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Characterization of Soil Organic Matter in Aggregates and Size-Density Fractions by Solid State ^{13}C CPMAS NMR Spectroscopy

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ABSTRACT

Understanding the changes in soil organic matter (SOM) composition during aggregate formation is crucial to explain the stabilization of SOM in aggregates. The objectives of this study were to investigate (i) the composition of SOM associated with different aggregates and size-density fractions and (ii) the role of selective preservation in determining the composition of organic matter in aggregate and size-density fractions. Surface soil samples were collected from an Alfisol on the Northern Tablelands of NSW, Australia with contrasting land uses native pasture, crop-pasture rotation and woodland. Solid state ^{13}C cross-polarization and magic angle spinning (CPMAS) Nuclear Magnetic Resonance (NMR) spectroscopy was used to determine the SOM composition in macroaggregates (250-2000 μm), microaggregates (53-250 μm), and <53 μm fraction. The chemical composition of light fraction (LF), coarse particulate organic matter (cPOM), fine particulate organic matter (fPOM) and mineral associated soil organic matter (mSOM) were also determined. The major constituent of SOM of aggregate size fractions

was O-alkyl carbon, which represented 44-57% of the total signal acquired, whereas alkyl carbon contributed 16-27%. There was a progressive increase in alkyl carbon content with decrease in aggregate size. Results suggest that SOM associated with <53 μm fraction was at a more advanced stage of decomposition than that of macroaggregates and microaggregates. The LF and cPOM were dominated by O-alkyl carbon while alkyl carbon content was high in fPOM and mSOM. Interestingly, the relative change in O-alkyl, alkyl and aromatic carbon between aggregates and SOM fractions revealed that microbial synthesis and decomposition of organic matter along with selective preservation of alkyl and aromatic carbon plays a significant role in determining the composition of organic matter in aggregates.

Keywords: soil aggregate, O-alkyl carbon, alkyl carbon, aromatic carbon, selective preservation

INTRODUCTION

The dynamics of soil organic matter (SOM) in soil aggregates is one of the key factors controlling the stabilization of carbon in soil. Relatively undecomposed plant residue that is occluded within aggregates is usually referred to as particulate organic matter (POM) and termed light fraction (LF) if not incorporated (Six et al. 2002). The decomposition of POM in microaggregates (< 250 μm diameter) is slow but deterioration of macroaggregates (> 250 μm) by changes in land use (e.g. forest to cropland) typically leads to more rapid loss of POM from the soil (John et al. 2005). The different decomposition rates of POM and SOM associated with mineral particles in macroaggregates and microaggregates are believed to produce structurally different organic molecules because of the progressive loss of the more biologically labile

components and a concomitant increase in the proportion of more resistant components in the remaining material (Wang et al. 2004).

Solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has been used successfully to study the chemical nature of SOM in bulk soils (Mahieu et al. 1999). This spectroscopic technique has also been used to study LF and POM isolated from bulk soil. However, the study of SOM stabilization in soil aggregates demands more specific chemical characterization of physically isolated SOM fractions (Kögel-Knabner et al. 2006). Separation of macroaggregates and microaggregates is considered a better methodical approach to isolate physical SOM fractions without mixing the SOM fractions in soil aggregates (Torn et al. 2009). However, the methods of isolation of SOM fractions vary widely and are not sensitive enough to capture small variations in different SOM pools (Denef et al. 2009).

Golchin et al. (1994a, 1994b, 1995) fractionated bulk soil into LF and POM and showed that a higher proportion of O-alkyl carbon was associated with the former while the latter contained more alkyl carbon than other density fractions. Similar variations in chemical forms of POM and LF were also reported by Skjemstad et al. (1986, 1987, 1996, 2001, 2008) and Baldock et al. (1989, 1990a, 1992, 1997). However, Six et al. (2001) observed no biochemical differences between extracts of these two fractions with solution NMR. In contrast to the differences in the chemical composition of LF and particulate organic matter, Helfrich et al. (2006) and Gartzia-Bengoetxea et al. (2011) observed a similar alkyl and O-alkyl carbon content in different aggregate size ranges which they proposed was indicative of similar decomposition rates for both fractions in the various aggregate size ranges. However, Steffens et al. (2011) reported higher

alkyl carbon concentration in small aggregate size classes and in POM fractions in grazed semi-arid steppe soils. It is therefore possible that SOM in different aggregate sizes can have a different chemical composition and decomposition rate.

