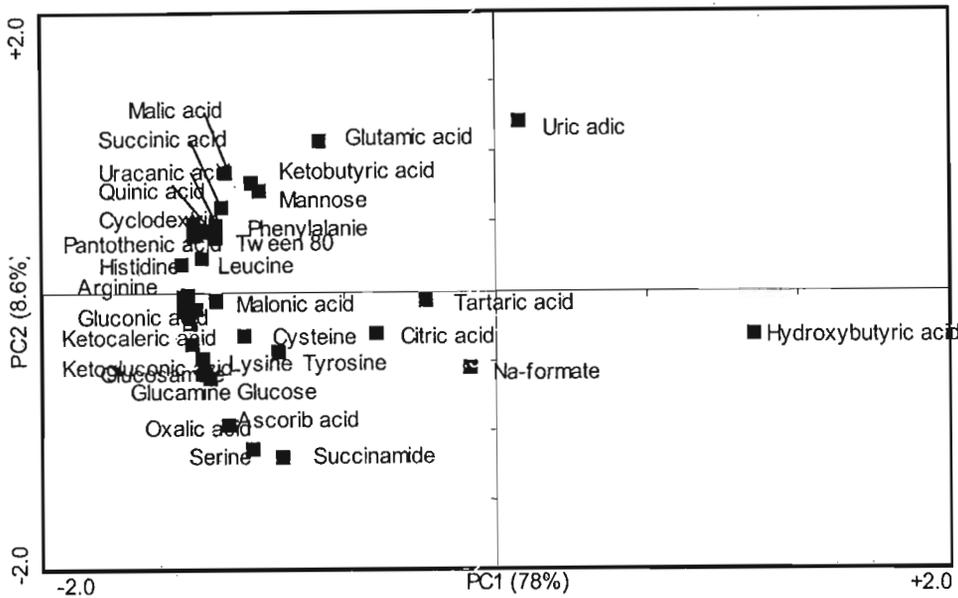


**Figure 8.2:** Redundancy analysis ordination bi-plot of (a) Biolog data and (b) SIR data under sugarcane residue management practices. Analyses represent redundancy analysis of average well color development at 120 h. The percent of the variation explained by the two ordination axes are included on the bi-plot. Six site variable showing correlations with variation along PC1 and PC2 axes shown. Grass = unfertilized grass; T = green cane harvesting with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

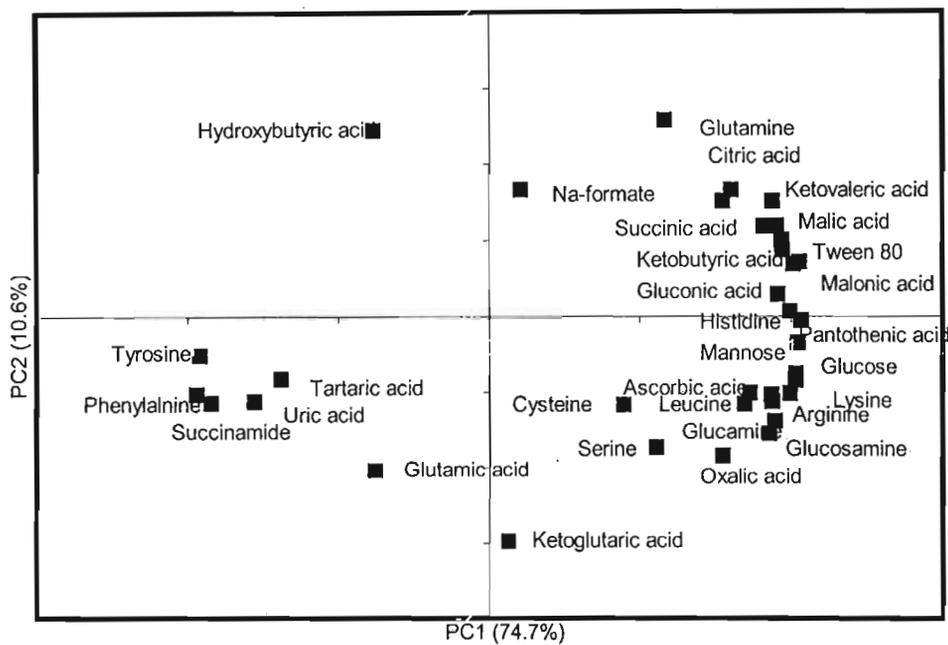


**Figure 8.3:** Redundancy analysis ordination bi-plot of (a) Biolog data and (b) SIR data under agricultural land management practices. Analyses represent redundancy analysis of average well color development at 120 h. The percent of the variation explained by the two ordination axes are included on the bi-plot. Six site variable showing correlations with variation along PC1 and PC2 axes shown. KIK = kikuyu pasture; VEL = undisturbed grassveld; RYE = rye grass; MAI = maize; SUG = burnt sugarcane.

(a)



(b)



**Figure 8.4:** Ordination bi-plot of the loading scores of the individual substrate responses on the first two principal components for (a) sugarcane residue management practices and (b) agricultural land management practices. The percent of the variation explained by the two ordination axes are included on the bi-plot.

**TABLE 8.1:** Redundancy analysis (RDA) of Biolog data showing the effects of sugarcane residue management practices at Mount Edgecombe.

Variable	Eigenvalue	% of explained variance	Monte Carlo test		Environment	Correlations	
			F	P		RDA Ax1	RDA Ax2
Organic C	0.58	63.8	22.21	0.005**		-0.815	-0.118
Mg	0.06	6.6	4.38	0.045ns		-0.345	0.423
K	0.03	3.3	1.84	0.327ns		-0.668	-0.134
P	0.15	16.5	8.96	0.01 ns		0.006	-0.845
Ca	0.05	5.5	3.11	0.285ns		-0.675	-0.315
PH	0.07	7.7	5.71	0.134ns		0.085	0.861
Sum of all canonical eigenvalues	0.909						
Total inertia	0.688						
RDA Ax1	0.71	68.2	3.245	0.005**	0.923		
RDA Ax2	0.085	9.4			0.125		
RDA Ax3	0.022	2.4			-0.065		
RDA Ax4	0.014	1.5			0.074		

**TABLE 8.2:** Redundancy analysis (RDA) of SIR data showing the effects of sugarcane residue management practices at Mount Edgecombe

Variable	Eigenvalue	% of explained variance	Monte Carlo test		Environment	Correlations	
			F	P		RDA Ax1	RDA Ax2
Organic C	0.57	62.6	16.9	0.005**		-0.832	-0.532
Mg	0.06	8.8	2.93	0.04 <sup>NS</sup>		-0.238	0.374
K	0.01	1.1	0.89	0.44 <sup>NS</sup>		-0.79	-0.454
P	0.16	17.6	15.29	0.005**		-0.183	-0.548
Ca	0.08	8.8	3.25	0.055 <sup>NS</sup>		-0.257	-0.082
PH	0.01	1.1	0.67	0.74 <sup>NS</sup>		0.202	0.476
Sum of all canonical eigenvalues	0.91						
Total inertia	0.356						
RDA Ax1	0.78	85.7	6.284	0.005**	0.985		
RDA Ax2	0.086	9.5			0.030		
RDA Ax3	0.043	4.7			-0.043		
RDA Ax4	0.029	3.2			0.002		

**TABLE 8.3:** Redundancy analysis (RDA) of Biolog data showing the effects of agricultural land management practices at Baynesfield Estate.

Variable	Eigenvalue	% of explained variance	Monte Carlo test		Environment	Correlations	
			F	P		RDA Ax1	RDA Ax2
Organic C	0.46	52.0	20.32	0.005**		0.883	-0.195
Mg	0.08	9.0	5.51	0.04ns		0.406	-0.426
K	0.02	2.2	1.34	0.255ns		-0.135	-0.918
P	0.11	12.4	4.62	0.02 ns		-0.448	-0.633
Ca	0.02	2.2	1.27	0.32ns		0.369	-0.774
PH	0.05	5.6	2.5	0.125 ns		0.196	-0.912
Sum of all canonical eigenvalues	0.889		5.27	0.009**			
Total inertia	0.432						
RDA Ax1	0.67	75.4	4.355	0.005**	0.929		
RDA Ax2	0.281	31.6			0.023		
RDA Ax3	0.021	2.4			-0.237		
RDA Ax4	0.18	2.0			-0.057		

