CHAPTER 5

RELATIONSHIPS BETWEEN LABILE CARBON DETERMINED BY $KMnO_4$ OXIDATION AND OTHER COMMON MEASUREMENTS OF SOIL ORGANIC CARBON

5.1. INTRODUCTION

The complex nature of organic matter in soil coupled with its association with the mineral matrix has led to the development of a wide range of approaches for its estimation. Recent advances in soil organic matter methodologies such as solid-state ^{13}C NMR spectroscopy (Preston, 1996), pyrolysis mass spectrometry (Schnitzer and Schulten, 1995), uv-photooxidation (Skjemstad *et al.*, 1993), and infrared spectroscopic techniques (Ladd *et al.*, 1993) have contributed significantly towards understanding the nature of organic carbon in soils. Such techniques however, are not readily accessible for monitoring purposes. Total organic carbon measurements such as the loss-on-ignition (Spain *et al.*, 1982), dichromate oxidation (Walkley and Black, 1934), and dry combustion techniques (Nelson and Sommers, 1982) have been widely employed for monitoring soil organic matter changes. A drawback with the use of total organic carbon measurements alone is that soil organic matter changes are gradual and short-term changes in organic carbon may be difficult to detect against a high background level.

Concern over the loss from soils of the labile carbon, which is more susceptible to management effects than the bulk organic matter, has fostered research on a wide range of approaches for its measurement. In the report of Johns and Skogley (1994), labile carbon was considered as those organic carbon components that are sorbed on carbonaceous resin capsules. Labile carbon determined as such was found to comprise mostly carbohydrates, hydroxyl and carboxyl groups, together with some metal-organic complexes. Some other workers have described labile carbon as the soil organic matter components that can be extracted by water (Garcia *et al.*, 1992; Liang *et al.*, 1995), while others have examined the soil microbial biomass carbon as a measure of labile carbon in soils (Amato and Ladd, 1988; Parkinson and Coleman, 1991; Degens and Sparling, 1996). While the use of the microbial biomass carbon in describing the labile organic carbon in soil has gained considerable interest, the question of reliable estimates remain unresolved

(Martens, 1995). Other workers have examined the "light-fraction, (LF)" as the labile component of soil organic matter (Magid *et al.*, 1996; Gregorich and Janzen, 1996). However, ^{13}C NMR analysis has shown that LF has a chemical composition comparable to that of litter and plant material (Skjemstad *et al.*, 1986) and may contain charcoal (Skjemstad *et al.,* 1990) which has been identified as resistant carbon (Skjemstad *et al.*, 1996). Since the loss of organic matter in soil is essentially an oxidative process, this study considered the labile carbon as that organic matter fraction that is readily oxidisable by a mild oxidising agent, 333 mM $KMnO_4$ solution.

In Chapter 4, it was shown that labile carbon obtained by ease of oxidation, together with the monitoring indicators calculated, can provide very meaningful index of organic matter turnover in soil. However, the relationship between such fractions and other commonly used measurements of soil organic matter is unclear. Also, an understanding of what components of soil organic matter are removed by such fractionation procedure is lacking. The study reported in this chapter therefore examines the components of organic matter removed by $KMnO_4$ oxidation and any possible relationship between $KMnO_4$ fractions and other commonly used measures of soil organic matter.

5.2. MATERIALS AND METHODS

5.2.1. Selection of soil samples

To obtain an understanding of the relationship between the $KMnO_4$-oxidisable carbon and other chemical fractions of soil organic matter, seven pairs of reference/cropped soils (labelled A/B) were selected from the 65 ground and sieved (< 500 μm) samples used in the study reported in Chapter 4 (Table 5.1). The seven pairs were selected to represent soils with a wide range in total and labile carbon concentration and with a wide variability in the relative losses of both total and labile carbon concentrations as a result of cultivation. From these seven pairs, two pairs were further selected for spectroscopic analysis. The selection of these two pairs was based on the highest relative loss in total and labile carbon concentration during cultivation.

Table 5.1: Description of the seven pairs of samples used in this study

Sample No.	History	Soil type	Location	Texture	C_T	C_L	C/N
					mg/g		
1A	Reference	Grey Clay	Gwydir Valley	Clay	22.4	3.6	14.1
1B	18 years	Grey Clay	Gwydir Valley	Clay	9.4	1.3	9.1
2A	Reference	Grey Clay	Emerald	Clay	33.1	6.3	12.2
2B	21 years	Grey Clay	Emerald	Clay	8.8	1.3	12.1
3A	Reference	Black Eearth	Macintyre Valley	Clay	16.1	4.0	12.3
3B	6 years	Black Earth	Macintrye Valley	Clay	12.8	2.0	11.4
4A	Reference	Red Brown Earth	Macquarie Valley	Silty Clay	11.4	1.9	10.6
4B	50 years	Red Brown Earth	Macquarie Valley	Silty Clay	8.2	1.4	10.2
5A	Reference	Grey Clay	Macquarie Valley	Clay	10.8	1.0	11.8
5B	4 years	Grey Clay	Macquarie Valley	Clay	10.7	1.2	11.3
6A	Reference	Grey Clay	Darling Downs	Clay	13.6	2.9	11.3
6B	40 years	Grey Clay	Darling Downs	Clay	9.3	1.8	13.1
7A	Reference	Grey Clay	Darling Downs	Clay	18.8	3.8	11.8
7B	50 years	Grey Clay	Darling Downs	Clay	11.2	1.8	11.9

5.2.2. Fractionation of humic substances

Fractionation of humic substances was carried out in triplicate using the procedure described by Anderson and Schoenau (1993) and Schnitzer *et al.* (1981). The procedure involved extracting the humic substances by dilute NaOH solution in an atmosphere of N_2 followed by acidification of the alkaline extract to separate the humic acid (HA) from the fulvic acid (FA). This fractionation procedure and the fractions obtained have been reviewed earlier in this thesis (Section 2.3.2 and Section 2.5.5).

(a) Extraction procedure

From each of the seven pairs of samples selected, 15 g was weighed into 250 mL plastic centrifuge bottle followed by addition of 150 mL of 0.5 M HCl. The mixture was allowed to stand at room temperature for one hour with occasional stirring and then centrifuged for 15 minutes at 6000 rpm (RCF = 1500 g). The supernatant was poured off in a fume hood and discarded. This step was recommended (Anderson and Schoenau, 1993) as a pre-treatment to remove floating plant

debris and inorganic forms of C, N, P, and S before extraction of the humic materials. The remaining acid in the soil was removed by adding a further 150 mL of deionised water into the centrifuge bottle and centrifuged at 1500 g for 15 minutes. The supernatant discarded. Using a dispenser, 150 mL of 0.5 M NaOH solution was then added to the soil in the centrifuge bottles and N_2 gas flushed into the centrifuge bottles from a gas cylinder for a few seconds before the cap was quickly tightened. The mixture was then tumbled in an end-over-end tumbler at 12 rpm for 18 hours. At the end of the tumbling period, the samples were centrifuged for 15 minutes at 1500 g to separate the alkaline extract from the soil residue. The supernatant from each soil was carefully decanted into clean 250 mL centrifuge bottles and stored in a cool room at 4°C for analysis. The residue of the extraction (humin) was washed with 150 mL deionised water, dried and stored for further analysis.

(b) Separation into humic acid (HA) and fulvic acid (FA)

Using a burette, 6 M HCl was added slowly into the alkaline extract while the pH was monitored progressively using a pH meter (Expandable ion Analyzer EA 940-Orion Reseach). The initial pH of the alkaline extracts ranged from 12.8 to 13.2. The pH decreased progressively with addition of HCl, and at a pH of 9, the flow of acid was stopped by closing the burette and a 5 mL aliquot taken from the extract. This aliquot was used for measurement of the E4/E6 ratio. The 6 M HCl was then allowed to pour into the extract until a pH of 1.5 was attained. The extracts were then stored in a coolroom (4°C) for 24 hours to allow the humic acid to precipitate from the solution. After 24 hours, the dark brown precipitate of HA was clearly distinguishable from the yellow FA solution. The extract was then centrifuged at 1500 g for 15 minutes to separate all suspended HA from the FA solution. The clear FA was then carefully poured into clean 250mL centrifuge bottles.

(c) Determination of E4/E6 ratio

The E4/E6 ratio is a measure of the particle or molecular weight of humic materials (Chen *et al.*, 1977) and is defined as the ratio of absorbances of solutions of humic substances at 465 and 665 nm. The procedure recommended by Chen *et al.* (1977) was to dissolve the purified humic material in 0.05 M $NaHCO_3$. However, Schnitzer *et al.* (1981) recommended that any solution of humic materials can be used provided that the solution is adjusted to pH 9. This procedure was adopted in this study. Using a solution of 0.5 M NaOH adjusted to pH 9 as a reference, the absorbances of the 5 mL aliquot taken from the extracts were read on a split-beam spectrophotometer at wavelengths of 465 and 665 nm. The ratio of the absorbances at the two wavelengths was then calculated to represent the E4/E6 ratio.

(d) Determination of carbon concentration of the humic fractions

The carbon concentration of the FA was determined spectrophotometrically using a procedure similar to the acid-dichromate oxidation described by Sims and Haby (1971). Essentially,

the organic carbon present in the FA was oxidised by dichromate in the presence of concentrated sulfuric acid. The heat of dilution generated by the addition of concentrated sulfuric acid to the mixture ensures complete oxidation of the FA. During the process, the dichromate undergoes changes according to the following half reaction:

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$$

The appearance of Cr^{3+} in the reaction media is directly proportional to the quantity of FA oxidised. Therefore, analysis of the reaction media for Cr^{3+} concentration provides a measure of the organic carbon in the FA.

Working standards were prepared by dissolving various quantities of sucrose to give solutions containing carbon equivalents of 10, 20, 30, 40, 50, and 60 μg C/mL. A 1 mL aliquot was transferred from the FA solution into a 30 mL centrifuge tube and diluted to 10mL using deionised water. After gentle stirring, 10 mL of 1 N $K_2Cr_2O_7$ was added followed by 5 mL of concentrated H_2SO_4 (96 % w/v, 36.8 N) using an automatic dispenser. The solution was mixed thoroughly and allowed to stand for one hour. The sucrose standard solutions also received the same treatment. Absorbance was read on a split-beam spectrophotometer at a wavelength of 600 nm and the carbon concentration of the FA calculated from the sucrose standard curves.

The carbon concentration of the humin in the soil residue was determined by ANCA-MS as described for the whole soil in Chapter 4. The carbon concentration of the HA was calculated as the difference between total carbon (C_T) and the sum of FA and humin.

5.2.3. Determination of total and labile polysaccharides

Total polysaccharides were determined in triplicates using the procedure described by Lowe (1993a). The method is based on the hydrolysis procedure described by Ivarson and Sowden (1962) and Chesire (1979). Essentially, all polysaccharides were hydrolysed by 12 M H_2SO_4 in an autoclave for one hour at 103 kPa and the saccharide monomers released were determined by spectrophotometry using the phenol-sulfuric acid reagent.

From each of the samples, 1 g was weighed in triplicate in 250 mL centrifuge bottles. Using a dispenser, 4 mL of a 12 M H_2SO_4 solution was added to each bottle, stirred gently, and allowed to stand at room temperature for four hours. The solution was then diluted to 0.5 M by adding 92 mL deionised water and placed in an autoclave (American Cyclomatic Control, Athertons Pty Ltd., Sydney) for one hour at a pressure of 103 kPa and a temperature of 110°C. The mixture was then removed, allowed to cool and centrifuged at 1500 g for 20 minutes. The supernatant was transferred into 250 mL volumetric flasks. The soil residue was washed with 50 mL deionised water, centrifuged

for a further 20 minutes, and the washings added to the first hydrolysates in the 250 mL volumetric flasks. The extracts were then made to volume using deionised water.

The standard curves were prepared using anhydrous D-glucose. The stock solution was prepared by dissolving 250 mg glucose in 250 mL deionised water in a 500 mL beaker. The solution was then transferred to a 1 L volumetric flask and made up to volume using deionised water. This gave a stock solution containing 100 μg C/mL. From the glucose stock solution, working standards were prepared by pipetting 0, 10, 20, 30, 40, 50, and 60 mL into 100 mL volumetric flasks and made to volume with deionised water. This gave working standards of 0, 10, 20, 30, 40, 50, and 60 μg C/mL. The stock solution was stored in a refrigerator and the working standards were prepared fresh each time polysaccharides were to be determined.

Colour development was based on the phenol-sulfuric acid reagent described by Dubois *et al.* (1956) and Doutre *et al.* (1978). The basic principle is that when treated with phenol in the presence of sulfuric acid, simple sugars, polysaccharides, and their derivatives, including the methyl ethers, give an orange-yellow colour whose intensity is directly proportional to the concentration of saccharide present. The reaction is reported to be sensitive and the colour stable (Dubois *et al.*, 1956). From each of the glucose working standards and sample hydrolysates, a 2 mL aliquot was pipetted into 30 mL centrifuge tubes followed by the addition of 2 mL of 5 % (w/v) solution of phenol and 10 mL of concentrated H_2SO_4 (96 % w/v, 36.8 N). The tubes were tightly stoppered and vigorously shaken for a few seconds before being placed in an oven at 30°C for 25 minutes. Absorbance was read at 490 nm in a split-beam spectrophotometer. Total polysaccharide concentration was calculated from the calibration curve and expressed as mg C/g sample.

The determination of labile polysaccharides followed the same procedure as for total polysaccharides, except that hydrolysis was carried out with 0.5 M H_2SO_4 only. The standard curve for labile polysaccharides was prepared using working standards of 0, 2, 4, 6, 8, 10, and 12 μg C/mL. Non-labile polysaccharides were calculated as the difference between total and labile polysaccharides.

5.2.4. Determination of water-soluble phenolics

Water-soluble phenolics were determined using the method described by Lowe (1993b). From each of the 14 samples selected, 5 g soil was weighed into a 250 mL centrifuge bottle. Using a dispenser connected to a 2.5 L Winchester bottle, 50 mL of deionised water was added to the soil giving a soil:solution ratio of 1:10. The solution was tumbled in an end-over-end tumbler for 4 hours and then centrifuged at 1500 g for 30 minutes. The clear supernatant was decanted in 250 mL centrifuge bottles.

Stock solution (100 ppm) was prepared by dissolving 100 mg vanillic acid in 250 mL of deionised water in a 500 mL beaker on a warm magnetic stirrer. The solution was then transferred into a 1L volumetric flask, allowed to cool and then made up to volume. This gave a solution containing 100 µg/mL vanillic acid. From the stock solution, working standards containing 0, 2, 4, 6, 8, and 10 µg/mL vanillic acid were prepared by diluting 0, 2, 4, 6, 8, 10 mL of the stock solution to 100 mL with deionised water in a 100 mL volumetric flasks.

Colour development was based on the Folin-Ciocalteau reaction with phenols described by Box (1983). The Folin-Ciocalteau reagent contains phosphomolybdic and phosphotungstic acids which are reduced to a blue colour in alkaline solution in the presence of phenolic substances. From the vanillic acid working standards, 10 mL aliquots were dispensed into a 30 mL centrifuge tube using a Gilson pipette, followed by the addition 10 mL of deionised water. 3 mL of a 20 % solution of Na_2CO_3 was then added followed by 1 mL of Folin-Ciocalteau reagent. The mixture was vigorously hand-shaken for a few seconds and allowed to stand for 1 hour at room temperature for colour development. The samples were treated in a similar manner. Absorbance was read at 750 nm against a blank in a split-beam spectrophotometer. The concentration of phenolics in the extracts was then determined from the calibration curve and initially expressed as µg/g vanillic acid equivalent. Further calculations showed that vanillic acid contains *ca.* 57 % carbon, and thus the results were multiplied by 0.57 to give µg C/g.

5.2.5. Determination of light fraction carbon (LF-C)

Isolation of the light fraction (LF) from each of the seven pairs of samples was done in duplicate following the same procedure described in Chapter 4. The total carbon concentration of the LF was determined using an ANCA-MS in the same manner as described for the soil aggregate fractions in Chapter 4. By multiplying the proportion of carbon in the LF and the mass of LF isolated, the amount of carbon present in the LF per unit mass of soil (LF-C) was determined and expressed as mg/g and as % of C_T. The amount of $KMnO_4$-oxidisable carbon in the LF was determined in the same manner as for the whole soil described in Chapter 4 and expressed as mg/g, % of LF-C, % of C_L, and % of C_T.

5.2.6. Determination of soil microbial biomass carbon

From the seven pairs of soil samples, three pairs of reference/cropped samples reflecting large, medium and small differences in C_T and C_L were identified. These three pairs were then collected from the bulk unprocessed samples described in Chapter 3, and all visible litter and root material removed before sieving through a 2000 µm sieve. Because the samples had been kept dry, and measurements of microbial biomass carbon are most commonly carried out on field moist samples (Anderson and Domsch, 1989; Voroney *et al.*, 1993), measurements of microbial biomass

carbon was done on incubated and non-incubated samples. Each sample was weighed into six pots (10 cm diameter and 5 cm deep) such that each pot contained 80 g of < 2000 μm sieved soil. The six pots for each sample were split into two equal sets; one set for incubation and the other set without incubation. The set of samples to be incubated was kept continuously moist at 70 % field capacity for four weeks in an oven at 20°C. The non-incubated set was also placed in the same oven at the same temperature but without moisture. This meant that for each sample, there were two treatments (incubated and non-incubated) replicated three times.