The degree of SOM decomposition can be assessed by the extent of chemical alteration of organic macromolecules during the process of microbial decomposition. Golchin et al. (1994a, 1994b) showed that O-alkyl carbon (e.g. carbohydrate) content decreased with increasing degrees of SOM decomposition. The decrease in O-alkyl carbon is associated with an increase in alkyl carbon content due to selective preservation and microbial synthesis during SOM decomposition (Golchin et al. 1998). Baldock et al. (1992) reported that alkyl carbon in the small particle size fraction was accumulated mainly due to the selective preservation of alkyl carbon compounds during decomposition. Other investigations highlighted the importance of microbial synthesis of O-alkyl and alkyl carbon in fine particle size fractions (e.g. Guggenberger et al. 1995, 1999; Six et al. 2006). The objectives of this study were to investigate (i) the composition of SOM associated with different aggregate and size-density fractions and (ii) the role of selective preservation in determining the composition of organic matter in aggregate and size-density fractions.

MATERIALS AND METHODS

Study Area and Soil Sampling

The study was conducted in the Armidale region (elevation 980 m) on the Northern Tablelands of New South Wales (NSW), Australia (Figure 1). Soil samples were collected from the surface (0-10 cm) of an Alfisol (equivalent to Dermosols in Australian Soil Classification) (Soil Survey Staff 2010; Isbell et al. 1996) with three contrasting land uses: (i) native pasture (ii) crop pasture rotation and (iii) woodland. The native pasture sites were composed solely of native perennial grasses such as Red Grass (*Bothriochloa macra*), Wire Grass (*Aristida ramosa*), Wallaby Grass (*Austrodanthonia* spp.), etc. Recently, a sown crop at Kirby was fescue (*Festuca arundinace*). The woodland consisted of *Eucalyptus* spp. dominated by Blakely's red Gum (*Eucalyptus blakelyi*) and Yellow Box (*Eucalyptus melliodora*). The parent material of these soils was tertiary basaltic igneous rock, and the description of the sites and chemical characteristics of the soils are given in Table 1. Soil samples were collected from each land use by selecting 3 separate blocks (50 × 50 m) along the slope of each paddock. In each block 10 random samples were collected and then composited into one.

Aggregate Preparation

Soil samples were dried at 40° C to constant moisture content and crushed to 2-4 mm aggregates by applying vertical force with a soil crusher. A 10g subsample of dried 2-4 mm aggregates was placed in a 250 mL beaker. Distilled water (100 mL) was added and left for 2 minutes for

aggregate slaking to take place without applying external force. The slaked soil aggregates were then stirred at 300 rpm for 3 minutes on a magnetic stirrer using a 3.5 cm magnetic flea to impart energy to the slaked aggregates. This low energy stirring after slaking was applied to dislodge smaller aggregates from larger aggregates. The soils were then wet-sieved for 15 minutes by a Yoder apparatus (Yoder Scientific Apparatus & Instruments, Yoder, IN) with 15 strokes per minute and 20 mm oscillation depth. The sieves used during sieving were 2000, 1000, 500, 250, 125 and 53 μm . The aggregates retained on each sieve were dried to constant moisture content at 40°C. The collected aggregates were then recombined to generate three fractions, (i) 250-2000 μm , (ii) 53-250 μm , and (iii) <53 μm . In the following, 250-2000 μm and 53-250 μm aggregates are referred to as macroaggregates and microaggregates, respectively. The soil that passed through 53 μm sieve during wet sieving is termed as <53 μm fraction. The <53 μm fraction might contain both unaggregated and aggregated <53 μm soil particles.