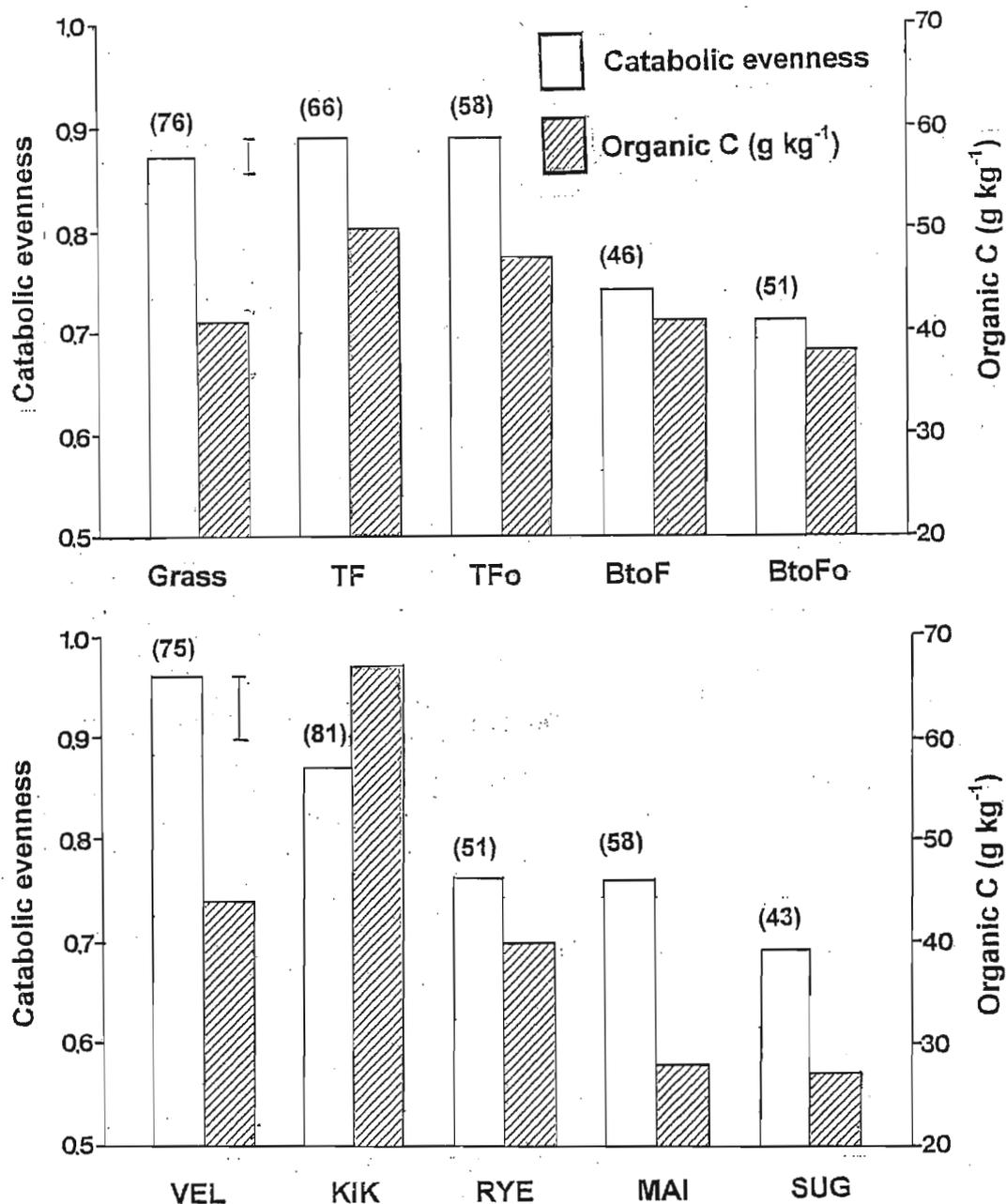
TABLE 8.4: Redundancy analysis (RDA) of SIR data showing the effects of agricultural management practices at Baynesfield Estate.

Variable	Eigenvalue	% of explained variance	Monte Carlo test		Environment	Correlations	
			F	P		RDA Ax1	RDA Ax2
Organic C	0.58	63.8	16.9	0.005**		0.968	0.089
Mg	0.02	2.2	2.93	0.04 <sup>NS</sup>		0.225	-0.509
K	0.04	4.4	0.89	0.44 <sup>NS</sup>		-0.286	-0.202
P	0.13	14.3	15.29	0.005**		-0.632	-0.499
Ca	0.08	8.8	3.25	0.055 <sup>NS</sup>		0.305	-0.327
PH	0.10	11.0	0.67	0.74 <sup>NS</sup>		0.066	-0.581
Sum of all canonical eigenvalues	0.909						
Total inertia	0.473						
RDA Ax1	0.747	82.2	5.442	0.005**	0.986		
RDA Ax2	0.160	11.7			-0.047		
RDA Ax3	0.058	6.4			0.044		
RDA Ax4	0.028	3.1			-0.025		

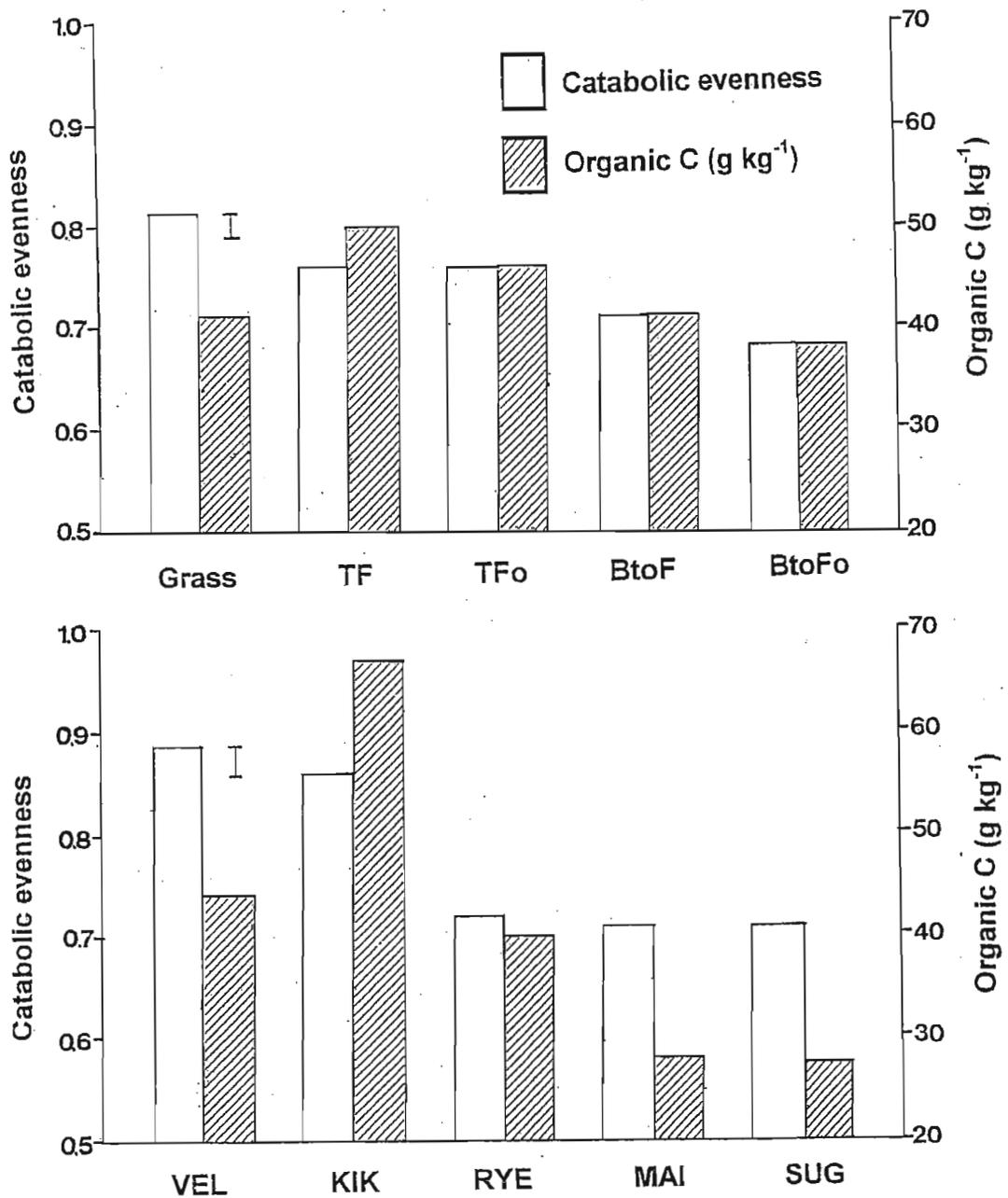
For both Biolog and SIR catabolic evenness (Figures 8.5 and 8.6) followed the same trend. Under sugarcane residue management practices, catabolic evenness followed the order:  $BtoFo \leq BtoF < TFo = TF = \text{Grass}$ . Catabolic evenness under agricultural land management practices followed the order:  $SUG \leq MAI \leq RYE < KIK < VEL$ . Since organic C explained the greatest amount of variance using RDA, the values for organic C are also shown on Figures 8.5 and 8.6. Regression analysis revealed that linear correlation coefficient between catabolic evenness and organic C were 0.79\*\*, 0.93\*\*, 0.94\*\*, and 0.43\*\* for the Biolog residue management, Biolog land management, SIR residue management and SIR land management practices respectively. Nevertheless although trends for catabolic evenness tended to follow those for organic C (Figure 8.5 and 8.6) there were some notable exceptions. That is, in the residue management practices grass had a similar evenness to the TFo and TF treatments yet the latter had substantially greater organic C contents. For the land management practices, veld had a higher evenness than kikuyu pasture yet kikuyu had a markedly higher organic C content.

In Figure 8.5 (Biolog data) values for richness are shown in parenthesis but for the SIR data (Figure 8.6) no such values are shown since for all soils tested richness was the same (i.e. = 36) because there was a catabolic response to all added substrates. For Biolog data richness followed the order:  $BtoF \leq BtoFo < TFo \leq TF < \text{Grass}$  and  $SUG < RYE \leq MAI < VEL < KIK$  for residue management and land management practices respectively.