Measurement of microbial biomass carbon was based on the substrate-induced respiration method described by Anderson and Domsch (1978) and Anderson *et al.* (1981). Approximately 70 g of each soil on a dry weight basis was weighed into respirometer pots followed by the addition of water to bring the moisture concentration of the soil to 70 % field capacity. The pots were further incubated at 20°C for approximately 60 hours and then placed on the respirometer. Basal respiration rates, calculated as CO_2/hr/100 g dry soil, were determined over 48 hours using a Respicond III™ respirometer (Nordgren Innovations AB, Terrängvägen. 3A, S-903 38 Umeå, Sweden). This respirometer has been described in detail by Nordgren (1988). The principle involved is that CO_2 is absorbed in a solution of potassium hydroxide (KOH) to form carbonate ions, which lower the conductivity of the solution. The change in conductivity is calibrated against CO_2 absorption to provide an integrated measure of respiration. After measurement of basal respiration, 3.0 mg glucose/g dry soil was added to the soil and mixed thoroughly. The added glucose stimulated a flush of microbial activity which was monitored as substrate-induced respiration for the following five hours, and used in the equation recommended by Anderson *et al.* (1981):

$$\text{mg Microbial Carbon} = 40.04 \times (\text{mg } CO_2\text{/hr/100 g dry soil}/1.964) + 0.37$$

The results obtained from the microbial biomass measurements were analysed using the ANOVA program of Burr (1980) and mean differences determined using the Duncan's Multiple Range Test.

5.2.7. Near Infrared Reflectance and solid-state ^{13}C CPMAS NMR Analyses

From the seven pairs of samples, two pairs of reference/cropped samples were selected as already outlined in Section 5.2.1 in order to determine what components of soil organic carbon are lost due to oxidation by 333 mM $KMnO_4$. To achieve this, the study utilised the techniques of near infrared reflectance (NIR) and solid-state cross-polarisation magic-angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR) spectroscopy. Each of the selected samples was oxidised using the same procedure described in Chapter 4 for obtaining the labile carbon. At the end of the oxidation period the samples were washed free of $KMnO_4$ using deionised water, filtered through

0.1 μm Gelman membrane filters and then dried in an oven at 30°C. To be able to isolate any possible effects that washing of the soil may have on the organic carbon concentration, the unoxidised samples were also washed with the same volume of water, filtered and dried in the same oven as the oxidised samples. The samples were then manually ground using a clay mortar and pestle and then sieved to < 500 μm. The sieved soils were then scanned through an NIR spectrophotometer from a wavelength of 400 nm to 2400 nm before NMR analysis.

^{13}C NMR analysis was carried out at the CSIRO Division of Soils using the same procedure as described by Skjemstad *et al.* (1996). Prior to NMR analysis, samples were treated with 2 % HF as described by Skjemstad *et al.* (1994) to remove magnetic materials, freeze-dried and ground to < 200 μm. The samples were then packed into 7 mm diameter zirconia rotors and CPMAS spectra were obtained at 50.309 MHz on a Varian Unity 200 spectrometer with a 4.7 T wide-bore Oxford superconducting magnet using a Doty Scientific Magic-Angle Spinning probe. Instrument conditions were identical to those reported by Skjemstad *et al.* (1994). The proportional contribution of the functional groups (carbonyl, o-aryl, aryl, o-alkyl, and alkyl) was calculated by integration of the area under each peak. The limiting chemical shift values for each region were not strictly adhered to, particularly where large and small bands occurred adjacent to one another. In this case, 'natural valleys' between bands were judged as more appropriately representing the chemical shift boundaries.

5.2.8. Data analysis

The relationships between the various carbon fractions and the $KMnO_4$-oxidisable carbon were determined using the statistical program on Microsoft Excel (Version 5).

5.3. RESULTS

5.3.1. Humic substances extracted from the selected soils

The total amount of humic substances extracted (HA + FA) ranged from 6.3 to 12.6 mg C/g in the reference soils and from 4.2 to 7.0 mg C/g in the cropped soils (Table 5.2). These ranges for the humic materials extracted represented 38.1 to 58.2 % of C_T in the reference soils and 47.7 to 65.5 % of C_T in the cropped soils. With the exception of the HA/FA ratios and the E4/E6 ratios, all humic substances were higher in the reference soils than in most of the cropped soils. There was a narrow range in the E4/E6 ratio, ranging from 6.9 to 8.3 in the reference soils and from 6.4 to 8.4 in the cropped soils. In most of the soils studied, the extracted humic materials consisted more of FA than HA (Table 5.2).

While the amount of FA extracted ranged from 2.9 to 8.4 mg C/g in the reference soils, and 2.0 to 5.2 mg C/g in the cropped soils, the extracted HA ranged from 1.4 to 4.9 mg C/g in the reference soils and from 1.8 to 3.1 mg C/g in the cropped soils. These ranges meant that HA comprised 8.6 to 31.3 % of C_T and 14.1 to 28.8 % of C_T in the reference and cropped soils, respectively. In all these measurements, variability, as shown by the coefficient of variability, was higher in the reference soils than in the cropped soils.

Table 5.2: Concentration of humic substances in selected reference and cropped soils.

Sample	Humic Acid (HA)	Fulvic Acid (FA)	Humin	HA/FA	HA + FA		E4/E6
No.	mg C/g			Ratio	mg C/g	% of C_T	Ratio
1A	2.7a	8.4a	11.3a	0.32b	11.1a	49.6b	7.4a
1B	1.9b	4.0b	3.5b	0.49a	5.9b	62.8a	6.4b
2A	4.9a	7.7a	20.5a	0.63b	12.6a	38.1b	8.3a
2B	2.2b	2.0b	4.6b	1.10a	4.2b	47.7a	8.4a
3A	1.4a	7.7a	7.0a	0.18b	9.1a	56.5a	6.9a
3B	1.8a	5.2b	5.8b	0.35a	7.0b	54.7a	7.7a
4A	2.2a	4.3a	4.9a	0.51a	6.5a	56.9b	7.2a
4B	1.9a	3.5b	2.8b	0.55a	5.4a	65.5a	7.2a
5A	3.4a	2.9a	4.5a	1.17a	6.3a	58.2a	7.6 a
5B	3.1a	3.2a	4.5a	0.97b	6.3a	58.3a	7.5a
6A	1.4a	5.9a	6.4a	0.23b	7.3a	53.3b	6.9a
6B	1.8a	4.0a	3.6b	0.45a	5.8b	61.8a	7.0a
7A	2.1a	7.0a	9.7a	0.30b	9.1a	48.3b	7.0a
7B	2.2a	4.2b	4.8b	0.51a	6.4a	57.3a	7.2a

For any pair of soils, means in a column followed by the same letter are not significantly different ($P < 0.05$ LSD)

5.3.2. Polysaccharides and water-soluble phenolics in the selected soils

The concentration of total polysaccharides (P_T) ranged from 1.2 to 5.5 mg C/g in the reference soils, and from 1.2 to 2.0 mg C/g in cropped soils (Table 5.3). These ranges in P_T represented 10.5 to 19.9 % of C_T in the reference soils and 11.5 to 21.5 % of C_T in the cropped soils. The labile polysaccharides (P_L) ranged from 0.1 to 0.4 mg C/g in the reference soils, and from 0.1 to 0.2 mg C/g in the cropped soils. The labile polysaccharides comprised between 5.6 to 12.0 % of the total polysaccharides in the reference soils, and between 6.7 to 11.0 % of the total polysaccharides in the cropped soils (Table 5.3). Although the proportion of C_T present as polysaccharides did not, on average, differ significantly between the reference and the cropped soils, the reference soils contained, on average, twice the concentration of polysaccharides in the cropped soils. The concentration of water-soluble phenolics was generally small, ranging from 3.6 to 22.7 μg C/g in the reference soils, and from 3.1 to 11.7 μg C/g in the cropped soils (Table 5.3).

Table 5.3: Concentration of polysaccharides and water-soluble phenolics in the selected reference and cropped soils.

Sample	Total Polysaccharides (P_T)		Labile Polysaccharides (P_L)		Non-labile Polysaccharides (P_{NL})		Water-Soluble Phenolics (WSP)
No.	mg C/g	% of C_T	mg C/g	% of P_T	mg C/g	% of P_T	μg C/g
1A	3.0a	13.2a	0.25a	8.3a	2.75a	91.7a	18.7a
1B	1.2b	13.0a	0.10b	8.3a	1.10b	91.7a	6.8b
2A	5.4a	16.2a	0.45a	8.3a	4.95a	91.7a	15.2a
2B	1.2b	14.0a	0.10b	8.3a	1.10b	91.7a	3.8b
3A	2.5a	15.7a	0.30a	12.0a	2.20a	88.0a	17.7a
3B	1.5b	11.5b	0.13b	8.7b	1.37b	91.3a	8.4b
4A	1.2a	10.5b	0.13a	10.8a	1.07a	89.2a	22.7a
4B	1.4a	17.0a	0.10a	7.1b	1.30a	92.9a	11.7b
5A	1.8a	17.3a	0.10a	5.6a	1.70a	94.4a	3.6a
5B	1.5a	13.8b	0.10a	6.7a	1.40a	93.3a	5.8a
6A	2.5a	18.4b	0.30a	12.0a	2.20a	88.0a	6.1a
6B	2.0a	21.5a	0.22a	11.0a	1.78b	89.0a	3.1b
7A	3.7a	19.9a	0.33a	8.9a	3.37a	91.1a	15.8a
7B	1.7b	15.5b	0.13b	7.6a	1.61b	92.4a	10.1b

Means of three replicates. In any pair of soils, means in a column headed by the same letter are not significantly different ($P < 0.05$ LSD)

As in the case of the humic substances, variability in the polysaccharides and water-soluble phenolics was higher in the reference soils than in the cropped soils. The coefficients of variability for total polysaccharides, labile polysaccharides, non-labile polysaccharides, and water-soluble phenolics were 41.4, 44.6, 48.2, and 48.3 %, respectively, in the reference soils, and 18.7, 32.8, 17.8, and 44.7 %, respectively, in the cropped soils.

5.3.3. Light fraction (LF) isolated from the selected soils

The total concentration of LF in the soil ranged from 8.0 to 26.9 mg/g in the reference soils, and from 6.9 to 14.4 mg/g in the cropped soils (Table 5.4). On average, the concentration of LF in the reference soils was almost double the concentration of LF in the cropped soils. While the carbon concentration of the LF from the reference soils ranged from 16.9 to 51.3 %, the carbon concentration of the LF from the cropped soils ranged 10.2 to 15.9 %.

Table 5.4: Concentration of light fraction and light fraction carbon in the selected reference and cropped soils.

Sample	Light Fraction (LF)		Light Fraction Carbon (LF-C)		Light Fraction Labile Carbon (LF-C_L)			
No.	mg/g	% C	mg C/g	% of C_T	mg C/g	% of LF-C	% of C_L	% of C_T
1A	15.1a	45.4a	6.9a	30.7a	2.5a	36.9a	70.4a	11.3a
1B	6.9b	15.9b	1.1b	11.7b	0.2b	20.4b	17.3b	2.4b
2A	26.9a	51.3a	13.8a	41.7a	4.6a	33.0a	72.4a	13.7a
2B	9.6b	12.4b	1.2b	13.5b	0.3b	21.4b	19.7b	2.9b
3A	19.8a	20.4a	4.0a	25.0a	1.5a	37.9a	38.1a	9.5a
3B	14.4b	15.2b	2.2b	17.1b	0.4b	18.7b	19.5b	3.2b
4A	11.6a	24.5a	2.8a	25.0a	0.9a	31.3a	45.7a	7.8a
4B	9.3b	10.6b	1.0b	12.1b	0.2b	19.6b	13.7b	2.4b
5A	8.0b	16.9a	1.4a	12.6a	0.2a	13.6b	19.2a	1.7a
5B	13.2a	10.2b	1.3a	12.5a	0.2a	16.6a	19.1a	2.1a
6A	10.7a	20.4a	2.2a	15.9a	0.7a	32.8a	24.3a	5.2a
6B	7.6b	10.5b	0.8b	8.6b	0.2b	22.5b	9.9b	1.9b
7A	15.8a	29.2a	4.6a	24.5a	1.4a	30.6a	37.4a	7.5a
7B	6.9b	15.2b	1.0b	9.4b	0.3b	24.7b	14.1b	2.3b

Means of duplicate determinations. In any pair of soils, means in a column followed by the same letter are not significantly different ($P < 0.05$ LSD)

On average, the carbon concentration of the LF from the reference soils was more than double the carbon concentration of the LF from the cropped soils (Table 5.4). The ranges in the carbon concentration of the LF meant that the LF contained between 12.6 and 41.7 % of the C_T in the reference soils, and between 8.6 and 17.1 % of the C_T in the cropped soils. The proportion of C_T in the reference soils present in the LF was, on average, more than double the proportion of C_T present in the LF in the cropped soils. This distribution of carbon in the light fraction meant that as much as 1.4 to 13.8 mg C_T/g is present as LF-C in the reference soils, while only 0.8 to 2.2 mg C_T/g was present as LF in the cropped soils (Table 5.4).

The $KMnO_4$-oxidisable carbon in the LF (LF-C_L) ranged between 0.2 and 4.6 mg C/g in the LF from the reference soils, while in the LF from the cropped soils the range was only 0.2 to 0.4 mg C/g (Table 5.4). These ranges in LF-C_L represent between 13.6 to 37.9 % of the carbon in the LF from the reference soils, and 16.7 to 24.7 % of the carbon in the LF from the cropped soils. On average, the LF-C_L represented about 31 % of the carbon of the LF from the reference soils, and about 21 % of the carbon of the LF from the cropped soils.

The relative proportion of $KMnO_4$-oxidisable carbon in the LF (LF-C_L) was higher than the proportion of $KMnO_4$-oxidisable carbon in the whole soil (C_L). In the whole soil, the C_L ranged from 10.5 to 20 % with a mean of 15.8 % of C_T in the reference soils, and from 11.5 to 21.5 % with a mean of 15.2 % of C_T in the cropped soils (Table 5.1). This means that about 19.2 to 72.4 % of the C_L in the reference soils is present in the LF, and about 10 to 20 % of the C_L in the cropped soils is present in the LF (Table 5.4). However, the LF-C_L constitutes only from 1.7 to 13.7 % with a mean of 8.1 % of the C_T in the reference soils, and from 1.9 to 3.3 % with a mean of 2.5 % of the C_T in the cropped soils (Table 5.4).

5.3.4. Microbial biomass carbon of incubated and non-incubated soils

The microbial biomass carbon in the three pairs of soils selected ranged from 117 to 359 µg C/g in the non-incubated soils and from 93 to 341 µg C/g in the incubated soils (Figure 5.1). In both the incubated and non-incubated soils, the microbial biomass carbon in the reference soils was almost double that in the cropped soils. After four weeks of incubation, the microbial activity was considerably lower in the incubated soils than in the non-incubated soils, although these differences were only found to be significant in three samples (Figure 5.1).

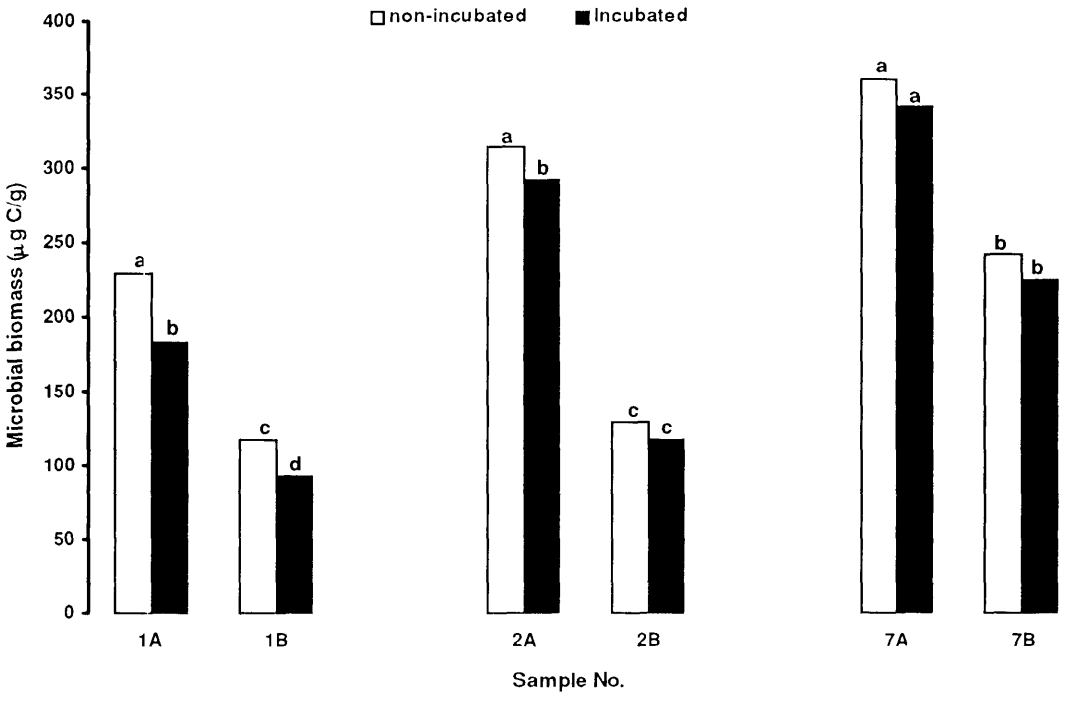

Figure 5.1: The concentration of soil microbial biomass carbon in three pairs of reference/cropped soils under different incubation treatments. Columns within a pair of soils headed by the same letter are not significantly different (P <0.05 DMRT)

5.3.5. Linear relationships between $KMnO_4$ carbon fractions and other organic carbon measurements

While the HA fraction showed no significant relationship to either C_L or C_{NL}, the FA fraction was significantly related to C_L but not to C_{NL} (Table 5.5). On the other hand, the humin fraction was found to be significantly related to C_{NL} but not to C_L. However, both C_L and C_{NL} were found to be significantly related to the sum of HA and FA, but only C_L was significantly related to the HA/FA ratio while C_{NL} showed no relationship to the HA/FA ratio.

Although C_L showed strong positive relationships to the total, labile and non-labile polysaccharides, C_{NL} was only related to the total and non-labile polysaccharides but not related to the labile polysaccharides (Table 5.5). There was no significant relationship between the water-soluble phenolics and either C_L or C_{NL}. While both the gross light fraction (LF) and the light fraction carbon (LF-C) were significantly related to C_L, C_{NL} was only significantly related to the LF-C but not to the LF. The C_L concentration was found to be significantly related to the microbial biomass carbon in the soils that were non-incubated, while there was no relationship between C_{NL} and microbial biomass carbon in either the incubated or non-incubated soils (Table 5.5).