Size and Density Fractionation

Macroaggregates and microaggregates were fractionated into SOM size and density fractions as described by Six et al. (1998) with few modifications (Figure 2). For example, sodium polytungstate was replaced with sodium iodide (NaI) for the density fractionation (Sohi et al. 2001) and LF was defined as SOM, which had a density <1.6 g cm^{-3} (Paul et al. 2008). Sub-samples of macroaggregates and microaggregates were placed into a 50 ml centrifuge tube to which a solution of NaI (30 mL, 1.6 g cm^{-3}) was added. The soil suspension was then slowly mixed by reciprocal shaking at 10 strokes per minute and then the tubes were swirled gently for 30 seconds. The solids that adhered to the lid were washed down with another aliquot of NaI

solution (15 mL) and centrifuged for 30 minutes at 1250 g. The floating materials were collected on a nylon filter and washed thoroughly with deionised water to remove the NaI. After filtration, the LF was transferred to an aluminum cup and dried at 40°C. The heavy fraction ($>1.6 \text{ g cm}^{-3}$) was then dispersed with 0.5% sodium hexametaphosphate by shaking for 18 hours in a reciprocal shaker. The dispersed heavy fraction was then passed through 250 and 53 μm sieves depending on the size of the aggregates to separate POM. The POM that retained on 250 and 53 μm sieves was termed as coarse POM (cPOM) and fine POM (fPOM), respectively. The heavy fraction that passed through a $<53 \mu\text{m}$ sieve was termed as mineral associated soil organic matter (mSOM). The content of each sieve (POM and sand) and mSOM were dried at 40°C and weighed.

Removal of Paramagnetic Materials

Soil macroaggregates, microaggregates and $<53 \mu\text{m}$ fractions were treated with 0.3 M hydrofluoric acid (HF) in a ratio of 1:40 to remove paramagnetic materials such as oxides and hydroxides of iron and manganese. The samples treated with HF were shaken reciprocally for 18 hours and then centrifuged at 4000 g for 25 minutes. This procedure was repeated 3-5 times depending on the extraction of iron (Fe)/manganese (Mn) from the sample. After the extraction, HF treated samples were washed with deionized water three times and dried at 40°C (Simpson and Preston 2008).

Solid State ^{13}C CPMAS NMR Spectroscopy

All aggregates and SOM size-density fractions were analyzed by solid-state NMR spectroscopy (Bruker Avance III wide bore 300 MHz spectrometer, Germany) at the University of New South

Wales, Australia. The ^{13}C spectra were acquired at 75 MHz with cross-polarization and magic angle spinning (CPMAS) at 12 kHz spinning speed, using a 4 mm CPMAS probe. Soil samples (~40-80 mg) were packed into zirconia rotors closed with polychlorotrifluoroethylene (Kel-F) caps. A ramped ^1H -pulse starting at 50% and increasing to 100% power was used during a contact time of 1.5 ms. The recycle delay was 1s for all spectra and 5000 to 60,000 scans were taken for each sample. Processing of the data was achieved with Topspin 3.0 (Bruker, Germany). The raw free induction decay (FID) was line broadened with a window function of 200 Hz, followed by phase and baseline corrections. The ^{13}C chemical shifts were calibrated relative to the carbonyl of glycine at 176 ppm as an external reference. Relative contributions of the various carbon groups were determined by integration of the signal intensity in their respective chemical shift regions. The region from 220 to 160 ppm was assigned to carbonyl (aldehyde and ketone) and carboxyl/amide carbon. 160 and 110 ppm were assigned to olefinic and aromatic carbon. The O-alkyl carbon signals were assigned to the region of 110 to 45 ppm. Alkyl carbon was detected in the region 45 to -10 ppm (Steffens et al. 2011). The total signal intensities were normalised to 100 to compare each type of carbon in different aggregate and SOM fractions. The ratios of alkyl carbon to O-alkyl carbon for each aggregate and SOM fractions were calculated to assess the degree of decomposition of SOM (Baldock et al. 1997; Kölbl and Kögel-Knabner 2004). An example of acquired spectra is shown in Figure 3.