Values for Shannon's diversity index are presented in Table 8.5. For Biolog data Shannon's diversity index followed the order:  $BtoFo \leq BtoF < \text{Grass} < TFo = TF$  and  $SUG < MAI \leq RYE < KIK < VEL$  for residue management and land management practices respectively.



**Figure 8.5:** Catabolic evenness of Biolog data and organic C content under (a) sugarcane residue management practices and under (b) agricultural land management practices. LSD ( $P < 0.05$ ) shown for treatment differences for evenness. Grass = unfertilized grass; T = green cane harvesting with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied; KIK = kikuyu pasture; VEL = grassveld; RYE = rye grass; MAI = maize; SUG = burnt sugarcane. Values for organic C are taken from chapters 3 and 7.



**Figure 8.6:** Catabolic evenness of SIR data and organic C content under (a) sugarcane residue management practices and under (b) agricultural land management practices. LSD (*P* < 0.05) shown for treatment differences for evenness. Grass = unfertilized grass; T = green cane harvesting with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied; KIK = kikuyu pasture; VEL = grassveld; RYE = rye grass; MAI = maize; SUG = burnt sugarcane. Values for organic C are taken from chapters 3 and 7.

**Table 8.5:** Values for Shannon's diversity index under different sugarcane residue management practices and different agricultural land management practices for the Biolog and SIR methods of measuring catabolic diversity.

Treatment	Biolog	SIR
Residue management		
Grass	3.98	4.02
TF	4.08	4.76
TFo	4.06	4.44
BtoF	3.91	3.88
BtoFo	3.86	3.56
Land management		
Veld	4.03	4.36
Kikuyu pasture	3.94	3.77
Ryegrass pasture	3.79	3.28
Maize	3.51	2.94
Sugarcane	3.48	2.46

## 8.4 Discussion

In general, it is not possible to interpret the functional diversity of microbial communities from estimates of community structure and species composition based on culture - based, molecular or biochemical methods (Pankhurst, 1997). This limitation exists primarily because soil microorganisms can be present in soil in resting or dormant stages which are not functionally active (Degens *et al.*, 2000). For this reason, direct measurements of functional diversity in soil microbial communities (as have been made here) are likely to provide information more relevant to the functioning of soils than the species diversity (Garland and Mills, 1991; Degens and Harris, 1997).

Since alteration of the community structure (i.e. an enrichment effect) occurs during incubation of Biolog plates, it has been pointed out that the substrate utilization profile reflects the resulting community produced, not the original microbial communities from which the samples were obtained (Smalla *et al.*, 1998; Bassio and Scow, 1995). Biolog test plates have been shown to primarily select for a small proportion of the total community largely made up of fast-growing copiotrophic bacteria such as *Pseudomonas*, *Enterobacter*, *Pantoea* and *Salmonella* (Smalla *et al.*, 1998). As a consequence, the colour development observed cannot be interpreted in terms of the number of utilizers or the metabolic potential of the original microbial community (Haack *et al.*, 1995). Other potential drawbacks of the Biolog method include the fact that the C sources tested are not necessarily those found in the soil and they are present in high concentrations (Campbell *et al.*, 1997), certain bacterial strains are unable to oxidize the C sources provided (Zak *et al.*, 1994; Haack *et al.*, 1995), the triphenyl tetrazolium chloride indicator dye can be toxic (Friedel *et al.*, 1994) and the pH of the medium is buffered at 6.5 (Yao *et al.*, 2000).

In order to overcome some of the drawbacks of the Biolog method, Degens and Harris (1997) developed a physiological approach to measuring the catabolic diversity of microbial communities without prior extraction or culturing of the microorganisms. The catabolic response profiles are determined by measuring

short-term utilization of a range of substrates added to soils. The short-term assay time captures the catabolic patterns of active microorganisms, rather than those in resting or dormant states. Thus, the assay provides a measure of the catabolic functional diversity of the soil microbial community. The technique has been shown to be sufficiently sensitive to distinguish changes in catabolic diversity that had developed in field sites over many years due to differences in land use and management (Degens and Vojvodic-Vokovic, 1999; Degens *et al.*, 2000) as well as changes occurring over short periods due to addition of organic substrates (Degens, 1998).

In view of the known drawbacks of the Biolog method it is not surprising that in this study the SIR method separated treatments more effectively than the Biolog method. That is, using the Biolog method, neither ryegrass and maize nor veld and kikuyu pasture were separated effectively and there was little separation due to fertilizer application (Figures 8.3 and 8.4). The greater effectiveness of the SIR method in separating between annually-tilled treatments, permanent grassland sites and fertilized and unfertilized sites is presumably related to the fact that the catabolic response of the whole soil microbial community is measured directly.

Nevertheless, the Biolog method revealed substantial differences in substrate utilization patterns due to land-use and trash management. Similarly, differences in Biolog substrate utilization patterns have been observed in rhizosphere and non-rhizosphere soil (Benizri *et al.*, 2002), the rhizosphere of different plants (Garland, 1996), different land uses (Zak *et al.*, 1994; Bending *et al.*, 2000; Yan *et al.*, 2000), different crop rotations (Lupwayi *et al.*, 2001), flooding and return of crop residues (Bassio and Scow, 1995).

For both the Biolog and SIR methods, the main separation using PCA was on the PC1 axis and this could be related to differences in soil organic C content. The importance of organic C content in influencing catabolic diversity has also been noted by others. Degens *et al.* (2000), for example, noted that functional diversity as estimated by the variation (evenness) in catabolic SIR profiles decreases where

there is a depletion in soil organic C as a result of arable land use. That is, catabolic evenness was greatest in soils under indigenous vegetation and least under arable crops. This was explained by the fact that the more easily decomposable organic C fractions are lost preferentially when land use leads to organic matter decomposition (Bremer *et al.*, 1994; Carter and Stewart, 1996). These labile fractions support a substantial proportion of the heterotrophic microbial biomass and their loss could therefore lead to a disproportionate decline in catabolic functions.

Within agricultural land uses, a similar phenomenon was observed in this study. That is, for both Biolog and SIR data, the preharvest burnt sugarcane had a lower catabolic evenness than the trashed plots and under the different land uses, SIR evenness followed the order: burnt sugarcane = maize < rye grass pasture < kikuyu pasture < grassveld. Similarly, for the Biolog data, kikuyu pasture had a greater evenness than the other land uses.

Nonetheless, the undisturbed sites (grass in the trash management site and veld at the land management site) had similar or greater catabolic evenness than the trashed sugarcane or kikuyu pasture treatments respectively yet the latter had appreciably higher organic C contents. It would, however, be expected that habitats with high and varied production of litter would result in high catabolic diversity (Giller, 1996). That is, heterogeneity of food resources will generally lead to a diverse microbial community. Certainly, all the agricultural land uses are monocultures whilst the undisturbed grassland areas contain a number of different species and therefore will have more heterogeneous litter inputs.

It is generally recognized that soil microbial activity is not distributed homogeneously within the soil, but is concentrated in "hot spots" of biotic activity including around decomposing litter, the rhizosphere and the drilosphere (the portion of the soil influenced by earthworms) (Beare *et al.*, 1995). This patchy distribution allows for structural and functional diversity of the community and for trophic specialization. It has been suggested that the mixing action of tillage will fragment microbial hot spots and microsites into a relatively homogeneous mixture and this will favour a reduction

in structural and functional diversity (Beare *et al.*, 1995; Lupwayi *et al.*, 2001).

Nonetheless, under maize and annual ryegrass, soil is tilled each year whilst under burnt sugarcane it is only tilled every 14 - 20 years. Sugarcane soils, however, had a similar SIR catabolic evenness as maize and a lower Biolog evenness and richness than both maize and ryegrass. A possible explanation for this is that, as noted in chapter 6, the inter-row area under burnt sugarcane is effectively fallow. The sparse inputs of organic residues to the soil presumably lead to a low metabolic diversity under burnt sugarcane.

Reductions in heterotrophic catabolic diversity accompanying a loss of soil organic matter may, in turn, reduce the capacity of the soil to decompose organic matter (Degens, 1998). In general, communities with reduced species diversity will be more variable and less resilient to environmental stress (Giller *et al.*, 1997). Reductions in catabolic evenness could therefore result in a less resilient and more unstable decomposition function (Degens *et al.*, 2000) particularly during disturbances or unexpected periods of stress (e.g. seasonal temperature or moisture extremes). If this is the case then land uses resulting in substantial losses of organic matter such as maize, annual ryegrass and preharvest burnt sugarcane will generate soil less resilient to stress and disturbance. By contrast, the substantial increases in catabolic evenness and richness induced by a change from preharvest burning to green cane harvesting may increase the resilience of the sugarcane agro-ecosystem.

## 8.5 Conclusions

Both the Biolog and SIR methods detected substantial differences in the ability of microbial communities to metabolize different C substrates as affected by land use and trash management. The SIR method, however, was more successful at separating treatments within broadly similar land uses (e.g. between annually tilled soils or between fertilized and unfertilized soils). It therefore seems a better alternative particularly if the aim is to investigate the effects of short-term changes in soil management of metabolic diversity. In addition, it overcomes many of the

shortcomings of the Biolog method because substrates are added directly to the soil.

The catabolic evenness and richness of soil communities under preharvest burnt sugarcane was low compared to that under undisturbed vegetation or permanent pasture. Conversion from burning to green cane harvesting greatly increased catabolic evenness and richness and therefore presumably also tended to increase the resilience of the soil to stress and disturbance particularly in relation to decomposition functions.