Table 5.5: Linear relationships between $KMnO_4$ carbon fractions and other organic carbon measurements

Other organic carbon	C_L			C_{NL}		
measurements	Slope	Intercept	r^2	Slope	Intercept	r^2
Humic acid (HA)	-0.76	3.81	0.19ns	0.82	8.66	0.02ns
Fulvic acid (FA)	0.52	-0.31	0.91**	1.46	3.43	0.56ns
Humin	0.36	0.13	0.54ns	1.32	2.96	0.96**
HA/FA	-2.47	3.53	0.59*	-4.41	12.84	0.19ns
HA + FA	0.50	-1.31	0.76*	1.73	-1.57	0.91**
Polysaccharides						
Total	1.15	-0.07	0.71*	3.39	3.81	0.63*
Labile	10.80	0.24	0.84**	23.55	6.21	0.40ns
Non-labile	1.25	-0.03	0.68*	3.82	3.66	0.64*
Water-soluble phenolics[A]	0.10	1.11	0.39ns	0.29	7.43	0.32ns
Light fraction	0.21	-0.20	0.60*	0.56	4.04	0.44ns
Light fraction carbon	0.49	1.02	0.70*	1.75	6.30	0.91**
Microbial biomass C						
Non-incubated	15.54	-0.59	0.59*	52.73	2.04	0.45ns
Incubated	14.81	-0.07	0.54ns	48.60	4.14	0.39ns

Linear relationships were determined using C_L and C_{NL} as the dependent variables. ns = not significant; * = significant at $P < 0.05$; ** = significant at $P < 0.01$. [A] Water-soluble phenolics are in μg C/g.

5.3.6. CPMAS ^{13}C NMR spectra of $KMnO_4$ oxidised and non-oxidised samples

The ^{13}C NMR spectra of the oxidised and non-oxidised samples from the two pairs of reference (1A and 2A) and cropped (1B and 2B) soils are presented in Figures 5.2 and 5.3. To facilitate the interpretation of ^{13}C NMR data, spectra are commonly divided into four broad chemical shift regions. Peaks in the 0 - 50 ppm region generally represent carbon present in alkyl functional groups (eg., $-CH_3$, $-CH_2-$). This generally includes carbon in straight chain, branched and cyclic alkanes, and alkanoic acids (Hatcher *et al.*, 1994). About 50 % of alkyl carbon is usually attributable to extractable lipids and an additional 20 % to bound lipids extractable after acid hydrolysis. The remaining may be ascribed to cutin-like macromolecules (Zech and Guggenberger, 1996). Peaks in the 50 - 110 ppm region generally represent carbon present in o-alkyl groups ($-OCH_3$). This includes carbon in carbohydrates, ether, α-carbon of amino acids, carbon occurring in five- or six-membered rings bonded to -OH, and aliphatics containing carbon bonded to -OH (Skjemstad and Dalal, 1987; Guggenberger *et al.*, 1995b). Polysaccharides (cellulose and hemicellulose) account for most of the

o-alkyl resonances, though minor contributions are also due to oxygen-substituted lignin side chains, lignin methoxyl groups, and ester carbon in cutin and waxes (Zech and Guggenberger, 1996). Peaks in the 110 - 160 ppm region indicate the presence of carbon in aromatic compounds (Hatcher *et al.*, 1994). Aromatic carbon in soil is mainly due to lignin (Kögel *et al.*, 1988), though other aromatic compounds such as phenolic acids, and alkenes may also be present. Peaks in the 160 - 200 ppm region indicate the occurrence of carbon in carbonyl compounds. This includes carboxyl groups, esters, amides, ketonic and quinonic carbon (Newman *et al.*, 1980; Hatcher *et al.*, 1994).

A comparison of the ^{13}C NMR spectra of the oxidised and non-oxidised soils reveals a number of features (Figures 5.2 and 5.3). Firstly, in the non-oxidised soils, resonances due to the o-alkyl groups (50 - 110 ppm) dominate the spectra. The next largest peaks are the alkyl (0 - 50 ppm) and then the aromatics (110 - 160 ppm). The differences in the intensity of these peaks are clearer in the reference soils than in the cropped soils. The sharpness of the peaks at around 30 - 32 ppm indicates a predominance of $-CH_2-$ structures within the alkyl species (Oades *et al.*, 1987). In all the reference and cropped soils, the intensity of the peaks at 0 - 50 ppm (alkyl C) was higher in the oxidised samples than in the non-oxidised samples. In the reference soils (1A and 2A), a significant reduction in the intensity of the resonance at 50 - 110 ppm (o-alkyl C) due to oxidation was apparent (Figures 5.2 and 5.3). This was also accompanied by substantial reductions in the intensity of the peaks around 110 - 160 ppm (aromatic C) and 160 - 200 ppm (carbonyl C). This observation for the reference soils was however, not apparent in the spectra of the cropped soils (Figures 5.2 and 5.3). In both the cropped soils (1B and 2B), there was an increase in the proportion of the alkyl C and a decrease in the proportion of carbonyl C in a similar manner as for the reference soils. However, in both the cropped soils, the proportion of aromatic C increased following oxidation by $KMnO_4$.

The relative proportions of the different functional groups between the oxidised and non-oxidised samples, as calculated from the area under each peak, reveal major differences (Table 5.6). In both the reference soils (1A and 2A), there was a lower proportion of carbonyl C, aromatic C, and o-alkyl C in the oxidised soil than in the non-oxidised soil, while the proportion of alkyl C was higher in the oxidised than in the non-oxidised soil (Table 5.6). This trend was however, not observed in both of the cropped soils (1B and 2B) wherein the aromatic C was higher in the oxidised than in the non-oxidised soil (Table 5.6). It therefore appears that in native or uncropped soils, oxidation by $KMnO_4$ resulted in declines in the proportions of carbonyl C, aromatic C, and o-alkyl C, but increases the proportion of alkyl C. Generally, oxidation with $KMnO_4$ resulted in reductions in the proportions of o-alkyl C and carbonyl C. A comparison of the relative changes in all the functional groups due to oxidation shows that, on average, the greatest reduction was in the aromatic group, followed by the o-alkyl and then the carboxyl group. The alkyl group generally increased in proportion after oxidation.

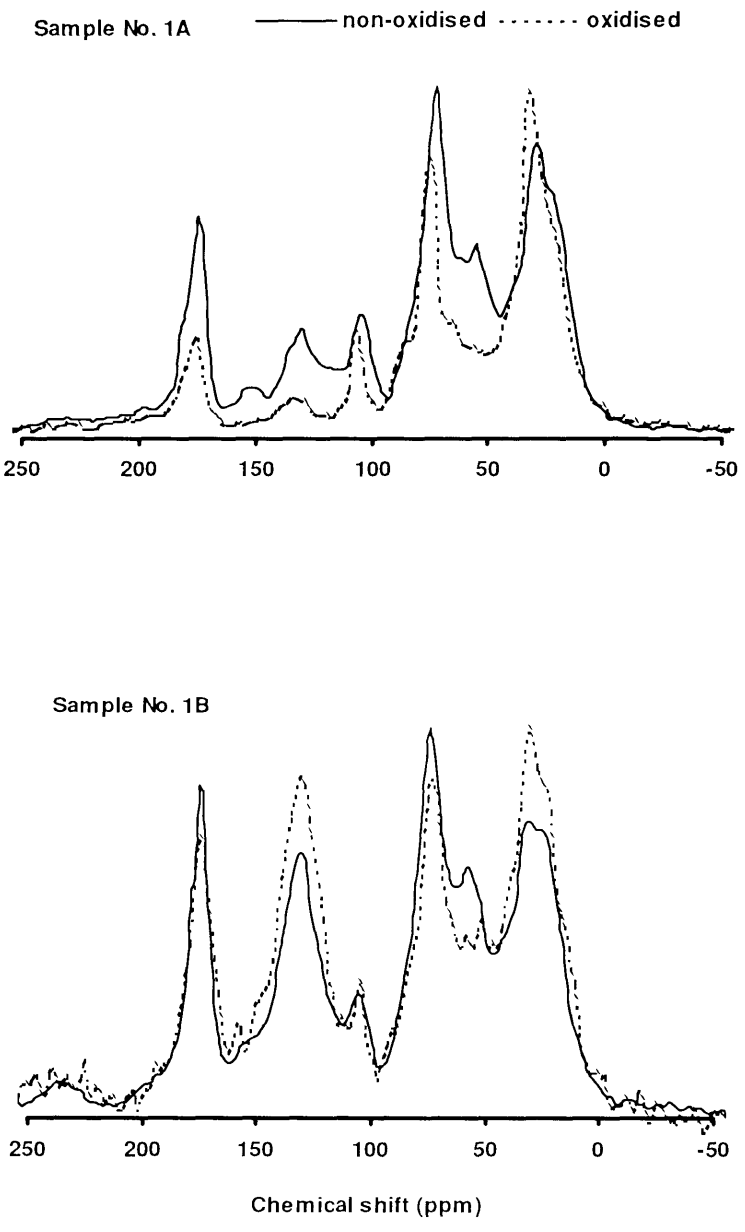

Figure 5.2: A comparison of CPMAS ^{13}C NMR spectra of non-oxidised and the $KMnO_4$-oxidised soil (Samples 1A and 1B)

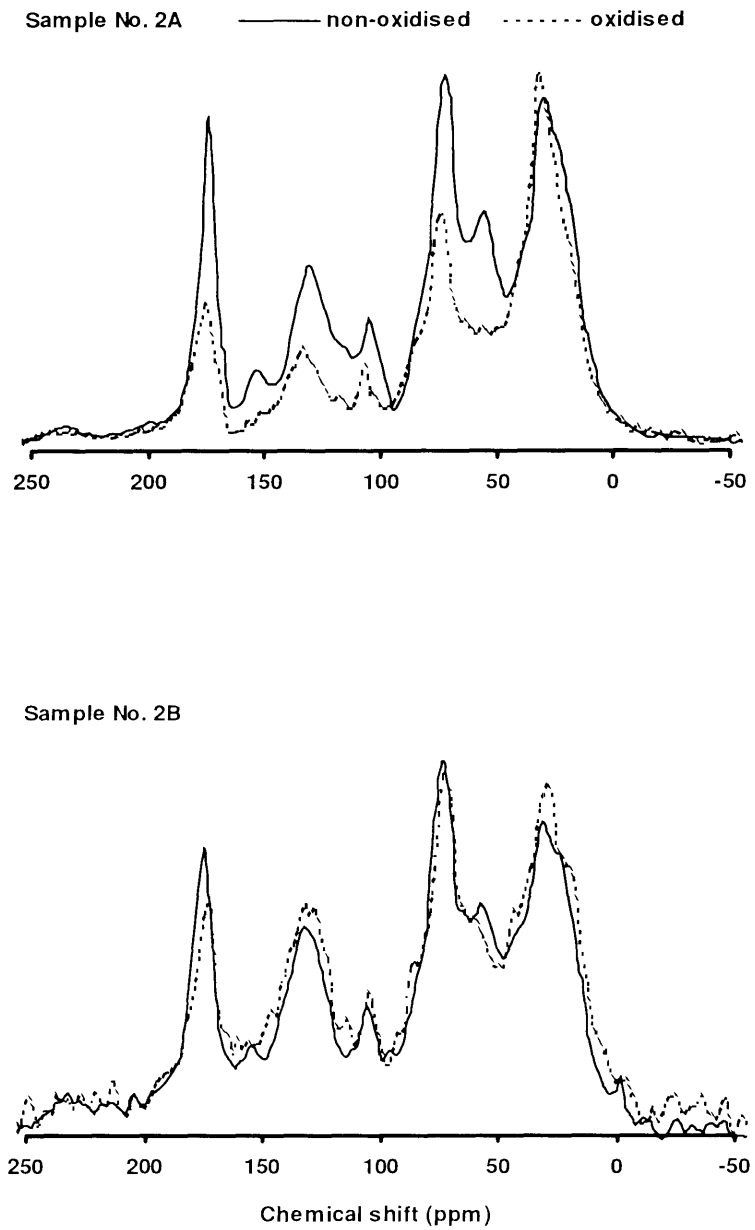

Figure 5.3: A comparison of CPMAS ^{13}C NMR spectra of the non-oxidised and the $KMnO_4$-oxidised soil (Samples 2A and 2B).

Table 5.6: Relative proportion of different carbon species in non-oxidised and $KMnO_4$ oxidised soil

		Carbonyl C	Aromatic C	O-alkyl C	Alkyl C	O-alkyl/Alkyl
Sample No.	Treatment		% of Spectra			
1A	Non-oxidised	14	14	42	31	1.35
	Oxidised	9	5	39	47	0.83
1B	Non-oxidised	15	23	35	27	1.30
	Oxidised	14	32	28	26	1.08
2A	Non-oxidised	13	18	38	31	1.22
	Oxidised	10	14	32	44	0.73
2B	Non-oxidised	17	23	35	25	1.40
	Oxidised	14	24	35	27	1.30

5.3.7. Near Infrared Reflectance (NIR) spectra of $KMnO_4$-oxidised and non-oxidised soil

The near infrared has been defined as that part of the electromagnetic spectrum lying between the visible region and the fundamental infrared region (Day and Fearn, 1982). Thus, it covers the range of wavelengths from 800 to 2500 nm. The NIR spectra of soils is often expressed as absorbance (log1/R, where R is the reflectance). The relationship between organic matter concentration and spectral absorbance in the near infrared region has been demonstrated by several workers (Bowers and Hanks, 1965; Al-Abbas *et al.*, 1972; Krishnan *et al.*, 1980; Dalal and Henry, 1986; Fritze *et al.*, 1994). It can be seen that for both pairs of soils, the absorbance of the reference soils was consistently higher than the absorbance of the cropped soils throughout the near infrared spectral region (Figure 5.4). The trends observed in the absorbance pattern were similar for the reference and cropped soil throughout the NIR spectra. When the soils were oxidised to remove the labile carbon, there was a shift in the absorbance pattern of the oxidised soil when compared to the unoxidised soil (Figures 5.5 and 5.6). It can be seen that in the pair of samples from the Gwydir valley (1A and 1B), the NIR spectrum of the non-oxidised sample was lower than that of the oxidised sample between the wavelengths of 400 and 1300 nm (Figure 5.5). The spectra of both the oxidised and non-oxidised samples remained close together until at a wavelength of 1600 nm, after which the NIR spectrum of the oxidised soil fell lower than that of the non-oxidised soil. A closer examination of the spectra of the oxidised soils and the non-oxidised soils at wavelengths beyond 1600 nm reveals that differences were greater in the reference soil (1A) than in the cropped soil (1B). The spectra of the oxidised and non-oxidised soils from the second pair of samples (2A and 2B) were very similar to those obtained in the first pair of samples (Figure 5.6), with some slight variations. In the second pair of samples, the NIR spectra of the oxidised soil became lower than that of the non-oxidised soil at a lower wavelength (1100 nm) than the first pair of samples. Also, the differences between the spectra

of the oxidised and non-oxidised soil were more pronounced in the reference soil of the second pair (2A) than in the cropped soil (2B).

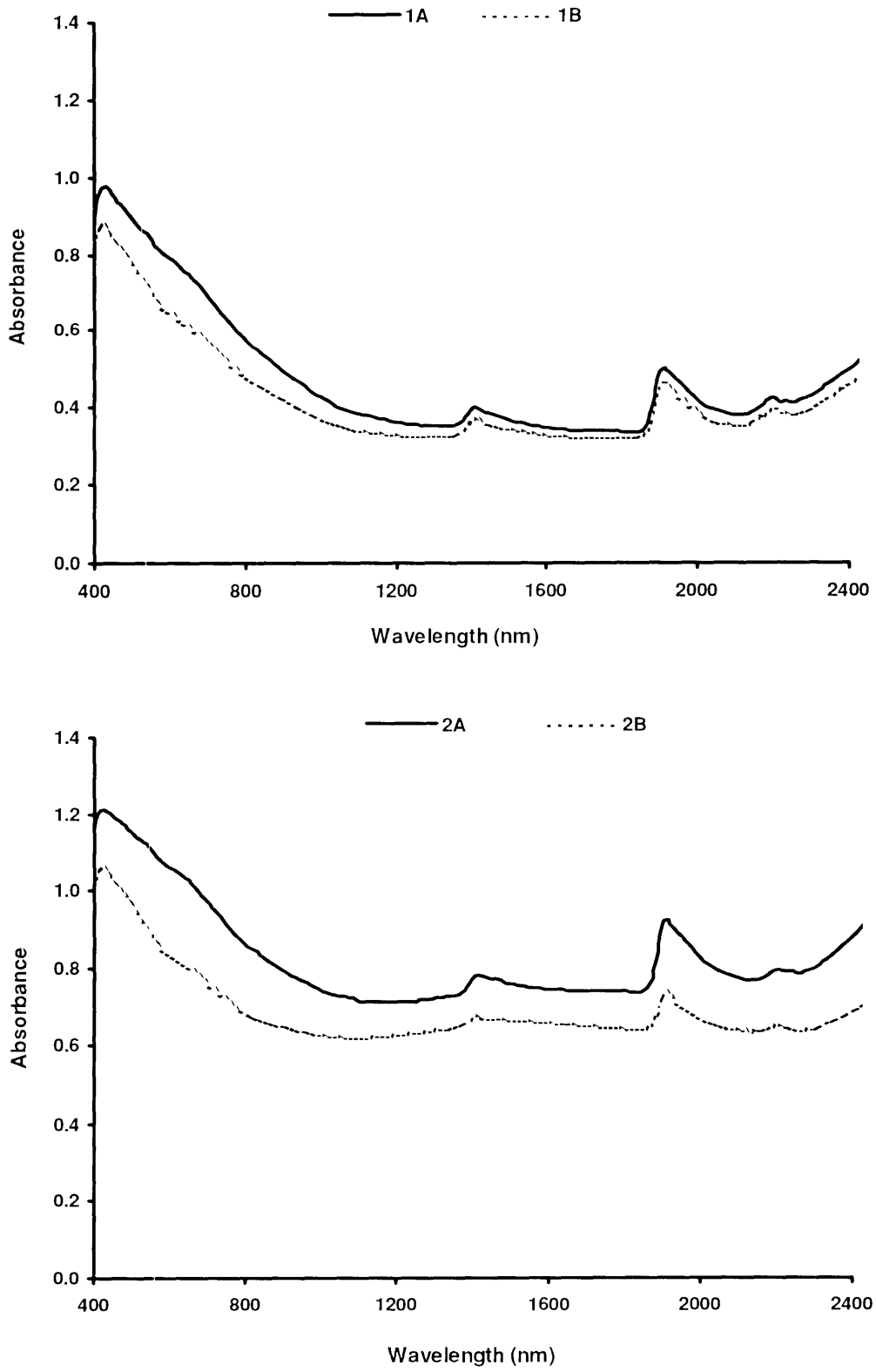

Figure 5.4: Near Infrared Reflectance (NIR) spectra of two pairs of reference and cropped soils