Statistical analysis was not performed because replicated samples were not analyzed. The difference in chemical composition of SOM in different aggregate sizes were described on the basis of relative contents (percent of total NMR signal acquired) of specific functional groups in aggregates and SOM fractions.

RESULTS

Chemical Composition of SOM in Aggregates

Carbon associated with macroaggregates, microaggregates and $<53 \mu\text{m}$ fraction was dominantly O-alkyl carbon, which represented 44-57% of the total signal acquired. The alkyl carbon contributed 16-27% of aggregate associated organic carbon. A relatively small proportion of the signal, i.e. 13-19%, was derived from aromatic carbon. Carboxyl carbon contributed only 7-13% of the organic carbon signal in aggregates (Figure 3 and Table 2).

Macroaggregates had more O-alkyl but less alkyl carbon under woodland whereas microaggregates had highest O-alkyl and alkyl carbon under native pasture. In the $<53 \mu\text{m}$ fraction, O-alkyl and alkyl carbon were found to be highest under native pasture and crop-pasture rotation, respectively. Moreover, in both microaggregates and the $<53 \mu\text{m}$ fraction, less alkyl carbon was observed in woodland. The macroaggregates and microaggregates had the highest aromatic carbon content in woodland but the $<53 \mu\text{m}$ fraction had most aromatic carbon in the crop-pasture rotation. Both microaggregates and the $<53 \mu\text{m}$ fraction had highest carboxyl carbon amounts in woodland, whereas the lowest content of these carbon forms were found in native pasture and crop-pasture rotation.

Chemical Composition of Size and Density Fractions

O-alkyl, alkyl and aromatic carbon contributed 41-63%, 13-19% and 16-31% of the total signal acquired from LF and cPOM (Table 3). A relatively small proportion of these SOM fractions

was composed of carboxyl carbon. In fPOM and mSOM of macroaggregates and microaggregates, O-alkyl carbon contributed 43-55% and 40-49%, respectively (Table 3). Fine POM and mSOM in microaggregates had higher alkyl, aromatic and carboxyl carbon contents compared to those of macroaggregates.

The O-alkyl and alkyl carbon contents of LF, cPOM and mSOM were high both under native pasture and crop-pasture rotation compared to woodland. Alkyl carbon content in fPOM, however, was high in woodland soils. The woodland SOM fractions contained more aromatic carbon than material from other land uses with an exception in mSOM under crop-pasture rotation. The mSOM under crop-pasture rotation contained highest most aromatic carbon compared to native pasture and woodland (Table 3).

Degree of SOM Decomposition

The alkyl to O-alkyl carbon ratio, which is indicative of the degree of SOM decomposition, was 18% and 20% higher in microaggregates and the <53 μm fraction than in macroaggregates (Table 2). The ratio in native pasture was highest in macroaggregates but crop-pasture rotation had the highest ratio in microaggregates and the <53 fraction. The alkyl carbon/O-alkyl carbon ratio of LF was similar to cPOM, which varied from 0.26-0.30 and from 0.24-0.44, respectively. The fPOM of microaggregates had 8% higher alkyl/O-alkyl carbon ratios than that of macroaggregates. In both aggregate sizes, fPOM in woodland had the highest alkyl/O-alkyl carbon ratio. Mineral associated SOM of microaggregates had 8% higher alkyl/O-alkyl carbon ratio than macroaggregates. The highest ratio of alkyl to O-alkyl carbon in native pasture and woodland was found in macroaggregates and microaggregates mSOM, respectively (Table 3).