## CHAPTER NINE

### 9.1 General conclusions

The long-term production of sugarcane has been found to result in a phenomenon called “yield decline”. Loss of soil productivity is thought to be a contributing factor to yield decline. As a result, research has focused on losses of soil organic matter and changes in soil organic matter quality. The loss of soil organic matter is not only related to repeated tillage but also the effect of preharvest burning of sugarcane fields.

The long-term trial (59 years), BT1 at Mount Edgecombe, South Africa, provided an ideal opportunity to study the effect of crop residue management practices on soil organic matter quality. The experimental treatments consisted of: (i) green cane harvested with retention of a trash blanket (100% cover on the soil surface) (T), (ii) burnt with tops left scattered on the soil surface (67 % cover on the soil surface) (Bt) and (iii) burnt with tops removed (Bto). The treatments were either (a) unfertilized (Fo) or (b) fertilized annually with 140 kg N ha<sup>-1</sup>, 20 kg P ha<sup>-1</sup> and 140 kg K ha<sup>-1</sup> (F). The effects of the contrasting crop residue management practices on the soil nutrient availability, soil physical conditions, labile organic matter fractions and the size, activity and diversity of the soil microbial biomass were examined. An evaluation of these properties across a transect of the field was also carried out to examine the effect of the sugarcane crop on these fractions.

The return of large amounts of residues to the soil surface as a trash blanket significantly increased the soil organic C content in the surface 10 cm compared to that of the burnt treatments. This accumulation of organic matter was accompanied by recycling and accumulation of essential plant nutrients (N, P and K) in both organic and inorganic forms. Trash retention in combination with fertilizer application resulted in an additional accumulation of nutrient reserves. Fertilizer application induced increases in exchangeable P and K, non-exchangeable K and also some accumulation in soil organic P. This suggested

that the fertilizer recommendations under long-term green cane harvesting should be reduced compared to those made for preharvest burnt cane.

Nitrogen fertilizer application ( $140 \text{ kg N ha}^{-1}\text{yr}^{-1}$ ) and to a lesser degree, organic matter mineralization resulted in soil acidification to a depth of 30 cm. As a result of acidification there was a resultant increase in exchangeable acidity and exchangeable Al. However, this is not thought to be a problem for sugarcane since it is highly tolerant of acidic conditions. The agronomic disadvantage of soil acidification is related to the displacement of the base cations and their leaching through the lower soil layers and the decrease in ECEC. Progressive acidification down the soil profile is difficult to ameliorate by surface liming. Thus, regular monitoring of soil pH and regular lime applications are desirable.

Aggregate stability increased as a direct result of the increased microbial biomass C and other labile organic matter fractions that are associated with the aggregation process under green cane harvesting. Fertilizer application induced lower aggregate stability than unfertilised conditions. This was attributed to an increase in the proportion of exchangeable cations present in monovalent form (due to applications of fertilizer K and leaching of Ca and Mg) favouring dispersion and an decline in aggregate stability. Such results demonstrate the interaction between soil chemical and soil physical properties.

Even though the total organic matter content only increased in the surface 10 cm in response to green cane harvesting, the labile soil organic matter fractions (e.g.  $\text{K}_2\text{SO}_4$  extractable C,  $\text{KMnO}_4$  extractable C, light fraction, microbial biomass C and N and readily mineralizable C and N) increased to a depth of 30 cm. This confirms other research indicating that these labile fractions are more sensitive indicators of soil management changes than organic C or total N content.

The size and the activity of the microbial biomass C were increased by the retention of trash under green cane harvesting. The increase in labile C to a depth of 30 cm resulted in a consequent increase in the size and activity of the microbial biomass and in enzyme activity to that depth. The increased cycling of

C, N, S and P induced by trash retention was reflected in the increased activity of enzymes involved in mineralization and cycling of these elements in the soil.

Fertilizer application had variable effects on microbial activity as a result of an interaction between fertilizer - induced increases in organic C, content and soil nutrient status versus fertilizer-induced soil acidification. The variable results suggested that measurements of the size and activity of the microbial community and activity of key enzymes are all required in order to understand the effects of various management practices on the functions of the soil microbial community.

New techniques were used to examine the soil microbial and functional diversity since the composition and diversity of the microbial community are increasingly being recognised as important ecological indicators of sustainable soil management practices. The accumulation of soil organic matter did not only increase the size of the microbial biomass, but also changed the composition and functioning of the microbial community. This is a direct result on the increased C supply and change in soil physical conditions. It was also found the soil community structure is dependent on the type of organic matter returned, e.g. increased labile C pools selectively stimulate bacterial communities and surface applied leaves and tops stimulate fungal growth. It was found that the microbial diversity and potential functioning of the microbial community was indeed improved under green cane harvesting when compared to burning.

Since sugarcane is a row crop and can remain in the ground for up to 10 years it was postulated that there would be a gradient of organic matter content from the row to the inter-row space because the root zone is concentrated in the row below the cane stems. Under burning, where the inter-row space is fallow, there was, indeed, a large gradient in organic matter content, soil microbial activity and aggregate stability across the field. Conversion to green cane harvesting resulted in the redistribution of root mass in the surface soil towards the inter-row space below the trash mulch. As a result of the large organic matter inputs via the trash itself, and in the form of root turnover, the organic matter status, size and activity

of the microbial community and aggregate stability are all appreciably increased in the inter-row space under green cane harvesting.

Green cane harvesting should be considered as an alternative management practices that would maintain and improve soil quality compared to that of preharvest burning. The conversion to green cane harvesting is an environmentally desirably change. It would reduce smoke and ash emissions and nutrient losses by volatilization during burning. It also causes an increase in soil organic matter content, an improvement in structure and soils nutrient status and an increase in the size, activity and taxonomic and functional diversity of the soil microbial community. Whilst the change will result in an increase in the cost of cane harvesting (since the bulk of the South African crop is had-harvested), this needs to be balanced against an improvement in the long-term sustainability of sugarcane monoculture. A reduced fertilizer bill, reduced erosion, reduced atmosphere pollution, better root growth in the surface soil, and a larger more active and more resilient soil microbial community are all benefits that need to be considered.

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Management treatment	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Organic P (mg kg <sup>-1</sup> )	pH (water)	Exchangeable cations (mmol <sub>e</sub> kg <sup>-1</sup> )				Exchangeable acidity	ECEC	Non-exchangeable K (mg kg <sup>-1</sup> )	Extractable P (mg kg <sup>-1</sup> )
					K	Ca	Mg	Na				
TF	55 d <sup>1</sup>	36 c	348 b	4.9 c	7.1 d	80 ab	50 b	3.1 b	5.5 b	146 cb	17.2 c	35 a
TFo	52 d	30 b	312 ab	5.7 a	4.0 b	121 c	62 c	3.0 ab	0.17 a	190 d	9.2 b	12 b
BtF	45 c	25 ab	308 a	5.2 b	4.9 c	69 a	40 a	2.7 ab	5.0 b	122 ab	11.8 bc	34 a
BtFo	40 ab	23 a	271 a	5.9 a	2.8 a	120 b	61 c	3.2 b	0.17 a	169 dc	5.2 a	9.5 b
BtoF	43 bc	23 a	304 a	5.1 bc	4.3 bc	66 a	33 a	2.6 a	5.2 b	111 a	7.6 ab	31 a
BtoFo	39 a	212 a	268 a	5.8 a	2.4 a	95 b	61 c	3.0 ab	0.13 a	162 c	4.1 a	7.6 b
Grass	44	25	324	5.7	4.4	97	56	3.5	0.1	161	6.1	8.0
Fertilizer effect <sup>2</sup>	*	*	*	*	*	*	*	ns	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.2:** Mean organic C, Total N, organic P, pH<sub>(water)</sub>, Exchangeable cations, exchangeable acidity, ECEC, non-exchangeable K and extractable P in the soil of the seven treatments in the 2.5 - 5 cm depth.

Management treatment	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Organic P (mg kg <sup>-1</sup> )	pH (water)	Exchangeable cations (mmol <sub>c</sub> kg <sup>-1</sup> )				Exchangeable acidity	ECEC	Non-exchangeable K (mg kg <sup>-1</sup> )	Extractable P (mg kg <sup>-1</sup> )
					K	Ca	Mg	Na				
TF	45 b 1	28 c	321 b	4.9 a	5.8 e	81 ab	48 b	2.2 a	4.8 b	142 cb	15.1 d	25 b
TFo	42 ab	26 bc	268 a	5.7 c	3.6 c	126 d	59 c	3.0 b	0.13 a	192 e	7.3 c	6.6 a
BtF	41 ab	22 ab	296 ab	5.2 b	4.4 d	72 a	42 ab	2.1 a	4.5 b	125 ab	6.8 c	20 b
BtFo	38 a	21 a	249 a	5.9 d	2.1 ab	105 c	57 c	2.4 a	0.13 a	166 d	3.5 ab	6.1 a
BtoF	40 a	22 ab	296 ab	5.1 ab	2.5 b	65 a	40 a	2.5 ab	4.3 b	114 a	5.4 b	24 b
BtoFo	37 a	20 a	264 a	5.8 d	1.6 a	91 bc	63 c	3.9 c	0.13 a	160 cd	2.1 a	6.3 a
Grass	39	23	278	5.7	2.7	92	66	2.7	0.1	164	3.6	9.0
Fertilizer effect <sup>2</sup>	*	*	*	*	*	*	*	*	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.3:** Mean organic C, Total N, organic P, pH<sub>(water)</sub>, Exchangeable cations, exchangeable acidity, ECEC, non-exchangeable K and extractable P in the soil of the seven treatments in the 5 - 10 cm depth.