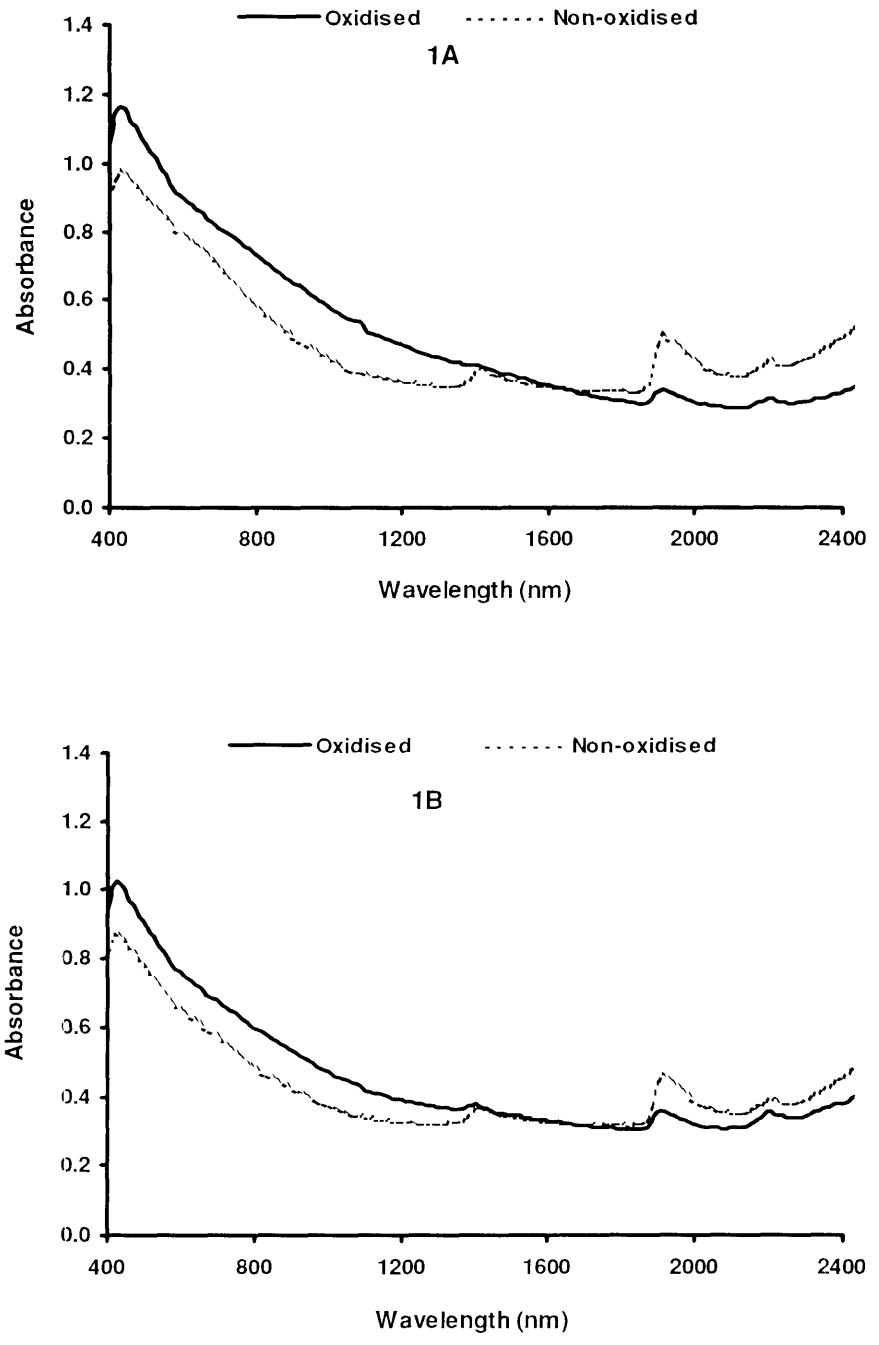

Figure 5.5: Near Infrared Reflectance (NIR) spectra of oxidised and non-oxidised soil from reference and cropped sites (1A and 1B)

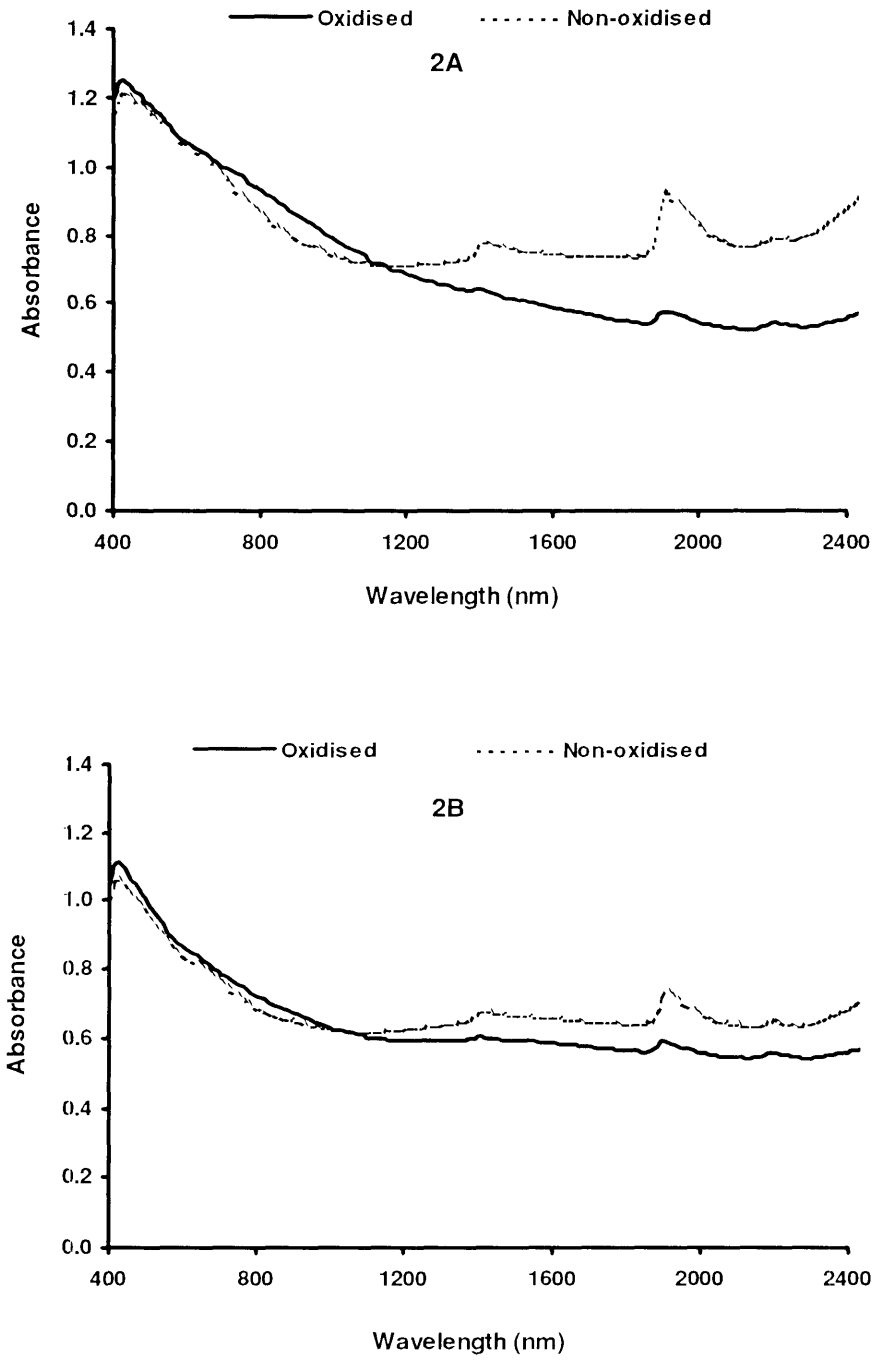

Figure 5.6: Near Infrared Reflectance (NIR) spectra of oxidised and non-oxidised soil from reference and cropped sites (2A and 2B)

5.4. DISCUSSION

5.4.1. Humic substances and their relationships to $KMnO_4$ carbon fractions

As mentioned earlier in this thesis (Chapter 2), the fractionation of humic substances into humic acid (HA), fulvic acid (FA) and humin was one of the earliest fractionation schemes used for studying soil organic matter. Because the fractions obtained by this scheme are defined by the procedure employed, their utility in the study of soil organic matter dynamics had been limited. In Table 5.2, it was shown that about 38 % to 65 % of the C_T was extracted as humic materials. This range is in close agreement with several earlier findings (eg Schnitzer and Vendette, 1975). Anderson and Schoenau (1993) reported that about 30 to 60 % of the total soil organic carbon is usually removed by alkali extractants. Górniak and Urban (1996) found extracted humic substances to vary between 27 and 65 % of the total soil organic carbon while Fujitake *et al.* (1996) found that humic substances comprised 22 to 85 % of the total soil organic carbon. It is interesting to note that with the exception of the HA/FA ratio, all humic fractions were lower in most of the cropped soils than in the corresponding reference soils (Table 5.2). Zaujec *et al.* (1996) indicated that the carbon concentration of HA increases as humification proceeds. Therefore, the higher HA/FA ratios observed in most of the cropped soils than in the reference soils (Table 5.2) indicates increased humification of soil organic matter as the soils are subjected to cultivation. It was also shown that in most of the soils, the concentration of FA in the humic extracts was more than the concentration of HA (Table 5.2). This finding is in agreement with the finding of Górniak and Urban (1996). Schulthess *et al.* (1996) suggested that alkaline solutions attack the HA compounds to produce FA-like products, and that the longer the soil organic matter is in contact with the alkaline solution, the greater will be the amount of FA that is formed. It has also been reported that the amount of total organic matter extracted did not increase after three hours, but the proportion of FA present in the extracting liquid continued to increase (Schulthess *et al.*, 1996). This possibly explains the higher FA concentration in the NaOH-extracted organic matter.

The linear relationships between the humic fractions and the $KMnO_4$ carbon fractions showed that the HA fraction was not related to either the C_L or the C_{NL}, but the FA fraction was significantly related to the C_L and not to the C_{NL} (Table 5.5). Although the actual chemical composition of the humic fractions is still debatable, much progress has been made. The labile components of soil organic matter are generally believed to consist of cellular biopolymers such as carbohydrates, amino acids, peptides and amino sugars (Piccolo, 1996). The HA fraction contains less of these components and is believed to be the most biologically resistant fraction of soil organic matter with strongly condensed aromatic structures surrounded by aliphatic side chain components (Anderson and Schoenau, 1993). Other reports have also considered the HA to be very stable in soil (Anderson, 1979), corresponding to the chemically stabilised organic matter postulated by Jenkinson and Rayner (1977) and Van Veen and Paul (1981). On the other hand, the FA fraction is believed to comprise mainly carbohydrates, microbial metabolites and younger material not highly associated with the

mineral fraction. These differences between HA and FA possibly explain why FA is related to the $KMnO_4$-oxidisable C (C_L) but not the HA (Table 5.5).

It is interesting to note that the humin fraction was significantly related to C_{NL} but not to C_L. Although the humin fraction is less understood, as compared to HA and FA, Hatcher *et al.* (1985) suggested that the humin fraction consisted of highly condensed HA, fungal melanins, and paraffinic structures. Also, Almendros and Gonzalez-Villa (1987) found a high proportion of polymethylene compounds (fatty acids) that seem to be inherited from the waxes of higher plants, and further suggested that during biodegradation, the lipid polymers are altered and incorporated into the humin fraction. Generally, soil humins are considered to be non-extractable humic type polymers that form strong associations with the soil mineral matrix and are not easily separated by the usual alkaline reagents. Therefore, the strong relationship between C_{NL} and humin (Table 5.5) shows that C_{NL} consists of the soil organic matter components that are stabilised due to chemical or physical associations within the soil mineral matrix.

While the HA/FA ratio showed no relationship to the C_{NL}, the relationship between the C_L and the HA/FA was significant, and it shows that C_L decreases as the HA/FA ratio increases (Table 5.5). This is not surprising, considering that the HA/FA ratio increases due to increased humification as the soils are subjected to cultivation. As already mentioned earlier in this thesis (Chapter 2), the HA/FA ratio is an indication of the extent of humification of the soil organic matter. In simple terms, humification is the biological, microbial or chemical conversion of organic residues to humus, which is the fraction most resistant to degradation (Tate, 1987). Although the pathways from fresh organic inputs to humus are not well elucidated, there has been some progress in understanding the process. Insam (1996) stated that either through microbial degradation or through microbial synthesis, phenolic units may be formed. These phenolic substances may further be transformed through hydroxylation, decarboxylation, and various oxidative mechanisms to form numerous mono-, di-, and tri-hydroxyphenols and benzoic acids. If these products are not further degraded, they may form highly active radicals or hydroxybenzoquinones which link with other phenolic units, peptides and amino acids to form the large humic acid molecules. From this, it can therefore be seen that increases in humification result in increases in the HA/FA ratio, which in turn is accompanied by a decrease in the amount of labile organic carbon.

5.4.2. Soil polysaccharides and their relationships to $KMnO_4$ carbon fractions

Measurements of soil polysaccharides have also been used extensively for studying soil organic matter dynamics. Because polysaccharides comprise 50 to 70 % of the dry weight of most plant tissue, they are often the most abundant materials added to the soil in the form of plant residues (Lowe, 1978). Interest in soil polysaccharides has mainly resulted from their suggested role in the development of soil structure, following the demonstration that bacterial polysaccharides could cause

aggregation of sand-clay mixtures (Lowe, 1978). A considerable amount of information is now available concerning the nature and concentration of polysaccharides in soils (Oades, 1972; Greenland and Oades, 1971). As reported in Table 5.3, the total polysaccharides in the soils studied constituted between 10 and 20 % of C_T in the reference soils and between 11 and 21 % of C_T in the cropped soils. These ranges are in close agreement with those reported earlier by Oades (1972) for Australian soils.

From the linear relationships (Table 5.5), it was seen that while the C_L concentration showed a strong relationship to the total, labile and non-labile polysaccharides, the C_{NL} was only related to the total and non-labile polysaccharides but not to the labile polysaccharides. This is consistent with the nature of polysaccharides in soil. It is known that total polysaccharides isolated from soil usually consist of mixtures of polysaccharides ranging from easily decomposable sugars to complex and resistant polysaccharides such as chitin. Among the total polysaccharides isolated are those bound through ester linkages to soil humic fractions (Chesire, 1979). Also included among the total polysaccharides isolated from the soil are simple sugars such as glucose, galactose, mannose, arabinose, xylose and rhamnose. It can therefore be seen that the more total polysaccharides, the higher the concentration of both labile and non-labile carbon in the extracts. Since the labile polysaccharides only include simple sugars such as those mentioned above, it is not surprising that they are only related to the C_L but not to the C_{NL} (Table 5.5). The strong relationship between the non-labile polysaccharides to both C_L and C_{NL} suggests that the non-labile polysaccharides comprise of both decomposable and resistant polysaccharide components. The decomposable polysaccharide components are made non-labile because of association with non-polysaccharide constituents. Lowe (1978) indicated that resistance to decomposition in some polysaccharides is partly a function of inaccessibility without necessarily involving biologically stable structures. It is therefore very likely that the non-labile polysaccharide consists of significant quantities of chemically simple and readily oxidisable sugars but made non-labile as a result of association with other soil constituents.

5.4.3. Water-soluble phenolics and their relationships to $KMnO_4$ carbon fractions

Water-soluble phenolics in soils have been studied mainly because of their known or postulated involvement in allelopathy (Jalal and Read, 1983), metal translocation and moderation of metal toxicity (Glass, 1973), their role as humus precursors (Insam, 1996), and as markers of vegetative origin of soil organic matter (Vance *et al.*, 1986). Simple phenols and phenolic acids can be present in the soil solution or in adsorbed or esterified forms, as well as occurring as structural units in complex humus polymers or plant residues (Lowe, 1993b), which together make up a large proportion of soil organic matter. The concentration of water-soluble phenolics in the soils studied in this thesis is generally small (Table 5.3), and no relationship was observed between the water-soluble phenolics and either the C_L or C_{NL} (Table 5.5). From the literature, it appears as if the importance of water-soluble phenolics is mainly in peats or heathland soils. The absence of any significant

relationship between the water-soluble phenolics and either the C_L or C_{NL} could most probably be due to the minute quantities present in the soils studied (Table 5.3).