DISCUSSION

Characterization of Aggregate Associated Soil Organic Matter

The signal from SOM associated with macroaggregates, microaggregates and the $<53 \mu\text{m}$ fraction was dominated by O-alkyl carbon, which is typically attributed to carbohydrates such as cellulose and hemicelluloses. The contents of O-alkyl carbon on average tended to be lower and alkyl carbon tended to be higher in microaggregates and the $<53 \mu\text{m}$ fraction compared with macroaggregates. Moreover, on average the aromatic carbon tended to increase in the microaggregates and $<53 \mu\text{m}$ fraction relative to the macroaggregates. The SOM composition of different aggregate sizes is determined by the composition of SOM fractions present in the aggregates. For example, the SOM composition in macroaggregates represents average composition of cPOM, fPOM and mSOM. The results of the present study showed that SOM fractions in macroaggregates (e.g. POM and mSOM) had higher O-alkyl carbon but lower alkyl and aromatic carbon content compared to that of SOM fractions in microaggregates. Moreover, the SOM in microaggregates and $<53 \mu\text{m}$ fraction was more decomposed than macroaggregates. Similar to the results of the current study, Baldock et al. (1992) demonstrated that the O-alkyl carbon content of SOM from coarse particle size fractions was higher compared to fine particle size fractions of soil. The authors concluded that the extent of decomposition of SOM associated with fine particle fractions was higher compared to that of coarse fractions. Golchin et al. (1998) stated that at a later stage of microbial decomposition, particulate organic matter can become occluded in microaggregates and decomposition products of particulate organic matter can

become adsorbed on $<53 \mu\text{m}$ particles and form mSOM. Therefore, the progressive increase in the degree of decomposition of SOM fractions from LF through POM to mSOM in the current study plays a significant role in changing SOM composition in different aggregates.

The Role of Selective Preservation of Specific Carbon Types on SOM Composition

Selective preservation means the relative increase over time in concentration of slowly decomposable organic carbon as the more easily decomposable organic matter reduces. For example, microbial decomposition of carbohydrates increases the proportion of alkyl carbon present in the organic matter (Hatcher et al. 1983; Baldock et al. 1992). The selective preservation against microbial decomposition of SOM containing a high proportion of alkyl carbon might be due to hydrophobicity of long chain lipids (Schulten and Schcitzer 1990; Bachmann et al. 2008). Similarly, aromatic carbon content tended to increase in small aggregate fractions and is probably also due to selective preservation of lignin, tannins, lipids (Kölbl and Kögel-Knabner 2004; Mueller and Kögel-Knabner 2009; Steffens et al. 2011).

We examined the selective preservation of alkyl and aromatic carbon in aggregates and size-density fractions of SOM. It was assumed that (i) the organic matter present in the macroaggregates was the substrate for organic matter in microaggregates and the $<53 \mu\text{m}$ fraction, (ii) the LF and cPOM supplied substrate for fPOM. The decomposition products of fPOM adsorb on $<53 \mu\text{m}$ particles and form mSOM and (iii) during the aggregate formation O-alkyl carbon was mainly lost by microbial decomposition and there was no microbial synthesis

of O-alkyl carbon. So, if the alkyl and aromatic carbon are selectively preserved during microbial decomposition and aggregate formation, the SOM associated with microaggregates and <53 μm would accumulate alkyl and aromatic carbon followed by the reduction of O-alkyl carbon in SOM fractions compared to macroaggregates. The percent reduction in O-alkyl carbon and accumulation of alkyl and aromatic carbon content between different aggregates and SOM fractions were calculated using Equation 1 and results are presented in Figure 4 and 5.

$$\frac{\text{Difference in content of specific carbon type between aggregates or SOM fractions}}{\text{Content of specific carbon type in large aggregate or SOM fraction}} \times 100 \quad [\text{Eq. 1}]$$