Management treatment	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Organic P (mg kg <sup>-1</sup> )	pH (water)	Exchangeable cations (mmol <sub>e</sub> kg <sup>-1</sup> )				Exchangeable acidity	ECEC	Non-exchangeable K (mg kg <sup>-1</sup> )	Extractable P (mg kg <sup>-1</sup> )
					K	Ca	Mg	Na				
TF	39 c 1	25 d	260 a	5.2 c	3.4 d	92 b	44 a	2 a	3.9 c	145 b	15 d	14 c
TFo	37 ab	22 c	251 a	5.8 a	1.9 b	106 c	54 b	2.6 b	0.1 a	165 c	6.9 c	6.4 a
BtF	38 bc	20 bc	254 a	5.4 cb	2.7 c	94 b	40 a	2 a	1.6 b	140 b	3.1 b	13 bc
BtFo	37 ab	20 bc	249 a	5.9 a	1.4 a	100 c	53 b	3 bc	0.13 a	158 c	1.0 a	5.6 a
BtoF	36 a	17 a	258 a	5.5 b	1.8 b	80 a	41 a	3 bc	1.5 b	127 a	2.6 ab	10 b
BtoFo	34 a	18 ab	260 a	5.9 a	1.1 a	95 b	57 b	3.4 c	0.1 a	157 c	2.0 a	5.5 a
Grass	34	20	269	5.9	2.5	110	66	2.7	0.1	159	1.6	8.3
Fertilizer effect <sup>2</sup>	**	ns	ns	*	*	*	*	*	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.4:**

Mean organic C, Total N, organic P, pH<sub>(water)</sub>, Exchangeable cations, exchangeable acidity, ECEC, non-exchangeable K and extractable P in the soil of the seven treatments in the 10 - 20 cm depth.

Management treatment	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Organic P (mg kg <sup>-1</sup> )	pH (water)	Exchangeable cations (mmol <sub>e</sub> kg <sup>-1</sup> )				Exchangeable acidity	ECEC	Non-exchangeable K (mg kg <sup>-1</sup> )	Extractable P (mg kg <sup>-1</sup> )
					K	Ca	Mg	Na				
TF	37 a	23 b	261 a	5.3 a	2.3 c	90 a	40 a	2.4 a	2.4 b	137 a	9.7 c	9.7 c
TFo	37 a	22 b	251 a	5.9 bc	1.7 b	108 b	55 b	3.0 a	0.1 a	168 b	4.1 b	6.6 a
BtF	36 a	22 b	260 a	5.7 b	1.6 b	92 a	36 a	2.5 a	0.2 a	133 a	0.9 a	7.9 b
BtFo	37 a	21 b	261 a	6.0 c	1.1 a	104 ab	58 b	3.1 ab	0.1 a	166 b	0.8 a	6.4 a
BtoF	36 a	17 a	250 a	5.7 b	1.4 ab	94 a	44 a	3.4 b	0.3 a	143 a	0.9 a	7.4 ab
BtoFo	37 a	17 a	258 a	6.0 c	1.1 ab	101 a	61 b	3.7 b	0.1 a	167 b	1.5 a	6.5 a
Grass	33	14	260	6.0	1.2	102	56	3.3	0.1	163	1.0	5.1
Fertilizer effect <sup>2</sup>	ns	Ns	ns	*	*	*	*	ns	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.5:**

Mean organic C, Total N, organic P, pH<sub>(water)</sub>, Exchangeable cations, exchangeable acidity, ECEC, non-exchangeable K and extractable P in the soil of the seven treatments in the 20 - 30 cm depth.

Management treatment	Organic C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Organic P (mg kg <sup>-1</sup> )	pH <sub>(water)</sub>	Exchangeable cations (mmol <sub>c</sub> kg <sup>-1</sup> )				Exchangeable acidity	ECEC	Non-exchangeable K (mg kg <sup>-1</sup> )	Extractable P (mg kg <sup>-1</sup> )
					K	Ca	Mg	Na				
TF	37 a 1	21 b	244 b	5.4 a	2.0 c	88 a	38 a	2.2 a	1.5 b	132 a	6.5 c	8.7 b
TFo	36 a	20 ab	254 b	5.9 b	1.6 b	102 b	58 b	3.0 b	0.1 a	165 b	3.7 b	5.9 a
BtF	37 a	20 ab	226 a	5.7 b	1.2 a	90 a	38 a	2.5 ab	0.1 a	131 a	0.5 a	6.7 a
BtFo	37 a	20 ab	239 ab	6.0 b	1.0 a	104 b	59 b	3.1 bc	0.1 a	167 b	0.6 a	6.4 a
BtoF	36 a	18 a	249 b	5.8 b	1.1 a	95 a	42 a	3.2 c	0.2 a	141 a	1.1 a	5.9 a
BtoFo	37 a	18 a	222 a	6.0 b	1.0 a	98 b	64 b	4.0 d	0.1 a	167 b	1.0 a	6.0 a
Grass	33	13	219	6.0	1.2	95	58	0.1	0.1	158	0.8	3.1
Fertilizer effect <sup>2</sup>	ns	Ns	ns	*	*	*	*	ns	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.6:** Mean quantities of NaHCO<sub>3</sub> - Pi, NaOH(I) - Pi, HCl - Pi and NaOH(II) - Pi and NaHCO<sub>3</sub> - Po, NaOH(I) - Po, NaOH(II) - Po, exchangeable Al, CuCl<sub>2</sub> - , NH<sub>4</sub>OAc - and Oxalate extractable Al in the 0 - 2.5 cm depth.

Management treatment	Pi (mg kg <sup>-1</sup> )				Po (mg kg <sup>-1</sup> )			Extractable Al (mmolc kg <sup>-1</sup> )			
	NaHCO <sub>3</sub>	NaOH(I)	HCl	NaOH(II)	NaHCO <sub>3</sub>	NaOH(I)	NaOH(II)	Potassium chloride	Copper chloride	Ammonium acetate	Ammonium oxalate
TF	19 b <sup>1</sup>	7.8 b	1.1 a	111 b	17 b	73 b	40 b	4.8 b	14 b	5.5 a	51 a
TFo	2.7 a	2.6 a	0.8 a	61 a	9.2 a	46 a	41 b	0.2 a	8.1 a	3.2 a	37 a
BtoF	17 b	7.4 b	0.8 a	99 b	11 ab	40 a	22 a	4.2 b	13 b	4.6 a	36 a
BtoFo	2.5 a	2.6 a	0.9 a	54 a	6.9 a	33 a	21 a	0.1 a	7.9 a	2.9 a	35 a
Grass	3.4	3.1	1.0	61	12	41	26	0.1	10	4.2	27
Fertilizer effect <sup>2</sup>	*	*	ns	*	*	*	ns	*	*	*	ns

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.7:** Mean quantities of NaHCO<sub>3</sub> - Pi, NaOH(I) - Pi, HCl - Pi and NaOH(II) - Pi and NaHCO<sub>3</sub> - Po, NaOH(I) - Po, NaOH(II) - Po, exchangeable Al, CuCl<sub>2</sub> - , NH<sub>4</sub>OAc - and Oxalate extractable Al in the 2.5 - 5 cm depth.

Management treatment	Pi (mg kg <sup>-1</sup> )				Po (mg kg <sup>-1</sup> )			Extractable Al (mmolc kg <sup>-1</sup> )			
	NaHCO <sub>3</sub>	NaOH(I)	HCl	NaOH(II)	NaHCO <sub>3</sub>	NaOH(I)	NaOH(II)	Potassium chloride	Copper chloride	Ammonium acetate	Ammonium oxalate
TF	9.0 b1	5.8 b	1.0 a	90 a	11 a	73 b	33 a	5.2 b	16 a	5.9 a	58 a
TFo	1.8 a	2.4 a	0.8 a	48 a	8.4 a	46 a	30 a	0.2 a	8.6 a	4.0 a	38 a
BtoF	8.6 b	5.6 b	0.9 a	92 a	8.4 a	40 a	24 a	4.6 b	15 a	5.1 a	34 a
BtoFo	2.0 a	2.3 a	0.9 a	46 a	7.9 a	33 a	21 a	0.1 a	8.4 a	3.2 a	33 a
Grass	2.9	2.6	1.0	56	8.1	41	23	0.1	12	4.6	32
Fertilizer effect <sup>2</sup>	*	*	ns	*	**	*	**	*	**	**	ns

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 3.8:** Mean quantities of NaHCO<sub>3</sub> - Pi, NaOH(I) - Pi, HCl - Pi and NaOH(II) - Pi and NaHCO<sub>3</sub> - Po, NaOH(I) - Po, NaOH(II) - Po, exchangeable Al, CuCl<sub>2</sub> - , NH<sub>4</sub>OAc - and Oxalate extractable Al in the 5 - 10 cm depth.