5.4.4. The light fraction (LF) and relationships to $KMnO_4$ carbon fractions

The importance of the light fraction (LF) as an estimate of the labile organic carbon in soils was emphasised earlier in Chapter 4. Although it generally occupies a small portion of the soil mass, the LF has been reported to contain a substantial portion of the total soil organic carbon because of its high carbon concentration. It was shown in Table 5.4 that the LF isolated from the soils in this study contained from 8.6 % up to 41 % of the total soil organic carbon. This range is in close agreement with ranges reported in several earlier reports (eg Gregorich and Ellert, 1993; Tiessen and Stewart, 1983). However, despite its wide usage and its established sensitivity to management effects, the chemical composition of the LF is not well understood. This has been a major limitation in interpreting the relationships observed between the LF and the $KMnO_4$ carbon fractions in this study. Gregorich and Janzen (1996) mentioned that the organic matter in the LF is comprised primarily of plant debris, as evident by its cellular structure, but may also contain fungal hyphae, spores, seeds, charcoal and animal remains. Based on ^{13}C NMR analyses, Baldock *et al.* (1992) and Golchin *et al.* (1994) suggested that the largest portion of carbon in the LF is present as polysaccharides. Dalal and Henry (1988) also stated that the LF is highly enriched in polysaccharides. These findings were further buttressed by the report of Gregorich and Ellert (1993) which stated that the LF organic matter comprised of high concentrations of oligosaccharides, polysaccharides, and hemicellulose, and thus serves as a readily decomposable substrate for microorganisms in soil. It therefore follows that the significant relationship shown between the LF and the $KMnO_4$ labile carbon (Table 5.5) is most probably due to the polysaccharide concentration in the LF.

It has been stated in several parts of this thesis that the LF also contains charcoal and lignin which are known as resistant carbon fractions. This is an indication that, although the LF can be used as a measure of labile carbon in soil, only a certain proportion is decomposable within a short time. To be able to compare this decomposability with that of the whole soil organic matter, the $KMnO_4$-oxidisable carbon in the LF was determined. It is interesting to note that while the $KMnO_4$-oxidisable carbon in the whole soil represents, on average, 15.8 and 15.2 % of the C_T in the reference and cropped soils respectively, as much as 72 % of this oxidisable carbon is present in the soil as light fraction (Table 5.4). This observation, and the fact that the proportion of oxidisable carbon in the LF is about twice that in the whole soils, shows a very strong link between the $KMnO_4$-oxidisable carbon in the soil and the carbon present in the soil as light fraction.

5.4.5. Soil microbial biomass carbon and relationships to $KMnO_4$ carbon fractions

The carbon present in soil microbial biomass has been suggested as a useful and sensitive measure of changes in soil organic matter status (Powlson *et al.*, 1987; Sparling, 1992). Because this fraction is known to have a rapid turnover rate of 1 to 2 years (Jenkinson and Ladd, 1981), changes in this fraction can be detected long before they are detectable in the total organic carbon. Inspite of its usefulness, estimation of microbial biomass carbon poses strong limitations (Martens, 1995). In this study, one of the most common procedures, the substrate-induced respiration (Anderson and Domsch, 1978), was used. As was shown in Figure 5.1, the microbial carbon ranged from 117 to 359 μg C/g and 93 to 341 μg C/g for the non-incubated and incubated soils, respectively. These ranges are generally smaller than most ranges (1 - 5% of C_T) reported for microbial biomass carbon in soils (Sparling, 1992; Zech and Guggenberger, 1996). It is most likely that the storage of the soil in dry conditions at room temperature for a considerable length of time (> 1 year) contributed to the low levels of microbial carbon. However, it can be seen that some microbial spores were able to survive during the storage period. When these soils were moistened and incubated, there was a flush of microbial activity from the surviving microbes which utilised the cells of the dead microbes and other sources of labile carbon during the incubation period. Marumoto *et al.* (1982) observed that about 50 % of the total carbon was released as CO_2 from dead ^{14}C-labelled microbes during the first 28 days of incubation at 22°C. Since there was still a ready supply of labile carbon in the non-incubated (unwetted) soils, it was observed that microbial activity was higher in the non-incubated soils than in the incubated soils. Of particular interest is the significant relationship between the microbial carbon in the non-incubated soil and the $KMnO_4$-oxidisable carbon (C_L) which is not significant in the incubated soils. Since the C_L was measured on the non-incubated soil, this C_L must have included the carbon present in the soil microbial biomass, both dead and surviving, and thus both C_L and microbial carbon were strongly related. When the soil was incubated, some of the labile carbon present as dead microbes would have been lost due to the stimulated activity of the surviving biomass. As a result the relationship between the $KMnO_4$-oxidisable carbon (C_L) and the microbial biomass diminished.

5.4.6. ^{13}C nuclear magnetic resonance (NMR) and near infrared reflectance (NIR) spectra of $KMnO_4$ oxidised and non-oxidised soil

As a result of the broad nature of the peaks in the ^{13}C NMR spectra, only information on the carbon types (eg aromatic, carbonyl, alkyl, etc) could be obtained. Since each of these carbon types may contain a wide range of organic compounds, and that the same organic compound can be present in two or more of these broad peaks, interpretation of the ^{13}C NMR spectra can only be considered as semi-quantitative. It is clear that organic compounds such as benzenecarboxylic acids can be represented in both the aromatic and the carbonyl regions, while carbohydrates can be represented in both the o-alkyl and carbonyl regions of the ^{13}C NMR spectra. Nonetheless, the

^{13}C NMR spectra were useful in providing information as to which functional groups were more sensitive to oxidation by $KMnO_4$ solution.

The spectra obtained for the two pairs of soils (Figures 5.2 and 5.3) were similar to those obtained by Skjemstad and Dalal (1987) for Vertisols, with a dominance of aliphatic groups (o-alkyl and alkyl). This dominance of aliphatics is supported by the relatively high E4/E6 ratios presented in Table 5.2. Chen *et al.* (1977) and Anderson and Schoenau (1993) indicated that a high E4/E6 ratio reflects a low degree of aromatic condensation, and infers the presence of large proportions of aliphatic structures. It was also seen from the spectra (Figures 5.2 and 5.3) that the effect of oxidising the soil by $KMnO_4$ solution on the o-alkyl C, carbonyl C, and alkyl C was similar for all soils, reference or cropped. In all the soils, there was a reduction in the proportion of the o-alkyl C and carbonyl C, while the proportion of alkyl C increased (Figures 5.2 and 5.3). Considering that carbohydrates account for most of the o-alkyl C resonances, and that carbohydrates are polyhydroxy aldehydes and ketones, it is highly probable that the most profound effect of the $KMnO_4$ oxidation was on the carbohydrates in the soil. It can also be seen from the spectra that this effect was more pronounced on the reference soils than on the cropped soils (Figures 5.2 and 5.3). As mentioned earlier in this thesis (Section 2.5.7), the most noticeable change in the initial stages of decomposition is a decrease in the ratio of o-alkyl to alkyl C (Amalfitano *et al.*, 1995; Baldock and Preston, 1995). This can be seen in Table 5.6 that the ratio of o-alkyl to alkyl C was substantially reduced as a result of $KMnO_4$ oxidation. This change has often been associated with the loss of the most easily metabolisable carbohydrates and an accumulation of alkyl C. Kinchesh *et al.* (1995b) used ^{13}C NMR to study some Rothamsted soils and found that, in all sites, carbohydrate C was the form that was most subject to change. These findings suggest that the effect of $KMnO_4$ oxidation on soil organic matter are similar to the effects of cultivation on soil organic matter.

The increases in the proportion of alkyl C due to $KMnO_4$ oxidation could have resulted from several mechanisms. Firstly, the possibility exists that $KMnO_4$ oxidation has no effect on carbon present in the alkyl group while the other forms of carbon were oxidised. This can increase the proportion of carbon present as alkyl C. Alternatively, the oxidative action of $KMnO_4$ on alkyl C was less than that obtained on the other carbon types, and this can also increase the proportion of alkyl C. However, the most probable mechanism is one in which carbon is being transformed from other forms to alkyl C during oxidation. Although, studies utilising neutral $KMnO_4$ oxidation of soil organic matter are rare, Schnitzer and Vendette (1975) found that more than 50 % of the products resulting from alkaline $KMnO_4$ oxidation of an Arctic humic acid were alkyl in nature. Recent studies have shown that during humification, the proportion of alkyl C tends to increase in both temperate and tropical environments (Krosshavn *et al.*, 1992; Zech and Guggenberger, 1996). However, extractable as well as bound lipids, which form a significant component of the alkyl C, were found to decrease during humification (Zeigler, 1989), and thus do not account for the increase of alkyl C. Similar observations were reported for cutin and suberin (Zech and Guggenberger, 1996). Therefore, the

most probable mechanism for the increase in the proportion of alkyl C during humification was attributed to cross-linking of lipid material and cutin- or suberin-like polyesters during humification. Also, Wang and Huang (1992) showed that pyrogallol, a trihydric phenol, could easily be transformed abiotically in the presence of MnO_2, a soil constituent and a product of $KMnO_4$ oxidation, to structures showing ^{13}C NMR spectra very similar to oxidised humic acids. In this study, it was concluded that oxidative processes could oxidise lignin-like aromatic ring systems while preserving aromatic character and increasing aliphatic content (Wang and Huang, 1992). Thus, the changes occurring in the alkyl C in soil during $KMnO_4$ oxidation can be similar to the changes occurring in soil organic matter during humification.

It was seen from the ^{13}C NMR spectra (Figures 5.2 and 5.3) and the relative proportions of the different carbon species (Table 5.6) that the effects of $KMnO_4$ oxidation on the aromatic carbon differed between the reference and the cropped soils. In the reference soils (1A and 2A), there was a decrease in the proportion of aromatic C due to $KMnO_4$ oxidation, while in the cropped soils (1B and 2B), the proportion of aromatic C increased. It was also seen that in both pairs of soils, the proportion of aromatic C was higher in the cropped soils than in the reference soils (Table 5.6). In their study on the changes in carbon species distribution of humic substances with extent of humification in a humic Cambisol in Germany, Zech *et al.* (1994) found that aromatic carbon increased during humification. As pointed out earlier in this chapter (Section 5.4.1), humification increases as the soils are subjected to cultivation. The higher proportion of aromatic C in the cropped soils relative to the reference soils is therefore to be expected.

Although aromatic C in soil is mainly due to lignin (Kögel *et al.*, 1988), Zech *et al.* (1994) reported that increases in aromatic C during humification are due to non-lignin aromatic structures, because lignin determined by wet chemical methods was found to decrease with humification. Similar observations were reported by Inbar *et al.* (1992) and Kögel-Knabbner *et al.* (1992b) in which the proportion of aromatic C increased during humification while the lignin-derived C decreased. Since the aromatic structures do not diminish with increasing humification, this implies that the aromatic structures are retained from the original lignin in some altered form or derived from additional structures added to the soil by microorganisms. This increase in non-lignin aromatic structures could have been stimulated by catalytic effects of soil minerals, promoting the formation of recalcitrant aromatic structures (Wang *et al.*, 1986). Alternatively, such structures may have been derived from black carbon or charcoal (Haumaier and Zech, 1995). It can therefore be argued that in the cropped soils, there was a higher proportion of recalcitrant aromatic structures which were not affected by the $KMnO_4$ oxidation. Since the proportion of the o-alkyl C and the carbonly C were reduced by the $KMnO_4$ oxidation, the result was that the proportion of aromatic C increased in the cropped soils. Because the aromatic C in the reference soils was not as resistant as that in the cropped soils, a reduction in aromatic C was observed in the reference soils due to $KMnO_4$ oxidation.

With regards to the near infrared reflectance (NIR) spectra (Figures 5.4 to 5.6), the observations can only be considered as suggestive, since the use of NIR spectroscopy for qualitative studies of soil organic matter are limited. In a review of the history and development of NIR as an analytical technique, Day and Fearn (1982) pointed out that the merits of NIR as a quantitative technique are well proven whereas its use as a qualitative technique is highly limited. Thus, most studies utilising the technique of NIR have mainly been on quantitative analysis of soil organic matter (eg Al-Abbas *et al.*, 1972; Krishnan *et al.*, 1980; Dalal and Henry, 1986). However, Fritz *et al.* (1994) suggested that information on organic matter collected from near infrared spectra can be very useful for explaining biological variations in soil humus. Of particular interest in the NIR spectra obtained in this study is the differences observed between the two pairs of soils (Figure 5.4). It was seen that though the NIR spectra for the reference and cropped soils were similar in both pairs of soil, the differences between the absorbance of the reference and cropped soil were much higher in the second pair of soils (2A and 2B) than in the first pair of soils (1A and 1B). These differences possibly reflect the differences in total carbon concentration (C_T) between the reference and cropped soils. In the first pair of soils (1A and 1B), the difference in C_T between the reference and cropped soil was 58 %, while in the second pair of soils (2A and 2B), the difference in C_T was 73 % (Table 5.1). However, an examination of the spectra of the oxidised and non-oxidised soil showed that absorbance of the oxidised soil was higher than that of the non-oxidised soil at lower wavelengths, while the reverse occurred at higher wavelengths (Figures 5.5 and 5.6). These spectra suggest that during $KMnO_4$ oxidation, organic matter that absorbs at the higher wavelength regions was oxidised, and possibly converted to organic matter absorbing at lower wavelength regions.

5.5. CONCLUSIONS

The labile components of soil organic matter can be appropriately determined based on ease of oxidation by neutral $KMnO_4$ solutions. Labile carbon determined by this procedure is significantly related to fulvic acid, soil polysaccharides and soil microbial biomass carbon. The $KMnO_4$ oxidisable carbon mostly comprise of soil carbohydrates and some unidentified aromatic compounds. The association between C_L and fulvic acid, carbohydrates and microbial biomass carbon indicate that the term labile is appropriate for $KMnO_4$ oxidisable carbon, and that the un-oxidisable C_{NL} is related to soil humin and non-labile polysaccharides. Therefore, the partitioning of soil carbon into C_L and C_{NL}, as shown in this study, allows the separation of active and less active soil carbon to be used for monitoring carbon dynamics of agricultural systems.

CHAPTER 6

THE EFFECTS OF COTTON STUBBLE MANAGEMENT SYSTEMS AND COTTON ROTATION SEQUENCES ON SOIL ORGANIC CARBON FRACTIONS

6.1. INTRODUCTION

The importance of soil organic carbon to soil fertility and sustainable crop production is now well recognised and has been reviewed earlier in this thesis (Chapter 2). In Chapter 4, it was shown that cultivation has resulted in substantial losses of the organic carbon status of the soils used for cotton production. The effect of cultivation was found to be more pronounced in the labile carbon (C_L) and the carbon management index (CMI) than in the total carbon (C_T) and non-labile carbon (C_{NL}). Soil compaction, a major concern for cotton growers (Shaw, 1993), and many other soil problems have been associated with losses in soil organic matter levels. Wallace (1994) indicated that the decrease in soil organic carbon is a better indicator of soil degradation than was tons of soil loss.

Increasing public and grower concern about soil and environmental quality in relation to long-term sustainable cotton production has emphasised the need to develop and implement management strategies that maintain and protect soil resources. Because crop residues contain considerable quantities of carbon and nutrients (Maskina *et al.*, 1993), their management can markedly affect the input-output balance of plant nutrients and soil organic matter. In cotton cropping systems, considerable amounts of all nutrients are returned to the soil during defoliation. This source of carbon and nutrients is readily decomposed by soil micro- and macro-organisms, and represents a flush of nutrients into the system at a time when crop demand is low (Blair *et al.*, 1995a). A significant amount of nutrient ends up in the stalks and capsules, and these are the components that can be managed on the farm. Traditionally, these stalks have been mechanically slashed at ground level and incorporated into the soil with disc and chisel-ploughing (Rochester *et al.*, 1997). More recently, the practice of raking and burning of the stubble has become widespread as a means of easing tillage operations, irrigation management, and reduction of cotton disease incidence. This practice not only results in a loss of 1.4 Mg C/ha every year (Rochester *et al.*, 1997), but also contributes to the atmospheric CO_2 pool.

Although the incorporation of crop residues in soil is widely believed to improve soil organic matter levels, there have been some conflicting reports in the literature. For instance, Rasmussen and Collins (1991) reported the results of a comparative study of residue-burnt (RB) and residue-incorporated (RI) and showed that although the RI treatments gave rise to higher organic matter levels, the effects of burning crop residues on the soil organic matter concentration after 45 years of cropping was not significant. While Pikul and Allmaras (1986) generally found less organic carbon in soils where residue was incorporated, Moss and Cotterill (1986) found greater soil organic carbon in the surface soil after burning crop residues. Biederbeck *et al* (1980) found that after 20 years of residue burning in the Canadian Prairies, soil organic carbon was reduced by 15 to 20% compared to a chopped straw treatment. These conflicting reports were interpreted by Prassad and Power (1991) to be due to differences in the type of residue, the degree of burning, depth of sampling, tillage employed, and the soil type.

For very fine-textured soils such as Vertisols, little information is available on the comparative effects of residue burning and residue incorporation on soil organic matter levels. Dalal (1989) found incorporation of crop residues in Vertisols resulted in higher organic matter levels under cereal cropping systems. Similar studies comparing the effects of burning and incorporation of cotton stubble on soil organic matter levels on Vertisols are rare. An attempt towards understanding these effects was carried out by Rochester *et al.* (1997), but the major focus was on N fertiliser recovery and lint yield of cotton. One of the objectives of the study reported in this chapter is to examine the comparative effects of burning and incorporating cotton stubble on various soil organic carbon fractions.

Crop rotations are usually known to improve organic matter levels in soil, but the effects invariably depend on the sequence of rotation and other management practices. In Australian cotton cropping systems, the benefits of including rotations in the cropping systems are still unclear. In a survey of 157 cotton growers in New South Wales (Cooper, 1993), the benefit which most growers claimed from using a rotation crop was improved soil structure. However, most of the growers expressed the need for more research to show how different rotation crops compared, and to document the benefits from different rotation crops.

With the establishment of the Cooperative Research Centre for Sustainable Cotton Production (CRC-SCP) in 1993, a range of rotation treatments were established in irrigated and dryland cotton growing regions with the broad objective of identifying the most sustainable crop rotation sequence for cotton production under irrigated and dryland conditions. Crop sequences were selected based on recommendations made by local cotton grower organisations, and the proposed indices for evaluation of sustainability include soil quality, pest and weed incidence, yield and

economic returns. A second objective of the study reported in this chapter is to examine the comparative changes in various soil organic carbon fractions in the different rotation sequences in one of the irrigated sites, and to use the results as a basis for recommending the most sustainable system with regards to improvement of soil organic matter status.