It was observed that the percent decrease in O-alkyl carbon in microaggregates compared to macroaggregates might contribute to increase in alkyl carbon content in microaggregates under crop-pasture rotation and woodland. The percent reduction of O-alkyl carbon in microaggregates compared to macroaggregates was <1% under native pasture and did not contribute to the increase in alkyl carbon content in microaggregates. The percent decrease in O-alkyl carbon between microaggregates and <53 μm fraction, however, did not increase the alkyl carbon content under native pasture and woodland in <53 μm fraction. The percent reduction of O-alkyl carbon between aggregate sizes correlates with an increase in the aromatic carbon content of microaggregates and <53 μm fraction except under aromatic carbon content in crop-pasture rotation microaggregates. The aromatic carbon content in microaggregates was 2.5% lower than that of macroaggregates under crop-pasture rotation. The accumulation of aromatic carbon under woodland was low in microaggregates (2.8%) and <53 μm fraction (1.2%). So, the accumulation of aromatic carbon was not consistent across aggregate sizes and land uses.

Moreover, the percent changes of O-alkyl, alkyl and aromatic carbon in fPOM and mSOM between macroaggregates and microaggregates did not show a consistent decrease in O-alkyl carbon with an increase in alkyl and aromatic carbon content, indicating that selective preservation might not exclusively be responsible for accumulation of alkyl and aromatic carbon in different aggregate fractions (Figure 5). As we assume that macroaggregates and microaggregates are formed around decomposing plant debris, selective preservation should cause the aromatic carbon content to be higher in macroaggregates and microaggregates than in the LF and cPOM. But aromatic carbon content showed no consistent change between physical SOM fractions of different aggregate sizes. Moreover, the aromatic carbon content was decreased in mSOM compared to fPOM.

Therefore, the percent changes in O-alkyl carbon, alkyl and aromatic carbon between different aggregates and SOM fractions indicated that the selective preservation might not contribute solely to the accumulation of alkyl carbon in aggregates. Baldock et al. (1990b) showed that bacterial and fungal decomposition of glucose could synthesis alkyl carbon in soil. So, the enrichment of alkyl carbon in small aggregates and SOM fractions may be due to microbial synthesis of lipid-like molecules as well as selective preservation during the decomposition of SOM. Moreover, the percent reduction of alkyl carbon in small aggregate and SOM fractions compared to large aggregate and SOM fractions, also suggests decomposition of alkyl carbon during SOM decomposition. Therefore, the content of alkyl carbon in any aggregate or SOM fractions represents the net effect of preservation/synthesis and decomposition of alkyl carbon. Similarly, the percent reduction and accumulation of aromatic carbon from small aggregates and

SOM fractions might also suggest preservation and decomposition of aromatics in soil aggregates.

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Table 1. Site description and soil properties of studied Alfisol

| Soil | Land use | Geo-reference | History | Sand | Silt | Clay | Bulk Density | SOC | pH _w (1:5) |
|---------|-----------------------|---------------------------------|--|---------|-------|-------|--------------------|---------|-----------------------|
| | | | | % (w/w) | | | Mg m ⁻³ | % (w/w) | |
| Alfisol | Native Pasture | 30°26'02.70"S 151°38'01.38"E | Native grass, lightly grazed, >20 years under current management | 37.49 | 15.42 | 47.09 | 1.01 | 4.36 | 5.84 |
| | Crop/Pasture Rotation | 30°26'03.36"S 151°38'02.67"E | Crop/pasture rotation, sporadic fertilizer application and > 20 years under current management | 34.04 | 11.37 | 54.59 | 0.92 | 3.51 | 6.09 |
| | Woodland | 30°26'02.74"S 151°38'04.77"E | Eucalypt woodland, grass cover lightly grazed, >20 years under current management | 45.27 | 15.69 | 39.04 | 0.84 | 4.16 | 6.04 |