Management treatment	Pi (mg kg <sup>-1</sup> )				Po (mg kg <sup>-1</sup> )			Extractable Al (mmolc kg <sup>-1</sup> )			
	NaHCO <sub>3</sub>	NaOH(I)	HCl	NaOH(II)	NaHCO <sub>3</sub>	NaOH(I)	NaOH(II)	Potassium chloride	Copper chloride	Ammonium acetate	Ammonium oxalate
TF	4.5 b <sup>1</sup>	3.9 a	1.0 a	74 ab	11 b	58 b	30 ab	3.9 c	13 a	6.0 a	41 a
TFo	1.7 a	2.3 a	0.8 a	63 a	8.1 a	38 a	35 b	0.5 ab	7.3 a	6.1 a	34 a
BtoF	4.6 b	4.3 a	0.9 a	82 b	9.4 ab	30 a	26 a	1.5 b	11 a	5.8 a	33 a
BtoFo	1.5 a	2.2 a	0.8 a	47 a	7.9 a	29 a	23 a	0.1 a	7.6 a	3.4 a	30 a
Grass	2.6	2.5	0.9	54	8.3	34	23	0.1	7.3	4.0	32
Fertilizer effect <sup>2</sup>	*	**	ns	*	*	**	ns	*	**	**	ns

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.1:

Quantities of microbial biomass C, N, mineralizable C, N and K<sub>2</sub>SO<sub>4</sub> - extractable C in the 0 - 2.5 cm depth.

Management treatment	Microbial biomass C (mg kg <sup>-1</sup> )	Microbial biomass N (mg kg <sup>-1</sup> )	Mineralizable C (mg kg <sup>-1</sup> )	Mineralizable N (mg kg <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> - extractable C (mg kg <sup>-1</sup> )
TF	475 e <sup>1</sup>	74 d	455 c	74 d	329 d
TFo	384 d	52 c	455 c	57 c	289 bc
BtF	243 c	36 b	330 ab	46 b	230 b
BtFo	219 bc	29 b	304 ab	36 a	167 a
BtoF	195 b	28 b	275 a	39 a	161 a
BtoFo	152 a	19 a	271 a	34 a	158 a
Grass	316	44	378	26	430
Fertilizer effect <sup>2</sup>	*	*	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

**APPENDIX 4.2:** Quantities of microbial biomass C, N, mineralizable C, N and K<sub>2</sub>SO<sub>4</sub> - extractable C in the 2.5 - 5 cm depth.

Management treatment	Microbial biomass C (mg kg <sup>-1</sup> )	Microbial biomass N (mg kg <sup>-1</sup> )	Mineralizable C (mg kg <sup>-1</sup> )	Mineralizable N (mg kg <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> - extractable C (mg kg <sup>-1</sup> )
TF	398 f <sup>1</sup>	64 e	368 d	66 c	234 c
TFo	301 e	44 d	309 c	47 b	162 ab
BtF	180 d	29 c	234 b	42 b	186 b
BtFo	154 c	21 b	215 ab	32 a	150 a
BtoF	138 b	20 b	208 ab	28 a	154 a
BtoFo	96 a	12 a	199 a	25 a	149 a
Grass	234	32	281	26	168
Fertilizer effect <sup>2</sup>	*	*	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.3: Quantities of microbial biomass C, N, mineralizable C, N and K<sub>2</sub>SO<sub>4</sub> - extractable C in the 5 - 10 cm depth.

Management treatment	Microbial biomass C (mg kg <sup>-1</sup> )	Microbial biomass N (mg kg <sup>-1</sup> )	Mineralizable C (mg kg <sup>-1</sup> )	Mineralizable N (mg kg <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> - extractable C (mg kg <sup>-1</sup> )
TF	302 e <sup>1</sup>	44 e	263 c	59 c	196 b
TFo	236 d	33 d	246 c	40 b	134 a
BtF	150 c	22 c	211 b	36 b	143 a
BtFo	139 c	19 bc	191 b	27 a	134 a
BtoF	106 b	15 ab	150 a	25 a	136 a
BtoFo	81 a	8.8 a	144 a	24 a	140 a
Grass	141	18	191	23	148
Fertilizer effect <sup>2</sup>	*	*	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.4: Quantities of microbial biomass C, N, mineralizable C, N and K<sub>2</sub>SO<sub>4</sub> - extractable C in the 10 - 20 cm depth.

Management treatment	Microbial biomass C (mg kg <sup>-1</sup> )	Microbial biomass N (mg kg <sup>-1</sup> )	Mineralizable C (mg kg <sup>-1</sup> )	Mineralizable N (mg kg <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> - extractable C (mg kg <sup>-1</sup> )
TF	246 f <sup>1</sup>	36 e	215 c	49 d	167
TFo	189 e	24 d	209 c	33 c	128
BtF	127 d	18 c	161 b	30 c	136
BtFo	101 c	14 bc	138 ab	22 b	140
BtoF	84 b	11 ab	127 a	20 ab	128
BtoFo	59 a	7.4 a	131 ab	18 a	125
Grass	95	13	133	9	121
Fertilizer effect <sup>2</sup>	*	*	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.5: Quantities of microbial biomass C, N, mineralizable C, N and K<sub>2</sub>SO<sub>4</sub> - extractable C in the 20 - 30 cm depth.

Management treatment	Microbial biomass C (mg kg <sup>-1</sup> )	Microbial biomass N (mg kg <sup>-1</sup> )	Mineralizable C (mg kg <sup>-1</sup> )	Mineralizable N (mg kg <sup>-1</sup> )	K <sub>2</sub> SO <sub>4</sub> - extractable C (mg kg <sup>-1</sup> )
TF	130 d <sup>1</sup>	19 e	160 c	42 c	149
TFo	124 d	16 d	148 c	26 b	126
BtF	91 c	13 c	128 bc	22 ab	101
BtFo	70 bc	9.1 b	119 b	18 a	93
BtoF	61 ab	8.3 b	90 a	18 a	74
BtoFo	44 a	5.0 a	81 a	16 a	68
Grass	54	7.3	84	10	39
Fertilizer effect <sup>2</sup>	*	*	ns	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.6:

Mean quantities of Light fraction dry matter, C and N concentration, C and N held in Light fraction and C/N ratio in the 0 - 2.5 cm depth. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Light fraction dry matter (g kg soil <sup>-1</sup> )	Light fraction C (mg C kg soil <sup>-1</sup> )	Light fraction C concentration (g kg LF <sup>-1</sup> )	Light fraction N (mg N kg soil <sup>-1</sup> )	Light fraction N concentration (g kg LF <sup>-1</sup> )	Light fraction C/N ratio
TF	7.73 c <sup>1</sup>	1739 b	226 ab	66 c	8.6 bc	26 a
TFo	8.9 d	2172 c	245 bc	64 c	7.2 ab	34 c
BtF	7.2 bc	1879 b	261 c	66 c	9.1 c	29 ab
BtFo	8.15 cd	1693 b	210 a	53 bc	6.5 a	32 bc
BtoF	6.0 ab	1308 a	219 ab	46 ab	7.7 abc	28 ab
BtoFo	5.0 a	1032 a	206 a	35 a	7.0 a	29 ab
Grass	12	2335	195	112	9.3	21
Fertilizer effect <sup>2</sup>	**	ns	**	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.7:

Mean quantities of Light fraction dry matter, C and N concentration, C and N held in Light fraction and C/N ratio in the 2.5 - 5 cm depth. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Light fraction <u>dry matter</u> (g kg soil <sup>-1</sup> )	<u>Light fraction C</u> (mg C kg soil <sup>-1</sup> )	Light fraction C <u>concentration</u> (g kg LF <sup>-1</sup> )	<u>Light fraction N</u> (mg N kg soil <sup>-1</sup> )	Light fraction N <u>concentration</u> (g kg LF <sup>-1</sup> )	Light fraction C/N ratio
TF	7.0 cd <sup>1</sup>	1400 c	200 a	61 d	8.7 c	0.45833
TFo	8.4 d	1924 d	229 b	57 d	6.8 a	34 d
BtF	6.3 bc	1493 c	237 b	48 c	7.6 b	31 c
BtFo	7.8 d	1474 c	189 a	54 c	6.9 a	27 b
BtoF	5.4 b	1075 b	199 a	38 b	7.0 a	28 b
BtoFo	3.8 a	722 a	190 a	25 a	6.6 a	29 b
Grass	11.6	2181	188	109	9.4	20
Fertilizer effect <sup>2</sup>	**	ns	Ns	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.8:

Mean quantities of Light fraction dry matter, C and N concentration, C and N held in Light fraction and C/N ratio in the 5 - 10 cm depth. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Light fraction dry matter (g kg soil <sup>-1</sup> )	Light fraction C (mg kg soil <sup>-1</sup> )	Light fraction C concentration (g kg LF <sup>-1</sup> )	Light fraction N (mg N kg soil <sup>-1</sup> )	Light fraction N concentration (g kg LF <sup>-1</sup> )	Light fraction C/N ratio
TF	6.7 de <sup>1</sup>	1280 dc	191 c	53 e	7.9 c	24 ab
TFo	7.4 e	1613 d	218 d	44 d	6.0 a	36 d
BtF	4.8 c	1061 bc	221 d	33 bc	6.9 b	32 c
BtFo	6.1 d	970 bc	159 b	37 c	6.1 a	26 b
BtoF	3.9 b	639 ab	164 b	27 b	7.0 b	0.45833
BtoFo	2.6 a	359 a	138 a	0.125	5.9 a	0.45833
Grass	11	1859	169	79	7.2	23
Fertilizer effect <sup>2</sup>	**	ns	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