6.2. MATERIALS AND METHODS

6.2.1. Stubble management experiment

(a) Experimental site and design

The experimental site, design and soil sampling procedure are the same as described by Rochester *et al.* (1997). The experiment was carried out on a Typic Pellustert, locally classified as Ug 5.2 (Northcote, 1979), a self-mulching grey cracking clay that averages 60 % of predominantly montmorillonite clay and 20 % of both silt and sand. The site is located at the Australian Cotton Research Institute near Narrabri, northern New South Wales, Australia (149° 40′E, 30°10′S), and is known to have previously supported cotton crops under minimum tillage systems for several consecutive years. The experiment was carried out for 3 years (1991/92, 1992/93, and 1993/94 seasons) using a split-plot design with 4 replications. The main plots were the 2 stubble management systems, burning and incorporation, which were split for 5 N rates (0, 50, 100, 150, and 200 kg N/ha). Each sub-plot was 4 rows wide and 18 m long. In all years, cotton was sown in mid-October and harvested in April. Irrigation and insect control were the same as for commercial cotton. The stubble operations were performed within 2 months of cotton harvesting in all years. Cotton stubble was either retained by slashing at ground level followed by shallow incorporation or removed by uprooting the standing stalk, raked and burnt. Nitrogen was applied in the form of Urea in September to a depth of 15 cm and 10 cm to the side of the crop row. The same stubble and N treatment were maintained in each sub-plot throughout the experiment.

(b) Soil analyses

Soil samples (0 - 30 cm) collected from each plot at the start and end of the experiment were dried at 40°C, ground and sieved to < 0.5 mm. Analysis for total carbon (C_T), total nitrogen (N_T) and $\delta^{13}C$ was performed in the same way as described in Chapter 4. The labile carbon (C_L) and non-labile carbon (C_{NL}) in each sample was determined as described in Chapter 4. Based on the initial and final concentrations of C_T, C_L and C_{NL}, a Carbon Management Index (CMI) was calculated in the same way as described in Chapter 4, except that the samples collected in 1993 from each treatment were used as a reference. Total polysaccharide (P_T) and labile polysaccharides (P_L) were determined using the same method described in Chapter 5. The light fraction was isolated from each sample using the

same procedure described in Chapter 4 and analysed for total carbon concentration and $\delta^{13}C$ in the same manner as for the light fraction in Chapter 4.

(c) Data analysis

Analysis of variance (ANOVA) was performed on the data using the NEVA (Version 3.3) ANOVA program for split-plot factorial experiments (Burr, 1980). Mean separation was determined using the Duncan's Multiple Range Test (DMRT).

6.2.2. Crop rotation experiment

(a) Background

Crop rotation experiments were established at two irrigated and one dryland site, by the Cooperative Research Centre for Sustainable Cotton Production (CRC-SCP). The common objective of all the rotation experiments was to evaluate the sustainability of selected rotation sequences under minimum tillage in cracking clay soils. Sustainability in this regard was considered using a systems approach to include all facets that contribute towards sustainable cotton production. This includes soil characteristics, weed, pest and disease incidence, yield and economic returns. In this thesis, only the effects of the rotation sequences on the soil organic carbon status in one irrigated site are reported.

(b) Experimental site

The experiment is located at "Auscott", a commercial cotton farm near Warren, Macquarie Valley, in New South Wales, Australia (147° 46'E, 31° 47'S), an area with a semi-arid climate. The site experiences four distinct seasons with a mild winter and hot summer. The hottest month is January (mean daily maximum and minimum temperatures of 33°C and 18°C, respectively) and the coldest month is July (mean daily maximum and minimum temperatures of 15°C and 3°C, respectively). Mean annual rainfall is 479 mm. The soil at the site is a deep uniform montmorillonitic Entic Chromustert (USDA, 1981), locally classified as Ug 5.25 (Northcote, 1979). Prior to the start of the experiment in June 1993, the field had grown cotton continuously for at least three years under intensive tillage practices (disc and chisel ploughing, and ridging every year, deep ripping every 2 to 5 years). Cotton stubble had usually been raked from the field and burnt. The whole of the field grew cotton in the 1992/93 cropping season which was harvested in April 30, 1993, and the rotation treatments were started in June 1993. The average cotton yield of the field was 6.55 bales/ha.

(c) Experimental design

The experiment consisted of seven rotation sequences (Table 6.1) and is expected to last for at least seven years. Each treatment was replicated three times and each replicate was 40 m (40 rows) wide and 700 m long. All the treatments use a two-year cycle with the entire field under cotton every second year. This was considered to be a shorter duration than what many

growers would use, but it helps to compare treatments, and to quantify any benefits to the subsequent cotton crop in two-year intervals. For instance, if some of the rotations had included some two-year and some three-year rotations, the whole field would only grow cotton every six years. Therefore, it would have been six years before any comparisons on the effects of the different rotations on the yield of the subsequent cotton crop could have been made.

(d) Site management

Following the harvest of the 1992/93 crop in April 30, 1993, the stubble in the continuous cotton (CC) plots was stalk-pulled on June 15, 1993, then raked and burned. In the long fallow plots (CLF), the cotton stubble was slashed on June 16, 1993 and left on the surface. In all the other treatments, wheat or field peas were sown into the standing cotton stubble using a Napier™ trash seeder. The following July, the cotton stubble was slashed and left on the surface. In the cotton-field pea rotation (CFP), the field pea was planted at a seeding rate of 102 kg/ha followed by the application of 85 kg/ha of $(NH_4)_2HPO_4$ fertiliser. Dry matter yield of the field pea tops, measured in September 30, 1993, was 3.49 Mg/ha. On October 10, 1993, the field pea was sprayed with Roundup™ to kill the peas and stop seed set. In December 1993, the field pea residue was incorporated with a disc hiller.

Table 6.1: Description of the cotton rotation sequences in "Auscott-Warren"

	Summer	Winter	Summer	Winter	Summer
Rotation	92/93	93	93/94	94	94/95
CC	cotton	-	cotton	-	cotton
CLF	cotton	-	-	-	cotton
CFP	cotton	field peas	-	-	cotton
CWlo	cotton	wheat	-	-	cotton
CWhi	cotton	wheat+fertiliser	-	-	cotton
CWLL	cotton	wheat	lablab	-	cotton
CWLLF	cotton	wheat	lablab	fertiliser	cotton

CC = continuous cotton; CLF = cotton-long fallow; CFP = cotton-field peas;
CWlo = cotton-wheat (low input); CWhi = cotton-wheat (high input);
CWLL = cotton-wheat-lablab; CWLLF = cotton-wheat-lablab+fertiliser.

In all the rotations containing wheat in winter 1993 (Table 6.1), wheat seeds were sown on June 18, 1993, followed by the application of 85 kg/ha $(NH_4)_2HPO_4$ fertiliser. In the high-input wheat (CWhi), the seeding rate was 106 kg/ha with an application of 120 kg N/ha, while in the other wheat rotations, the seeding rate was 40 kg/ha with only 17 kg N/ha applied as urea. The high-input wheat rotation (CWhi) received one irrigation on September 6, 1993. Average tops dry matter yield, determined on October 26, 1993, was 9.45 Mg/ha for the high-input wheat and 3.19 Mg/ha for the

other wheat treatments. The wheat grain was harvested on December 12, 1993, after which the stubble was left standing until mid 1994 when the hills were reformed for the 1994/95 cotton crop.

In the summer of 1993/94, only one rotation (CC) had cotton while rotations CWLL and CWLLF had lablab (Table 6.1). Cotton was planted on September 30, 1993, in plots to which nitrogen had been applied as anhydrous ammonia (110 kg N/ha) and phosphorus as $NH_4H_2PO_4$ (45 kg P/ha). The dry matter yield of the cotton, determined on March 7, 1994, was 7.1 Mg/ha. Following the harvesting of the cotton on April 30, 1994, the cotton stubble was stalk-pulled on May 9, 1994, raked and burnt on May 11, 1994. The lablab for both rotations CWLL and CWLLF was sown on January 3, 1994 and later (April 16, 1994) sprayed with Roundup™ to stop growth. Average dry matter yield of the lablab, determined on April 28, 1994, was 1.1 Mg/ha. In May, the rows were reformed and lablab ploughed into the soil. In winter 1994, extra fertiliser was applied to the plots of rotation CWLLF at rates of 11 kg N/ha, 24.5 kg P/ha, and 73.4 kg K/ha.

In summer 1994/95, all plots were planted to cotton (Table 6.1) which was planted on October 3, 1994, following the application of N and P at the rates of 116 kg N/ha and 90 kg P/ha using anhydrous ammonia and $NH_4H_2PO_4$, respectively. Tops dry matter yields, determined on February 23, 1995, were 4.7, 5.4, 5.8, 5.2, 5.4, 5.2, and 5.3 Mg/ha respectively for the CC, CLF, CFP, CWlo, CWhi, CWLL, and CWLLF rotations. Soon after harvesting in April 1995, cotton stubble on the continuous cotton (CC) and the long fallow (CLF) plots was slashed and left on the surface. The cotton stubble on the other treatments was left standing until winter 1995 when the plots which had been sown to wheat, field peas, lablab and faba beans (replacing the lablab in rotation CWLLF) and was then slashed and left on the surface.

In winter 1995, field peas in rotation CFP were sown (June 9, 1995) into the standing cotton stubble at the same seed and fertiliser rate as in winter 1993 (Table 6.1). Tops dry matter yield, measured on September 29, 1995, was 1.3 Mg/ha. In October 1995, the field peas were sprayed with Roundup, as in 1993, and incorporated into the soil. Wheat was sown on June 9, 1995 into the standing cotton stubble at the same seed and fertiliser rates as in winter 1993. Tops dry matter yields, determined on September 29, 1995, were 1.5 and 1.4 Mg/ha, respectively for the CWhi and CWlo. After harvesting, the wheat stubble was left standing until winter 1996 when it was incorporated as the cotton hills were reformed. Both CWhi and CWlo received no irrigation in 1995 due to water shortage in the Macquarie Valley.

Growing lablab proved difficult at Warren, and following the poor performance of lablab in 1993/94, and discussion with Macquarie Valley growers, the lablab in rotation CWLLF was replaced by the faba beans in 1995 (Table 6.1). Fiord faba beans were sown on June 9, 1995, at a seeding rate of 100 kg/ha and a fertiliser rate of 85 kg P/ha and 77 kg N/ha using $(NH_4)_2HPO_4$. In October

1995, the faba beans, with an estimated dry matter yield of 1.0 Mg/ha, were sprayed with Roundup and ploughed into the soil.

In summer 1995/96, only two rotations had crops grown on them; CC and CWLL (Table 6.1). In CWLL, lablab was sown in January 1996 but because of water restrictions in the Macquarie Valley, it did not receive any irrigation and growth was poor. Tops dry matter yield determined in March 1996 was 235 kg/ha. In the CC rotation, cotton was sown using the same variety and management as in previous seasons. The tops dry matter, determined on March 19, 1996, was 5.9 Mg/ha.

(e) Soil sampling and analysis

Soil samples were collected to a depth of 30 cm from the experiment from each plot using a systematic sampling scheme at one-yearly intervals. Samples from each plot were collected in an area starting 100 m away from the head ditch and tail drain. In each 40-row plot, every second row from the tenth row was sampled until the middle of each plot. The samples were taken along three axes, a, b, and c, such that the terminal axes, a and c, were both 100 m away from the head ditch and tail drain, respectively. The central axis, b, was located midway between a and c. Soil samples (5 cm × 5 cm) were cut out from the centre of the cotton rows. This sampling scheme resulted in 63 samples from each plot which were bulked to give one composite sample per plot. Samples were collected from each plot in July 1993, 1994, 1995 and 1996 to monitor changes in soil organic carbon, total nitrogen, phosphorus and sulfur. Samples were air-dried, crushed gently and sieved to < 2 mm and all materials greater than 2 mm were discarded. The < 2 mm sieved soil was then finely ground to pass through a 0.5 mm sieve and then stored for chemical analysis.

Each sample was analysed for total carbon (C_T) and total nitrogen (N_T) using an ANCA-MS as described previously. Labile carbon (C_L) and non-labile carbon (C_{NL}) were analysed using the procedure of Blair *et al.* (1995b) described earlier. Monitoring indices, lability index, carbon pool index, and carbon management index, were calculated from the C_T, C_L, and C_{NL} using the same steps as in the stubble management experiment (Section 6.2.1).

(f) Data analysis

The various measurements were analysed as a split-plot with year as main plot and rotation sequence as sub-plot using the NEVA (Version 3.3) analysis of variance program for complete factorial experiment (Burr, 1980). The use of a split-plot analysis of variance follows the report of Campbell and Zentner (1997). Significant mean separation was calculated using the Duncan's multiple range test.

6.3. RESULTS

6.3.1. Effects of cotton stubble management systems on soil carbon fractions

(a) Effects of stubble incorporation and stubble burning on total nitrogen and soil carbon fractions.

The level of applied N had no significant effect on N_T, C_T, C_L, C_{NL}, total and labile polysaccharides for both stubble management systems on samples collected at the start (1991) and at the end (1994) of the experiment. Therefore, only mean values of these measurements are presented (Table 6.2). At the start of the experiment (1991), no significant differences were observed in N_T, carbon fractions and polysaccharides, which indicates the uniform nature of the experimental site. After 3 years, the stubble-incorporated plots were significantly higher in C_T, C_L and polysaccharides than the stubble-burnt plots. Total N and C_{NL} did not differ significantly between stubble management systems throughout the study.

Table 6.2: Effects of stubble incorporation and stubble burning on total nitrogen (N_T), soil carbon fractions (mg/g), and polysaccharides (mg C/g).

Residue	Year of		Carbon fractions			Polysaccharides	
treatment	sampling	N_T	C_T	C_L	C_{NL}	Total	Labile
Incorporated	1991	1.09a	10.05b	1.13b	8.92a	1.28b	0.11b
	1994	1.11a	11.10a	1.67a	9.43a	1.66a	0.14a
	Change	+0.02m	+1.05m	+0.54m	+0.51m	+0.33m	+0.03m
Burnt	1991	1.07a	9.67b	1.07b	8.60a	1.24b	0.10b
	1994	1.09a	10.08b	1.01b	9.07a	1.20b	0.10b
	Change	+0.02m	+0.41n	-0.06n	+0.47m	-0.04n	0.00n

Means of five nitrogen levels and four replications. Means in a column followed by the same letter are not significantly different ($P < 0.05$ DMRT).

The relative differences between the stubble management systems at the end of the experiment were more pronounced in the C_L and total polysaccharides (P_T) than in the other carbon and nitrogen measurements (Table 6.2). Initially, the C_L, P_T and P_L comprised 11.2, 12.7 and 1.1 % of the C_T respectively in the stubble-incorporated plots, and 11.0, 12.8 and 1.0 % of the C_T respectively in the stubble-burnt plots. At the end of the third year, the relative contributions of the C_L, P_T and P_L were 15.0, 14.9 and 1.3 % of the C_T respectively in the stubble-incorporated plots, and 10.0, 10.0, and 1.0 % of the C_T respectively in the stubble-burnt plots.

The C_T concentration after 3 years of stubble incorporation had increased by as much as 10 % of the initial concentration which was more than double the increase observed in the stubble-burnt plots (4.2 %). Assuming an average bulk density of 1.5 Mg/m^3 in the 0 - 30 cm depth under the minimum tillage system (see Constable *et al.*, 1992), these changes represent an increase of 1.6 Mg C/ha/year when stubble was incorporated as compared to an increase of 0.6 Mg C/ha/year when stubble was burnt. The increase observed in the stubble-incorporated plots is slightly higher than the estimated value of 1.4 Mg/ha/year in the report of Rochester *et al.* (1997). This is probably because total organic carbon in this study was measured by dry combustion while Rochester *et al.* (1997) measured organic carbon by wet oxidation, which normally leads to incomplete recovery. While the stubble-incorporated treatment resulted in an increase of 48 % in the C_L relative to the initial concentration, the C_L concentration in the stubble-burnt plots showed no significant change in the 3 years (Table 6.2). The increase observed in the C_L represents a dramatic change in the soil carbon concentration when compared to the changes in the other fractions. Both P_T and P_L showed increases of about 30 % and 27 % respectively in the stubble-incorporated plots but showed no significant change in the stubble-burnt plots (Table 6.2).

For a standard comparison of the changes in C_T, C_L and C_{NL} of the different stubble management systems, the Carbon Management Index (CMI) proposed by Blair *et al.* (1995b) was calculated (Table 6.3). The CMI incorporates the changes in C_T, C_L and C_{NL}, and has been previously shown to be a sensitive monitoring indicator of organic matter status in soil under different management systems (Lefroy and Blair, 1994).

Table 6.3: A comparison of Carbon Pool Index (CPI), Lability Index (LI), and Carbon Management Index (CMI) after 3 years of different stubble management systems

Management system	CPI	LI	CMI
Stubble incorporated	1.02a	1.38a	141a
Stubble burnt	1.02a	0.92b	94b

Means in a column followed by the same letter are not significantly different ($P < 0.05$ DMRT)

Incorporation of stubble resulted in an increase in the CMI of 41 % while burning resulted in a decrease in the CMI of 6 % (Table 6.3). Considering that the continuity of supply of carbon in soil depends on both the total pool size and the lability (an index of decomposability), the changes in the CMI indicate that cotton stubble retention will lead to a more sustainable management of soil organic matter than burning.

(b) Effects of stubble incorporation and stubble burning on the light fraction (LF) and light fraction carbon (LF-C).

The concentration of LF obtained did not differ significantly between plots at the start of the study (Table 6.4). After 3 years, the concentration of LF in the stubble-incorporated plots was significantly higher than the concentration of LF in the stubble-burnt plots at all N levels. The effect of applied N after 3 years was significant in the stubble-incorporated plots but not in the stubble-burnt plots. In the stubble-incorporated plots, the concentration of LF increased as the level of applied N increased up to 100 kg N/ha. The increases in the LF relative to the initial concentration in the stubble-incorporated plots were 22, 28, 57, 80, and 46 % at N levels of 0, 50, 100, 150 and 200 kg/ha, respectively. In the stubble-burnt plots, the LF decreased relative to the initial concentration by 18, 21, 6, 24 and 20 % at N levels of 0, 50, 100, 150 and 200 kg/ha, respectively. On average, the concentration of LF after 3 years in the stubble-incorporated plots was almost double that obtained in the stubble-burnt plots (Table 6.4).

Table 6.4: Effects of stubble management system on the light fraction (LF) and light fraction carbon (LF-C)

Residue treatment	Year of sampling	Light fraction (LF)	Light fraction carbon (LF-C)
		mg/g	mg C/g
Incorporated	1991	8.27b	1.03b
	1994	12.00a	2.35a
	Change	+3.73m	+1.32m
Burnt	1991	8.00b	1.02b
	1994	6.17c	1.13b
	Change	-1.83n	+0.11n

Means of five nitrogen levels and four replications. Means in a column followed by the same letter are not significantly different ($P < 0.05$ DMRT).

In order to understand how much of the total carbon in the soil was present as LF, the carbon concentration of the LF was determined by ANCA-MS. As with the LF, the amount of soil carbon present as light fraction (LF-C) did not differ significantly between plots at the start of the study (Table 6.4). After 3 years of different stubble management, the LF-C in the stubble-incorporated plots was significantly higher than that in the stubble-burnt plots. On average, the LF-C constituted 9.6 and 10.3 % of C_T in the stubble-incorporated and stubble-burnt plots, respectively, at the start of the study. After 3 years of different stubble management, the LF-C constituted, on average, 21 % and 11 % of the C_T in the stubble-incorporated and stubble-burnt plots, respectively. In the stubble-incorporated plots, the application of N significantly increased the LF-C when compared to the 0 kg N/ha. At all

levels of applied N, the concentration of soil carbon present as LF was significantly higher in the stubble-incorporated plots than in the stubble-burnt plots.

(c) Effects of stubble incorporation and stubble burning on the $\delta^{13}C$ of the whole soil and the light fraction

As with the other measurements, there was no significant difference in $\delta^{13}C$ between plots at the start of the study (Table 6.5). After 3 years of the different stubble management systems, the $\delta^{13}C$ values in the stubble-incorporated plots were consistently lower than in the stubble-burnt plots at all N levels. Application of N up to 150 kg/ha did not significantly affect the $\delta^{13}C$ value of the soil. Further increase in the N rate up to 200 kg/ha resulted in a decrease in $\delta^{13}C$ for both stubble management systems. In the stubble-incorporated plots, the $\delta^{13}C$ values decreased during the 3-year period while the $\delta^{13}C$ values in the stubble-burnt plots increased during the same period. The trends observed in the $\delta^{13}C$ of the LF were similar to those observed in the whole soil except that the $\delta^{13}C$ of the LF was consistently lower than the $\delta^{13}C$ of the whole soil (Table 6.5).

Table 6.5: Effects of stubble incorporation, stubble burning and nitrogen levels on the $\delta^{13}C$ (‰) of the whole soil and the light fraction

Residue treatment	Year of sampling	Whole soil	Light fraction
Incorporated	1991	-20.20b	-21.96b
	1994	-21.13c	-22.97c
	Change	-0.93n	-1.01n
Burnt	1991	-20.47b	-22.25b
	1994	-19.13a	-20.79a
	Change	+1.34m	+1.46m

Means of five nitrogen levels and four replications. Means in a column followed by the same letter are not significantly different ($P < 0.05$ DMRT).

6.3.2. Crop rotation effects on soil carbon fractions

(a) Effects on C_T

Because of the very small changes that took place during the successive one-year intervals, results were only presented between the initial samples (1993) and the final samples (1996). Generally, the total organic carbon concentration (C_T) of the field was low (Table 6.6). There was no significant difference in C_T between rotations at the start of the experiment in 1993 (Table 6.6). In 1996, some significant differences between the different rotations were observed. While there was no significant difference between the continuous cotton (CC), cotton-long fallow (CLF), and the cotton-field pea rotations (CFP), all these three rotations had significantly lower C_T than the low-input

wheat (CWlo) rotation by 1996. Although there was no significant difference between the C_T of the CC and the CLF rotations by 1996, the C_T in the CLF rotation was significantly lower than the C_T in all the rotations containing wheat in the sequence (Table 6.6). There was no significant difference between the C_T concentration of all rotations containing wheat in the sequence. Throughout the period of observation, the CLF rotation had the lowest C_T concentrations when compared to the other rotations (Table 6.6). When the C_T concentrations were compared between the whole period under investigation (1993 to 1996), all rotations containing wheat, except the CWhi rotation, showed significant increases in C_T while the changes in C_T of the CC, CLF, and CFP were not significant (Table 6.6).

Table 6.6: Changes in total carbon (C_T) and labile carbon (C_L) following different rotation sequences (mg/g)

Carbon fraction	Year of sampling	Rotation						
		CC	CLF	CFP	CWlo	CWhi	CWLL	CWLLF
C_T	1993	6.10cd	6.14cd	6.21cd	6.15cd	6.14cd	6.00d	6.29cd
	1996	6.21cd	5.69d	6.54bcd	7.76a	6.98abc	7.12ab	7.29ab
C_L	1993	0.80cd	0.82cd	0.85cd	0.85cd	0.86cd	0.85cd	0.82cd
	1996	0.82cd	0.75d	0.99ab	1.05a	1.00ab	1.05a	0.89bc

Means of three replications. Means in any measurement followed by the same letter are not significantly different ($P < 0.05$ DMRT)

(b) Effects on C_L

As with the C_T, there was no significant difference in labile carbon (C_L) concentration between the different rotations at the start of the study in 1993 (Table 6.6). By 1996, significant differences in C_L between the different cropping sequences were apparent. While there was no difference in the C_L concentration between the CC and CLF rotations, both of these rotations had lower C_L concentration than the CFP, CWlo, CWhi, and CWLL rotations. Although rotations containing wheat in the sequence had the highest C_L concentration, the C_L concentration in the CWLLF rotation was significantly lower than the C_L concentration in the CWLL, CWlo rotations, but was not significantly different from the C_L concentration in CC, CFP and CWhi rotations (Table 6.6). The lowest C_L concentration was observed in the CLF rotation, though this was not significantly different from the C_L in the CC rotation. When the overall changes between 1993 and 1996 were considered, there were significant increases in the C_L concentration in the CFP, CWlo, CWhi, and CWLL rotations (Table 6.6). Throughout the period under consideration, the C_L concentration in the CC, CLF, and CWLLF showed no significant change.

(c) Effects on the lability Index (LI)

The lability index (LI) provides a measure of decomposability of the soil organic carbon. Since the LI relates the ratio of C_L to C_{NL} at any stage to the same ratio at the start of the study, all treatments in 1993 had a value of 1.00 for the lability index (Table 6.7). By 1996, the LI in the CFP rotation, though not significantly different from the LI in the CC, CWhi and CWLL rotations, was significantly higher than the LI in the CLF, CWlo, and CWLLF rotations (Table 6.7). While no significant difference was observed between the LI in the CWlo, CWhi, and CWLL rotations, the LI in the CWLLF rotaion was significantly lower than the LI in CWhi and the CWLL rotations. However, throughout the monitoring period, the only significant increase in LI was observed in the CFP rotation (Table 6.7).

Table 6.7: Changes in lability index (LI), carbon pool index (CPI) and carbon management index (CMI) following different rotation sequences

Carbon	Year of	Rotation						
fraction	sampling	CC	CLF	CFP	CWlo	CWhi	CWLL	CWLLF
LI	1993	1.00bc	1.00bc	1.00bc	1.00bc	1.00bc	1.00bc	1.00bc
	1996	1.01ab	0.98bc	1.13a	0.98bc	1.03ab	1.05ab	0.93c
CPI	1993	1.00de	1.00de	1.00de	1.00de	1.00de	1.00de	1.00de
	1996	1.03cde	0.94e	1.07bcd	1.28a	1.15abc	1.20a	1.17ab
CMI	1993	100cd	100cd	100cd	100cd	100cd	100cd	100cd
	1996	104c	92d	120a	124a	118ab	126a	109bc

Means of three replications. Means in any measurement followed by the same letter are not significantly different ($P < 0.05$ DMRT)

(d) Effects on the carbon pool index (CPI)

The carbon pool index (CPI) expresses the total organic carbon (C_T) at any stage as a ratio of the C_T at the start of the study. Therefore, all rotations had a CPI of 1.00 at the start of the study in 1993 (Table 6.7). In 1996, the highest CPI was observed in the rotations containing wheat in the sequence while the least CPI was observed in the CLF and the CC rotations. The CPI in the CFP rotation, though not significantly different from the CPI in the CC, CWhi, and CWLLF rotations, was significantly lower than the CPI in the CWlo and the CWLL rotations (Table 6.7). Over the entire three-year period, the CPI had significantly increased in the CWlo, CWhi, CWLL, and CWLLF rotations. There was no significant change in the CPI of the CC, CLF, and CFP rotations throughout the period under consideration (Table 6.7).

(e) Effects on the carbon management index (CMI)

The carbon management index (CMI) is a product of the carbon pool index (CPI) and the lability index (LI). Because it takes into account the changes in both the total carbon (C_T) and the labile carbon (C_L) relative to a reference starting point, the CMI can be considered as a sensitive indicator of sustainability in terms of organic carbon status in the soil. Since both the CPI and the LI at the start of the experiment had a value of 1.00, the CMI was 100 in all plots in 1993 (Table 6.7). By 1996, the CMI in the CFP, CWlo, CWhi, and CWLL rotations was significantly higher than the CMI in the CC and CLF rotations. No significant difference was observed between the CC and the CWLLF rotations and between the CFP, CWlo, CWhi and CWLL rotations throughout the period under consideration. The least CMI was observed in the CLF rotation (Table 6.7). During the three-year period, the CMI in the CFP, CWlo, CWhi, and CWLL has increased significantly by 20%, 24 %, 18 %, and 26 % respectively, while no significant change occurred in the CMI of the CC, CLF, and CWLLF rotations (Table 6.7).

6.4. DISCUSSION

6.4.1. Effects of cotton stubble management systems on soil carbon fractions

The significantly higher concentration of all carbon fractions in the stubble-incorporated plots as compared to the stubble-burnt plots after 3 years (Table 6.2) demonstrates the potential of cotton stubble as a source of soil organic matter in Vertisols. Stubble management practice did not significantly affect the total N concentration of the soil, presumably because of the low N concentration of cotton stubble (Blair *et al.*, 1995a). Polysaccharides usually comprise 50 - 70 % of the dry weight of most plant tissue, and hence are the most abundant materials added to soil in the form of plant residues (Lowe, 1978). It is therefore not surprising that the amount of polysaccharides, both total and labile, was significantly higher with stubble incorporation than with stubble burning. The labile carbon is usually more sensitive to soil management than the total organic carbon (Bremer *et al.*, 1994) and therefore short-term changes in labile carbon can be useful for predicting long-term changes in soil organic matter. The labile carbon (C_L) in this study comprises all those organic matter components that can be readily oxidised by a mild oxidising agent (Blair *et al.*, 1995b). As would be expected, the relative differences between the stubble management systems were more pronounced in C_L than in the other organic carbon fractions (Table 6.2).

Although most published reports have been on crop residues other than cotton, earlier reports have shown that the effects of crop residues on soil organic matter levels are only weakly related to the type of residue applied. For instance, Larson *et al.* (1972) found that residues of alfalfa, cornstalks, oat straw, sawdust, and bromegrass produced similar effects on organic carbon in a Hapludoll in Iowa. Sauerbeck (1982) in Germany also concluded that different types of crop residues

had similar effects on soil organic matter. Therefore, the results obtained in this study can be appropriately compared to findings of previous studies on the effects of burning and retention of crop residues on soil organic matter. In close agreement with the results of this study, Collins *et al.* (1992), in a wheat cropping system, found that burning of residues resulted in a decline in soil microbial biomass C and N, which was attributed to the relatively smaller amount of organic matter returned to the soil in the residue-burnt soil. Also, Ladd *et al.* (1994) found that after 3 years of residue management in wheat rotation systems, organic C and total N concentrations were not significantly different between plots with residue retained on the surface and those with residue incorporated into the soil, but both were higher than plots where residue was burnt.

The significance of these results in terms of general soil quality and sustainability is not only limited to improvement of soil organic matter. Other results from the same study published earlier by Rochester *et al.* (1997) revealed that recovery of fertiliser N was reduced by 10 % in the stubble-burnt plots as compared to the stubble-incorporated plots. Also, cotton lint yield tended to decline in successive crops for both stubble burning and stubble retention. However, in the third crop, yield reduction in the stubble-burnt plots was significantly higher ($P < 0.06$) than yield reductions in the stubble-returned plots.

The light fraction (LF) has been used extensively to study the effects of soil management systems on soil organic matter (Skjemstad *et al.*, 1986; Janzen *et al.*, 1992; Barrios *et al.*, 1996) because it is known to be more sensitive than the total organic matter (Gregorich and Janzen, 1996). The importance of this fraction as a source of labile carbon in soil was confirmed by studies showing highly significant positive relationships between LF and C mineralisation (Bremer *et al.*, 1994; Janzen *et al.*, 1992). The LF mainly consists of plant debris, both fresh and partially decomposed, although other material may be present in significant quantities. Charcoal, for example, may be an important constituent of the LF in some soils (Skjemstad *et al.*, 1990). The ranges observed in LF-C are in close agreement with values reported by Bremer *et al.* (1994) for cultivated soils. The changes that occurred in the light fraction carbon (LF-C) suggest that a substantial proportion of the increase in C_T in the stubble-incorporated soil is due to the LF. This can be seen from the relative changes that occurred in the C_T (Table 6.2) and in the LF and LF-C (Table 6.4). While the C_T in the stubble-incorporated soil increased by only 10 % above the initial concentration, the LF-C has more than doubled relative to the initial concentration. The LF consists of the applied plant residues that have not undergone complete humification. Since cotton stubble is known to have a high C/N ratio (Blair 1993) it is expected to undergo humification at a slow rate such that in 3 years, a substantial amount can still be isolated in the LF. The increases in LF and LF-C as a result of increasing rates of N in the stubble-incorporated soil are due to increases in the yield of cotton with higher rates of N (Rochester *et al.*, 1997). It can be argued that the higher the yield of cotton, the more stubble that is returned to the soil, and therefore, more LF. In the soil in the stubble-burnt treatment, there was a consistent decline in the LF and LF-C, relative to the initial concentration at all levels of N. The level

of N applied did not increase the amount of LF in the stubble-burnt soil, because, despite the increases in yield with increasing rate of N, all the stubble was removed from the soil. In addition, the amount of LF initially present in the soil continuously undergoes decomposition at rates that can be hastened by the presence of fertiliser N (Jenkinson, 1981). Therefore, the reductions in the amount of LF and LF-C due to stubble burning indicate that some of the LF originally present in the soil has been lost as a result of enhanced decomposition.

The changes in the $\delta^{13}C$ of the whole soil and the LF (Table 6.5) as a result of burning or incorporating cotton stubble were very useful in understanding the dynamics of organic matter under the different management systems. The $\delta^{13}C$ has been used extensively to follow organic matter changes under different management systems (Balesdent and Mariotti, 1996; Conteh *et al.*, 1997b; Skjemstad *et al.*, 1990). The use of this measure is based on the fact that the $\delta^{13}C$ of soil organic matter closely corresponds to that of the native vegetation from which the organic matter is derived, and that addition of plant material with a different $\delta^{13}C$ to the soil will change the isotopic composition of the soil (Balesdent and Mariotti, 1996; Boutton, 1996). While the $\delta^{13}C$ of the LF was consistently lower than that of the corresponding whole soil, it was observed that in both the whole soil and the LF (Table 6.5), the $\delta^{13}C$ decreased with time in the stubble-incorporated soil but increased in the stubble-burnt soil. These changes can be explained through an understanding of the changes in $\delta^{13}C$ that normally accompany decomposition of organic matter in soil.

Because decomposition of plant residues in soil is normally a slow and lengthy process, as was mentioned earlier in Chapter 4, there is little direct evidence for evaluating the potential for isotope fractionation as plant carbon is incorporated into the soil organic matter. However, there have been numerous reports which indicate that the $\delta^{13}C$ of organic matter in the soil is higher than that of the current above ground or below ground inputs by approximately 1‰ or less in the surface soil, and by up to 3 ‰ with increasing depth in the profile (Balesdent *et al.*, 1993; Stout *et al.*, 1981). Ryan *et al.* (1995) found that the $\delta^{13}C$ value of soil microbial biomass (-24.2 ‰) was higher than that of the bulk soil organic matter (-26.6 ‰) from which the biomass was extracted. Also, Gleixener *et al.* (1993) demonstrated that fungi had $\delta^{13}C$ values that were 1.5 - 6.0 ‰ higher than those of the woody tissue that they decomposed. These studies, and many others, showed that as soil organic matter is repeatedly processed and reprocessed by the diversity of macro- and micro-organisms, the potential clearly exists for the residual organic carbon to undergo an increase in $\delta^{13}C$ with time.

Cotton stubble has $\delta^{13}C$ values that are consistent with C3 photosynthetic pathway (*ca.* -22 ‰ to -26 ‰). Because the whole soil contains more decomposed organic matter than the LF, it is therefore not surprising that the $\delta^{13}C$ values in the LF were consistently lower than those of the corresponding whole soil (Table 6.5). In the stubble-incorporated soil, the decrease in $\delta^{13}C$ of both

the whole soil and the LF is due to the addition of more fresh and partially decomposed plant residues which are lower in $\delta^{13}C$ than the soil. Since no fresh plant material was added to the stubble-burnt soil, the organic matter originally present in the soil undergoes continuous decomposition leading to higher $\delta^{13}C$ values with time in the stubble-burnt soil. These changes in $\delta^{13}C$ values strongly support the earlier observation that most of the increases observed in the C_T of the stubble-incorporated soil were due to increases in the amount of LF in the soil.