## APPENDIX 4.9:

Mean quantities of Light fraction dry matter, C and N concentration, C and N held in Light fraction and C/N ratio in the 10 - 20 cm depth. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatment	Light fraction dry matter (g kg soil <sup>-1</sup> )	Light fraction C (mg C kg soil <sup>-1</sup> )	Light fraction C concentration (g kg LF <sup>-1</sup> )	Light fraction N (mg N kg soil <sup>-1</sup> )	Light fraction N concentration (g kg LF <sup>-1</sup> )	Light fraction C/N ratio
TF	4.9 e <sup>1</sup>	838 d	171 c	33 d	6.8 d	25 a
TFo	4.3 d	774 d	180 cd	23 c	5.3 b	34 c
BtF	2.5 b	465 c	186 d	15 b	6.0 c	31 b
BtFo	3.2 c	435 c	136 b	17 bc	5.2 b	26 a
BtoF	2.2 b	306 b	139 b	12 b	5.5 bc	25 a
BtoFo	1.1 a	125 a	114 a	4.7 a	4.3 a	27 a
Grass	5.3	588	111	27	5.1	22
Fertilizer effect <sup>2</sup>	*	*	*	*	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 4.10: Mean quantities of Light fraction dry matter, C and N concentration, C and N held in Light fraction and C/N ratio in the 20 - 30 cm depth. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Light fraction dry matter (g kg soil <sup>-1</sup> )	Light fraction C (mg C kg soil <sup>-1</sup> )	Light fraction C concentration (g kg LF <sup>-1</sup> )	Light fraction N (mg N kg soil <sup>-1</sup> )	Light fraction N concentration (g kg LF <sup>-1</sup> )	Light fraction C/N ratio
TF	2.7 b	405 d	150 c	15 b	5.4 d	27 a
TFo	2.4 b	374 d	156 c	9.8 ab	4.1 bc	38 b
BtF	1.1 a	166 c	151 c	5.8 a	5.3 c	28 a
BtFo	1.3 a	169 c	130 b	5.2 a	4.0 b	33 ab
BtoF	0.98 a	117 b	119 b	3.9 a	4.0 bc	30 a
BtoFo	0.95 a	88 a	93 a	2.9 a	3.1 a	30 a
Grass	2.4	194	81	9.6	4	20
Fertilizer effect <sup>2</sup>	ns	*	*	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.1:

Mean quantities of basal respiration, metabolic quotient ( $qCO_2$ ), FDA hydrolysis, dehydrogenase and arginine ammonification rate in the 0 - 2.5 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Basal respiration: ( $\mu g CO_2-Cg^{-1}day^{-1}$ )	Metabolic quotient ( $\mu g CO_2-C mg^{-1} biomass day^{-1}$ )	FDA hydrolysis	Dehydrogenase	Arginine
			rate	activity	ammonification rate
			( $\mu mol product released g^{-1} h^{-1}$ )		
TF	17.7 d <sup>1</sup>	3.7 d	0.8 c	0.048 c	0.72 d
TFo	17.7 d	4.6 c	0.73 d	0.085 e	0.82 e
BtF	12.8 c	5.3 b	0.59 c	0.042 b	0.41 b
BtFo	11.8 b	5.4 b	0.59 c	0.067 d	0.62 c
BtoF	10.7 a	5.5 b	0.54 ab	0.028 a	0.24 a
BtoFo	10.5 a	6.9 a	0.45 a	0.037 b	0.29 a
Grass	14.7	4.6	0.8	0.044	0.48
Fertilizer effect <sup>2</sup>	ns	*	*	*	**

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.2:

Mean quantities of basal respiration, metabolic quotient (qCO<sub>2</sub>), FDA hydrolysis, dehydrogenase and arginine ammonification rate in the 2.5 - 5 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Basal respiration ( $\mu\text{g CO}_2\text{-Cg}^{-1}\text{day}^{-1}$ )	Metabolic quotient ( $\mu\text{g CO}_2\text{-C mg}^{-1}\text{biomass day}^{-1}$ )	FDA hydrolysis	Dehydrogenase	Arginine
			rate	activity	ammonification rate
			( $\mu\text{ mol product released g}^{-1}\text{ h}^{-1}$ )		
TF	14 e <sup>1</sup>	3.5 a	0.75 c	0.041 b	0.67 d
TFo	11 d	3.7 a	0.63 b	0.072 d	0.73 e
BtF	8.8 c	4.9 b	0.48 a	0.036 b	0.38 b
BtFo	8.0 cb	5.2 b	0.47 a	0.057 c	0.51 c
BtoF	7.4 ab	5.1 b	0.46 a	0.023 a	0.23 a
BtoFo	7.0 a	7.3 c	0.45 a	0.025 a	0.25 a
Grass	11	4.7	0.76	0.038	0.4
Fertilizer effect <sup>2</sup>	ns	*	ns	*	**

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.3: Mean quantities of basal respiration, metabolic quotient (qCO<sub>2</sub>), FDA hydrolysis, dehydrogenase and arginine ammonification rate in the 5 - 10 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Basal respiration ( $\mu\text{g CO}_2\text{-Cg}^{-1}\text{day}^{-1}$ )	Metabolic quotient ( $\mu\text{g CO}_2\text{-C mg}^{-1}\text{ biomass day}^{-1}$ )	FDA hydrolysis	Dehydrogenase	Arginine
			rate	activity	ammonification rate
			( $\mu\text{ mol product released g}^{-1}\text{ h}^{-1}$ )		
TF	10.3 d <sup>1</sup>	3.4 a	0.61 c	0.023 b	0.51 d
TFo	9.3 c	3.9 a	0.44 b	0.062 d	0.64 e
BtF	7.9 b	5.3 b	0.30 a	0.024 b	0.24 b
BtFo	7.2 b	5.2 b	0.28 a	0.051 c	0.44 c
BtoF	5.8 a	5.5 b	0.35 a	0.015 a	0.15 a
BtoFo	5.2 a	6.4 c	0.30 a	0.014 a	0.21 b
Grass	7.1	5	0.4	0.027	0.31
Fertilizer effect <sup>2</sup>	**	ns	ns	**	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

## APPENDIX 5.4:

Mean quantities of basal respiration, metabolic quotient ( $qCO_2$ ), FDA hydrolysis, dehydrogenase and arginine ammonification rate in the 10 - 20 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Basal respiration ( $\mu g CO_2-Cg^{-1}day^{-1}$ )	Metabolic quotient ( $\mu g CO_2-C mg^{-1} biomass day^{-1}$ )	FDA hydrolysis	Dehydrogenase	Arginine
			rate	activity	ammonification rate
			( $\mu mol product released g^{-1} h^{-1}$ )		
TF	8.2 d <sup>1</sup>	3.3 a	0.33 b	0.017 a	0.44 c
TFo	7.6 c	4.0 a	0.33 b	0.054 d	0.51 d
BtF	6.5 b	5.1 c	0.25 a	0.022 b	0.21 a
BtFo	4.5 a	4.4 b	0.24 a	0.043 c	0.34 b
BtoF	4.7 a	5.6 c	0.25 a	0.011 a	0.16 a
BtoFo	4.3 a	7.3 d	0.25 a	0.011a	0.18 a
Grass	4.9	5.2	0.26	0.013	0.28
Fertilizer effect <sup>2</sup>	ns	ns	ns	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.5:

Mean quantities of basal respiration, metabolic quotient (qCO<sub>2</sub>), FDA hydrolysis, dehydrogenase and arginine ammonification rate in the 20 - 30 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Basal respiration ( $\mu\text{g CO}_2\text{-Cg}^{-1}\text{day}^{-1}$ )	Metabolic quotient ( $\mu\text{g CO}_2\text{-C mg}^{-1}\text{biomass day}^{-1}$ )	FDA hydrolysis	Dehydrogenase	Arginine
			rate	activity	ammonification rate
			(μ mol product released g <sup>-1</sup> h <sup>-1</sup> )		
TF	6.5 d <sup>1</sup>	5.0 a	0.23 a	0.013 b	0.36 c
TFo	6.0 d	4.8 a	0.23 a	0.045 e	0.45 e
BtF	5.0 c	5.5 a	0.23 a	0.02 c	0.20 b
BtFo	3.8 b	5.4 a	0.24 a	0.034 d	0.28 c
BtoF	3.4 ab	5.6 a	0.25 a	0.01 a	0.11 a
BtoFo	3.0 a	6.8 b	0.24 a	0.01 a	0.12 a
Grass	3.2	5.9	0.23	0.011	0.2
Fertilizer effect <sup>2</sup>	ns	ns	ns	ns	**

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.6:

Mean quantities of acid- and alkaline phosphatase, aryl-sulfatase, Invertase, L-histadase and protease in the 0 - 2.5 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Acid phosphatase	Alkaline phosphatase	Aryl-sulfatase	Invertase	L-histadase	Protease
	(μmol product released g <sup>-1</sup> h <sup>-1</sup> )					
TF	9.0 e <sup>1</sup>	4.3 b	0.72 b	2.6 c	0.53 bc	1.3 d
TFo	6.2 d	4.4 b	1.53 c	2.4 c	1.11 d	0.9 c
BtF	4.1 c	2.0 a	0.30 a	2.3 c	0.29 a	0.6 b
BtFo	3.1 b	2.4 a	0.64 b	2.4 c	0.61 c	0.5 b
BtoF	2.7 a	2.2 a	0.18 a	1.8 b	0.22 a	0.2 a
BtoFo	2.4 a	2.3 a	0.20 a	0.9 a	0.47 b	0.2 a
Grass	5.8	4.9	0.62	2.5	0.62	0.7
Fertilizer effect <sup>2</sup>	*	ns	*	ns	*	ns

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.7:

Mean quantities of acid- and alkaline phosphatase, aryl-sulfatase, Invertase, L-histadase and protease in the 2.5 - 5 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Acid phosphatase	Alkaline phosphatase	Aryl-sulfatase	Invertase	L-histadase	Protease
	(μ mol product released g <sup>-1</sup> h <sup>-1</sup> )					
TF	5.4 d <sup>1</sup>	3.8 c	0.5 c	2.6 e	0.41 b	1.07 d
Tfo	5.0 d	3.9 c	1.15 d	2.4 d	1.05 c	0.84 c
BtF	4.0 c	1.3 a	0.28 ab	2.4 d	0.22 a	0.51 b
BtFo	2.8 b	2.0 b	0.42 cb	2.1 c	0.54 b	0.50 b
BtoF	2.6 b	2.2 b	0.13 a	1.7 b	0.19 a	0.23 a
BtoFo	1.8 a	2.4 b	0.19 a	0.8 a	0.38 ab	0.22 a
Grass	4.9	4.4	0.56	2.4	0.5	0.64
Fertilizer effect <sup>2</sup>	ns	ns	*	**	**	ns

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.8: Mean quantities of acid- and alkaline phosphatase, aryl-sulfatase, Invertase, L-histadase and protease in the 5 - 10 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Acid phosphatase	Alkaline phosphatase	Aryl-sulfatase	Invertase	L-histadase	Protease
	$(\mu \text{ mol product released g}^{-1} \text{ h}^{-1})$					
TF	3.2 c <sup>1</sup>	4.4 c	0.46 d	2.2 c	0.38 c	0.98 d
TFo	2.8 b	4.5 c	0.72 e	2.0 c	0.82 e	0.73 c
BtF	2.1 ab	1.4 a	0.18 b	2.0 c	0.15 a	0.4 b
BtFo	1.9 a	1.9 b	0.38 c	2.0 c	0.46 d	0.35 b
BtoF	2.4 b	1.5 ab	0.12 ab	1.4 b	0.12 a	0.12 a
BtoFo	2.1 ab	1.9 b	0.07 a	0.48 a	0.31 b	0.11 a
Grass	2.8	4.1	0.25	1.5	0.43	0.53
Fertilizer effect <sup>2</sup>	ns	ns	*	ns	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

## APPENDIX 5.9:

Mean quantities of acid- and alkaline phosphatase, aryl-sulfatase, Invertase, L-histadase and protease in the 10 - 20 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Acid phosphatase	Alkaline phosphatase	Aryl-sulfatase	Invertase	L-histadase	Protease
	(μ mol product released g <sup>-1</sup> h <sup>-1</sup> )					
TF	3.2 c <sup>1</sup>	3.3 c	0.31 bc	1.9 e	0.31 c	0.64 d
TFo	3.1 c	3.6 c	0.40 c	1.3 c	0.72 d	0.44 c
BtF	2.3 b	1.3 a	0.23 b	1.5 d	0.12 a	0.20 b
BtFo	2.2 ab	2.1 b	0.27 b	1.4 c	0.34 c	0.16 b
BtoF	2.2 ab	1.3 a	0.09 a	1.0 b	0.10 a	0.13 a
BtoFo	1.8 a	1.9 b	0.03 a	0.21 a	0.23 b	0.12 a
Grass	2.7	3.3	0.21	1.2	0.48	0.42
Fertilizer effect <sup>2</sup>	ns	*	Ns	**	*	*

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

APPENDIX 5.10:

Mean quantities of acid- and alkaline phosphatase, aryl-sulfatase, Invertase, L-histadase and protease in the 20 - 30 cm soil layer. T = green cane harvested with trash retention; Bt = burnt with harvest residues left on the soil surface; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K; Fo = no fertilizer applied.

Management treatments	Acid phosphatase	Alkaline phosphatase	Aryl-sulfatase	Invertase	L-histadase	Protease
	(μ mol product released g <sup>-1</sup> h <sup>-1</sup> )					
TF	3.2 d <sup>1</sup>	3.2 d	0.21 bc	1.6 d	0.24 b	0.46 d
TFo	2.9 cd	3.3 d	0.25 c	1.0 c	0.46 d	0.36 c
BtF	2.7 c	1.3 c	0.16 b	1.4 d	0.1 a	0.13 a
BtFo	2.0 b	1.9 b	0.17 b	1.1 c	0.28 c	0.25 b
BtoF	2.2 b	1.1 a	0.04 a	0.67 b	0.1 a	0.10 a
BtoFo	1.2 a	1.6 bc	0.04 a	0.2 a	0.16 b	0.10 a
Grass	2.3	2.3	0.13	0.8	0.31	0.37
Fertilizer effect <sup>2</sup>	*	*	Ns	*	*	ns

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

<sup>2</sup> Significance of fertilizer effect (\* =  $P \leq 0.05$ ; \*\* =  $P \leq 0.01$ ; ns = no significant difference).

## APPENDIX 6.1:

Mean quantities of organic C, microbial biomass C, K<sub>2</sub>SO<sub>4</sub>-extractable C, light fraction dry matter and basal respiration at increasing distances from the row centre (Row, 30 cm from the row centre and 60 cm from the row centre) in the 0 - 10 cm depth. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K.

Management treatment	Sample area	Organic C g kg <sup>-1</sup>	Microbial biomass C mg kg <sup>-1</sup>	K <sub>2</sub> SO <sub>4</sub> - extractable C	Light fraction dry matter g kg <sup>-1</sup>	Basal respiration μgCO <sub>2</sub> -Cg <sup>-1</sup> day <sup>-1</sup>
TF	Row	47.6 d	1014 f	563 e	27 d	21 e
BtoF	Row	42.9 c	567 d	273 c	14 c	12.6 c
TF	30 cm	42.2 c	664 e	388 d	15 c	15 d
BtoF	30 cm	38.4 a	356 b	189 b	8.4 b	8.4 b
TF	60 cm	41.3 b	455 c	285 c	9.5 b	12.1 c
BtoF	60 cm	38.0 a	268 a	147 a	6.0 a	7.0 a

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

APPENDIX 6.2:

Mean quantities of organic C, microbial biomass C, K<sub>2</sub>SO<sub>4</sub>-extractable C, light fraction dry matter and basal respiration at increasing distances from the row centre (Row, 30 cm from the row centre and 60 cm from the row centre) in the 10 - 20 cm depth. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K.

Management treatment	Sample area	Organic C g kg <sup>-1</sup>	Microbial biomass C mg kg <sup>-1</sup>	K <sub>2</sub> SO <sub>4</sub> - extractable C mg kg <sup>-1</sup>	Light fraction dry matter g kg <sup>-1</sup>	Basal respiration μgCO <sub>2</sub> -Cg <sup>-1</sup> day <sup>-1</sup>
TF	Row	42.4 c	634 e	460 e	16 e	13.5 e
BtoF	Row	40.2 b	267 c	213 c	12.2 d	6.2 b
TF	30 cm	40.0 b	446 d	250 d	9.2 c	9.0 d
BtoF	30 cm	38.8 a	216 b	152 b	6.7 b	5.3 ab
TF	60 cm	39.6 ab	262 c	192 c	5.7 b	7.5 c
BtoF	60 cm	38.0 a	147 a	100 a	4.0 a	4.7 a

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at  $P \leq 0.05$ .

APPENDIX 6.3:

Mean quantities of organic C, microbial biomass C, K<sub>2</sub>SO<sub>4</sub>-extractable C, light fraction dry matter and basal respiration at increasing distances from the row centre (Row, 30 cm from the row centre and 60 cm from the row centre) in the 20 - 30 cm depth. T = green cane harvested with trash retention; Bto = burnt with harvest residues removed; F = fertilized annually with N, P and K.

Management treatment	Sample area	Organic C g kg <sup>-1</sup>	Microbial biomass C mg kg <sup>-1</sup>	K <sub>2</sub> SO <sub>4</sub> extractable C mg kg <sup>-1</sup>	Light fraction dry matter g kg <sup>-1</sup>	Basal respiration μgCO <sub>2</sub> -Cg <sup>-1</sup> day <sup>-1</sup>
TF	Row	37.2 a	509 f	367 d	9.7 d	11.5 e
BtoF	Row	37.2 a	230 d	170 c	6.9 c	5.4 bc
TF	30 cm	37.6 a	274 e	165 c	6.4 c	7.3 d
BtoF	30 cm	37.5 a	170 b	121 b	3.9 b	4.5 b
TF	60 cm	37.5 a	211 c	130 b	3.8 b	7.0 d
BtoF	60 cm	36.2 a	89 a	74 a	2.8 a	3.4 a

<sup>1</sup> Mean values within one column followed by the same letter are not significantly different at *P* 0.05.

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