6.4.2. Effects of different rotation sequences on soil carbon fractions

As mentioned earlier in this chapter (Section 6.1), crop rotations have been reported in several studies as a means of improving organic matter status in soils (see Section 2.4.2). To the farmer, the selection of crops to be grown in the rotation is the most basic management decision. This decision is quite often constrained by many factors which may include climatic, economic, as well as land suitability (Paustian *et al.*, 1997). Also, the selection of crops to be grown in a rotation is quite often linked to other management factors such as tillage and soil fertility. However, in this thesis, the central focus is on how crop type and rotation sequence influence the carbon inputs and changes.

From the results presented in Section 6.3.2, the effects of the different rotation sequences on the various soil carbon fractions appear to be determined by three major factors; the crop type, the amount of dry matter returned to the soil, and the application of inorganic fertiliser. Although the relative contribution of these factors is hard to separate, it appears that the amount of residue returned to the soil is the dominant factor. This can be seen from an examination of the relative changes that occurred in all carbon fractions in the different rotation sequences in comparison to the dry matter yields of the different rotation crops. It is clear from the overall (1993 to 1996) increases in C_T, C_L (Table 6.6), CPI and CMI (Table 6.7) that the rotations with the most significant increases are those that included wheat in the rotation sequence. These are the rotations with the highest quantities of dry matter returned to the soil.

Although the overall increase in the C_T of the CWLLF rotation (Table 6.6) was significant, it is surprising that the C_L (Table 6.6) and the CMI (Table 6.7) in this rotation (CWLLF) showed no significant change considering that this rotation also contained wheat in the sequence (Table 6.1). A possible reason for this observation could be due to the extra fertiliser applied in winter 1994 and the replacement of the wheat+lablab by faba beans in winter 1995. The micro-organisms which decompose organic material added to soil usually obtain the necessary inorganic nutrients from two sources; those already present in the soil in inorganic forms and those in the added plant material itself (Jenkinson, 1981). The application of extra inorganic fertiliser in the CWLLF rotation (Table 6.1) could have resulted in a flush of microbial activity, rapidly breaking down the added plant material.

Because the soil microbes act more on the C_L, this fraction showed no significant increase which was ultimately reflected in the lack of significant increase in the CMI of the CWLLF rotation (Table 6.7). Since C_L generally comprises a small percentage of the C_T, the C_T was not significantly affected by the soil microbial action and therefore the overall increase in the C_T of the CWLLF rotation was found to be significant (Table 6.6). The effect of the applied fertiliser was also observed in the relative changes in the carbon fractions of the low-input wheat (CWlo) and the high-input wheat (CWhi) rotations. Although the total dry matter returned in the CWhi rotation was generally more than the total dry matter returned to the soil in the CWlo rotation, the overall increases in the C_T and CMI in the CWlo rotation were more than the overall increases in the C_T and CMI of the CWhi rotation. The rate at which the added organic material in the CWhi rotation was decomposed may have been much faster, due to the extra inorganic fertiliser added, than that in the CWlo rotation, such that despite the higher rates of residue returned in the CWhi than in the CWlo rotation, the increases in soil carbon fractions in the CWlo rotation were still higher than in the CWhi rotation.

The effects of the quantity of dry matter returned to the soil on soil organic carbon levels in crop rotations have been documented in several long-term studies. Zielke and Christenson (1986) found that changes in soil carbon for six rotations including corn, sugarbeet, navy beans, oats and alfalfa were closely correlated with the amount of residue returned to the soil. Similarly, Havlin *et al.* (1990) reported that rotation effects on soil organic carbon were directly related to the amount of residue produced. Also, Campbell and Zentner (1993) found that changes in soil organic matter in crop rotations reflected the residue production of each rotation.

Because of the difficulty in quantifying the below-ground residue production, generalisations are hard to make. However, some reports have indicated that for most annual crops, carbon inputs from root production usually account for 20 to 40 % of total dry matter production (Buyanovsky and Wagner, 1986; Paustian *et al.*, 1989; Van Veen *et al.*, 1989). Therefore, it also follows that since the rotations containing wheat in the sequence produced more above-ground dry matter, the below-ground residue production in these rotations would also be higher than in the other rotations. This contribution of the below-ground residue to the organic carbon levels is supported by the report of Campbell *et al.* (1991a) who found, in a wheat cropping system, similar concentrations of soil organic carbon in fertilised plots where the wheat straw was removed compared with fertilised plots where the wheat straw was retained. This was despite the fact that carbon inputs were estimated to be greater in the treatment with straw retention. One interpretation offered by the authors (Campbell *et al.*, 1991a) was that roots, rather than straw, were the primary source of carbon to build stable organic matter.

In addition to the quantity of residue returned to the soil, other characteristics such as the quality of the residue, may have contributed to the changes in organic carbon observed. Cereals,

such as wheat, are known to have a high lignin concentrations and high C/N ratios as compared to legumes such as field peas, faba beans or lablab (Tian *et al.*, 1992; Lefroy *et al.*, 1995). As a result of the high lignin concentration and high C/N ratios, wheat residue added to the soil decomposes slowly and thus increasing the carbon stabilisation efficiency. On the other hand, residues of field peas have a low lignin concentration and low C/N ratios. As a result, their decomposability is high and this is reflected in the significant increase in the lability index (LI) of the cotton-field pea rotation (Table 6.7).

Throughout the study period (1993 to 1996), there was no significant change in all the carbon fractions of the continuous cotton (CC) and the cotton-long fallow (CLF) rotations. From the site management description presented earlier in Section 6.2.2, it can be seen that in the CC rotation, all the stubble was removed and burnt in the first 2 cotton seasons of the experiment (summer 1993/94 and 1994/95). This means that for this rotation, the only residue returned to the soil during the first 2 cotton seasons was the leaf fall during defoliation. As already mentioned earlier, this source of carbon is readily decomposed by soil micro- and macro-organisms (Blair *et al.*, 1995a) and therefore could not make any significant contribution to the stable carbon pool in the soil. The cotton dry matter returned to the soil after the summer 1995/96 season may not have been enough to produce any significant effect.

In the cotton-long fallow rotation (CLF), the absence of a standing crop for an extended period could have contributed to the consistently low levels and the absence of any significant change for all carbon fractions in this rotation. In semi-arid agricultural systems, decreases in soil organic matter with increasing fallow frequency have been well documented (Campbell and Zentner, 1993; Biederbeck *et al.*, 1984). Most of these reports show a roughly linear decrease in soil carbon with increasing proportion of fallow in the rotation. According to Campbell *et al.* (1991a), rotations which include summer fallow tend to lead to lower organic carbon levels than continuously cropped rotations although some studies have failed to record any effect of summer fallow. While the changes in all the carbon fractions observed in the cotton-long fallow rotation were not statistically significant, it can be seen from the results in Section 6.3.2 that there was a consistent tendency towards declining levels of all the carbon fractions.

6.5. CONCLUSIONS

The results of this study show that management of cotton stubble significantly affects the organic matter status of Vertisols. Incorporation of stubble increases both the total carbon concentration and the Carbon Management Index while burning reduces the Carbon Management Index. Most of the increases in soil organic matter observed in a 3-year period are due to increases in the amount of light fraction. With regards to crop rotation options, it appears most probably that

rotating cotton with wheat is a more sustainable option with regards to long-term improvement of soil quality than continuous cotton or legumes alone. The inclusion of legumes in the rotation sequence appears to produce mainly short-term benefits probably as a result of their rapid decomposition rates. However, since observations have only been made for a relatively short time, subsequent monitoring of the organic carbon changes is recommended for a conclusive evaluation of the role of different rotation sequences on soil organic carbon status. In general, it can be concluded that for sustainable management of soil organic matter in Vertisols under cotton production, as much of the stubble produced in the system be returned to the soil rather than removed.

CHAPTER 7

GENERAL DISCUSSION

The development of sustainable cropping systems requires a conceptual framework as to what constitutes a sustainable cropping system. As mentioned earlier (Chapter 2), sustainability is a systems issue Although it is possible to talk about, for example, sustainable soil management, sustainability is influenced as much by the interactions between soil processes and those of plant and weed growth, pest and disease development, tillage and other labour activities, as it is by each of these factors individually. Therefore, the sustainability of a cropping system can be appropriately assessed through the use of indicators, which are partial indices that estimate some aspect of the broader concept. The selection of indicators can be achieved through a step-wise approach, by identifying a set of attributes that constitute components of a sustainable cropping system, and then develop techniques for monitoring these attributes.

The organic matter concentration in a soil is a key indicator of a sustainable cropping system because of its influence on the physical, chemical and biological health of a soil. Although the total organic matter concentration in agricultural soils rarely exceeds 5 % by soil weight, it is a key component of any ecosystem, and variations in its abundance and nature have profound effects on many of the processes that occur in the system. On some soils, such as the red brown earths, where illite may comprise 50 - 60 % of the clay fraction, declining soil organic matter can have drastic effects. Therefore, the variations in organic matter content of a soil induced by a cropping system is a significant indicator of the sustainability of that cropping system.

The quantity of organic matter in a soil is influenced by land management by altering the annual input from plants and animals, and by altering the rate at which these organic materials decompose. Soil organic matter can be managed through the retention of all stubble produced during the growing season or the incorporation of a rotation crop in the cropping system. The rotation crop should preferably be one that produces large quantities of dry matter, all of which should be returned to the soil. The success of any organic matter management strategy will however, depend on methods that can detect and monitor short-term changes in the organic matter quantity and quality. Because of the complex nature of organic matter in soil and its association with the mineral matrix, a wide range of approaches have been employed for its estimation. Most of these approaches were reviewed earlier in this thesis (Chapter 2). Since soil organic matter exists in a wide diversity of forms with considerable variability in decomposition rates, the loss of the labile fraction due to cropping is invariably greater than that assessed from the loss of the total soil organic matter. However, the

issue of what constitutes labile carbon in soil is unresolved. Since the loss of organic matter in soil is essentially an oxidative process, the study presented in this thesis considered the labile carbon as that fraction of soil organic matter that is readily oxidisable by a mild oxidising agent, 333 mM $KMnO_4$ solution.

Although it is now common knowledge that cultivation generally results in declines in soil organic matter concentrations, there is neither an accepted measure of organic matter quality nor has there been consensus as to whether the quality of organic matter has changed during cultivation. For instance, Skjemstad *et al.* (1986) and Oades *et al.* (1988) found that even though continuous cultivation resulted in a decrease in soil organic matter concentrations, the chemistry of organic matter in native and cropped soils, as shown by ^{13}C NMR spectra, was similar. Also, Preston *et al.* (1994) reported that despite a carbon loss of more than 50 % due to cultivation, changes in the nature of organic carbon in the size fractions, as shown by ^{13}C NMR spectra, were generally small. This is where the carbon fractions utilised in this thesis find their potential applications. It was shown earlier (Chapter 4) that cultivation has resulted in significant declines in all organic carbon fractions. However, in most of the soils examined, the relative losses in the labile carbon and the carbon management index (CMI) were higher than the relative losses in the total carbon and the non-labile carbon. These observations show that the $KMnO_4$ oxidisable carbon (C_L) and hence the CMI are very sensitive indicators of organic matter changes brought about by cultivation.

It is also known that ease of decomposition of any organic carbon fraction is not only due to its chemical composition, but also due to its location within the soil aggregates. The role of aggregates in the protection of soil organic matter from rapid decomposition was also demonstrated in the results presented in Chapter 4. It was seen that though there was a higher concentration of both C_T and C_L in the microaggregates (< 250 μm) than in the macroaggregates (> 250 μm), the rates of decomposition of both C_T and C_L were higher in the macroaggregates than in the microaggregates. However, in all the aggregate sizes, the relative losses of C_L were higher than the relative losses of C_T. These observations also support the hypothesis that the $KMnO_4$-oxidisable carbon (C_L) is a measure of labile carbon in soil and can be used for monitoring short-term changes in organic matter under different cropping systems.

Since labile carbon in soil has been determined by a wide range of methods, there was a need to examine how labile labile carbon determined by $KMnO_4$ oxidation relates to other common measurements of soil organic matter. It was shown in Chapter 5 that labile carbon determined by $KMnO_4$ oxidation is significantly related to fulvic acid, soil polysaccharides and soil microbial biomass carbon, but not related to the humic acid and the humin. Based on these relationships and from information obtained from the ^{13}C NMR spectra (Chapter 5), it appears that the $KMnO_4$-oxidisable carbon comprise mostly of carbohydrates together with some unidentifiable aromatic compounds.

From these results, it can be concluded that partitioning of soil carbon into C_L and C_{NL}, as shown in this thesis, will allow the separation of active and less active soil carbon to be used for monitoring soil organic carbon dynamics in agricultural systems. Since the continuity of supply of carbon in soil depends on both the total pool size and the decomposability, the carbon management index (CMI) can be considered to be a useful indicator of sustainable cropping systems.

Increasing public and grower concern with the cotton industry about soil and environmental quality in relation to long-term sustainable cotton production has emphasised the need to develop and implement management strategies that maintain and protect the soil resource. This is directly related to maintaining the quality and quantity of soil organic matter. As mentioned earlier in this thesis, the soils used for cotton production generally have high soil organic matter concentrations in their native state. This high level of soil organic matter was also a contributor to the physical and chemical fertility of the soil. However, the prevailing high temperature and moisture regimes under which cotton production occurs have resulted in significant losses in soil organic matter. This was illustrated in the data presented in Chapter 4. The continuation of this trend will threaten long-term sustainable cotton production. Reversing this trend will have to rely on efficient soil organic matter management strategies. This will have to be realised either through the retention of all cotton stubble produced during the growing season or the inclusion of a suitable rotation crop in the cropping system. However, the benefits of cotton stubble retention to soil organic matter in the soils used for cotton production are not well understood. In most cotton growing countries, cotton stubble is considered as an ecological burden rather than a useful material (Gould, 1993). Its slow decomposition in the soil and its poor digestibility, which are both probably due to its high lignin content, are suggested as the main obstacles to its reasonable utilisation.

Results from the experiment reported in Chapter 6 have shown that retention of cotton stubble increased the CMI by up to 40 % while removal of stubble led to a decline in the CMI within a period of 3 years. Maintenance of the stubble in the cropping system is therefore essential for the long-term sustainability of cotton cropping systems. However, as in many other cropping systems, management of stubble in cotton cropping systems will not only be influenced principally by the nature and quantity of stubble available and the immediate and future land use, but also by many other constraints (Lefroy and Strong, 1995). The availability of labour and equipment as well as the alternative uses for the stubble within the farming system are among such constraints. In cotton cropping systems, stubble management can be a difficult problem, especially under minimum tillage situations, because of its slow rate of decomposition and its interference with irrigation and other management practices. Nonetheless, burning or removal from the field for stock feed or ethanol production will surely hasten the decline in soil organic carbon and soil fertility. Hot burning can lead to the loss via volatilisation of up to half of the N and S in the stalk (Blair, 1993).

Further research is required on the rate of decomposition of cotton stalks and capsules, and on the fate of the nutrients released. The breakdown rate of these stubble can be manipulated in a number of ways including fine-slashing of the stubble or treatment with mild sulfuric acid if breakdown rates are to be enhanced. Sulfuric acid can be obtained at a cheap cost, it provides a source of S, and it can help check the increase in pH brought about by long periods of cultivation (see Chapter 3). The key issue however, is whether or not the breakdown rate should be increased, or whether these stubble represent a longer term supply of carbon and nutrients, and a resistant residue to enhance soil physical conditions. With regards to crop rotation options, it appears most probably that rotating cotton with wheat is a more sustainable option with regards to long-term improvement of soil quality than continuous cotton or legumes alone. The inclusion of legumes in the rotation sequence appears to produce mainly short-term benefits probably as a result of their rapid decomposition rates. However, since observations have only been made for a relatively short time, subsequent monitoring of the organic carbon changes is recommended for a conclusive evaluation of the role of different rotation sequences on soil organic carbon status.

7.1. FUTURE RESEARCH DIRECTIONS

(a) Soil test calibration and interpretation: The survey reported in Chapter 3 showed that most of the soils used for cotton production in Australia have adequate soil test levels. This was not observed on the cotton seedling growth in the nutrient omission trial under glasshouse conditions. The critical levels used for recommending nutrient amendments for Australian cotton are based on research conducted elsewhere, mostly on crops other than cotton. It is clear that appropriate interpretations of soil test values require proper correlations of test values with known field response for cotton under Australian conditions. Further research is required in this area for efficient management of nutrients under cotton.

(b) Relating soil carbon fractions to yield and soil structure: In the cracking clay soils used for cotton production, the role of soil organic matter in maintaining yield and soil structure is not well understood. The study reported in this thesis, and several previous studies, showed that cultivation of soils has resulted in significant losses in soil organic matter, but the ultimate effect on the yield of cotton and soil structure is not well documented. Also, the issue of how much soil organic matter is appropriate for heavy clay soils, such as black earths, to ensure that the chemical, physical and biological functions of the soil are adequate for sustained productivity is unresolved. There have been questions as to how a unit decline in soil organic matter relates to changes in soil structure, cation exchange capacity and yield. Further research is necessary to relate the quality and quantity of organic matter to other sustainability measurements.

(c) Detailed characterisation of organic matter under different cropping systems: With the current push towards stubble retention, information on the benefits of cotton stubble towards soil organic matter quantity and quality is still limited. Results from the study reported in this thesis have shown higher levels of organic matter in soil on which cotton stubble was retained as compared to soil on which cotton stubble was removed. Also, the short-term results from the cotton rotation experiment showed that rotations containing wheat in the sequence are more beneficial with regards to improvement of soil organic matter status than continuous cotton or legumes only. However, the nature of organic matter contributed to the soil in these management systems and the influence on cotton productivity are not known. Results from other studies (Skjemstad, 1997: Personal communications) indicated that a significant component of the organic carbon in soils under cotton is charcoal. There is a need to examine in detail the chemical and physical fractions of soil organic matter in soils under cotton, and to study the changes in these fractions under different cotton cropping systems. These changes should be related to other measurements, such as yields, nutrient uptake, and soil aggregate stability, in order to assess the relative contribution of different fractions of soil organic matter in maintaining yields and soil structural fertility.