Notes. SOC, Soil organic carbon

Table 2. Relative contents (% of total signal acquired) of alkyl C, O-alkyl C, aromatic C, carboxyl C and alkyl/O-alkyl C ratios of aggregate as revealed by ^{13}C CPMAS NMR spectroscopy

| Land use | Aggregates | Chemical Shift limits (ppm) | | | | Alkyl C/ O-Alkyl C |
|-----------------------|----------------------------|-----------------------------|------------|-----------|----------|-----------------------|
| | | 220-160 | 160-110 | 110-45 | 45-(-10) | |
| | | Carboxyl C | Aromatic C | O-Alkyl C | Alkyl C | |
| Native Pasture | | 7.09 | 13.12 | 54.09 | 25.69 | 0.47 |
| Crop-pasture rotation | Macroaggregates | 10.15 | 16.94 | 53.84 | 19.07 | 0.35 |
| Woodland | | 9.2 | 17.6 | 57.26 | 15.94 | 0.28 |
| Native Pasture | | 4.39 | 14.53 | 53.66 | 27.42 | 0.51 |
| Crop-pasture rotation | Microaggregates | 10.66 | 16.51 | 46.5 | 26.33 | 0.57 |
| Woodland | | 11.71 | 18.09 | 44.4 | 25.8 | 0.58 |
| Native Pasture | | 10.84 | 16.3 | 47.39 | 25.47 | 0.54 |
| | <53 μm fraction | | | | | |
| Crop-pasture rotation | | 9.97 | 18.95 | 43.77 | 27.3 | 0.62 |

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Woodland

13.43

18.31

44.46

23.8

0.54

Table 3. Relative contents (% of total signal acquired) of alkyl C, O-alkyl C, aromatic C, carboxyl C and alkyl/O-alkyl C ratios of SOM fractions as revealed by ^{13}C CPMAS NMR spectroscopy

| Land use | SOM Fractions | Chemical Shift limits (ppm) | | | | Alkyl C/ O-Alkyl C |
|------------------------|---------------|-----------------------------|------------|-----------|----------|-----------------------|
| | | 220-160 | 160-110 | 110-45 | 45-(-10) | |
| | | Carboxyl C | Aromatic C | O-Alkyl C | Alkyl C | |
| Native Pasture | LF | 4.08 | 16.56 | 62.67 | 16.69 | 0.27 |
| Crop-pasture rotation | | 5.41 | 17.52 | 61.16 | 15.92 | 0.26 |
| Woodland | | 15.39 | 30.79 | 41.25 | 12.57 | 0.30 |
| <i>Macroaggregates</i> | | | | | | |
| Native Pasture | cPOM | 8.19 | 16.32 | 61 | 14.49 | 0.24 |
| Crop-pasture rotation | | 9.65 | 16.58 | 54.56 | 19.21 | 0.35 |
| Woodland | | 15.06 | 24.62 | 41.94 | 18.41 | 0.44 |
| Native Pasture | fPOM | 9.2 | 18.73 | 54.98 | 17.09 | 0.31 |
| Crop-pasture rotation | | 9.58 | 14.66 | 52.54 | 23.22 | 0.44 |
| Woodland | | 10.64 | 19.51 | 47.21 | 22.63 | 0.48 |
| Native Pasture | mSOM | 10.54 | 8.61 | 49.25 | 31.6 | 0.64 |
| Crop-pasture rotation | | 15.84 | 16.67 | 46.26 | 21.24 | 0.46 |
| Woodland | | 14.28 | 17.81 | 42.61 | 25.29 | 0.59 |
| <i>Microaggregates</i> | | | | | | |
| Native Pasture | fPOM | 12.47 | 17.11 | 48.55 | 21.88 | 0.45 |
| Crop-pasture rotation | | 11.59 | 18.01 | 48.34 | 22.06 | 0.46 |
| Woodland | | 13.4 | 23.59 | 40.09 | 22.92 | 0.57 |
| Native Pasture | mSOM | 11.52 | 12.64 | 47.11 | 28.73 | 0.61 |
| Crop-pasture rotation | | 14.65 | 17.31 | 42.46 | 25.58 | 0.60 |
| Woodland | | 13.26 | 14.65 | 42.24 | 29.85 | 0.71 |

Notes. SOM, Soil organic matter; LF, Light fraction; cPOM, Coarse particulate organic matter; fPOM, Fine particulate organic matter; mSOM, Mineral associated soil organic matter

Figure 1. Location map of study area, NSW, Australia.

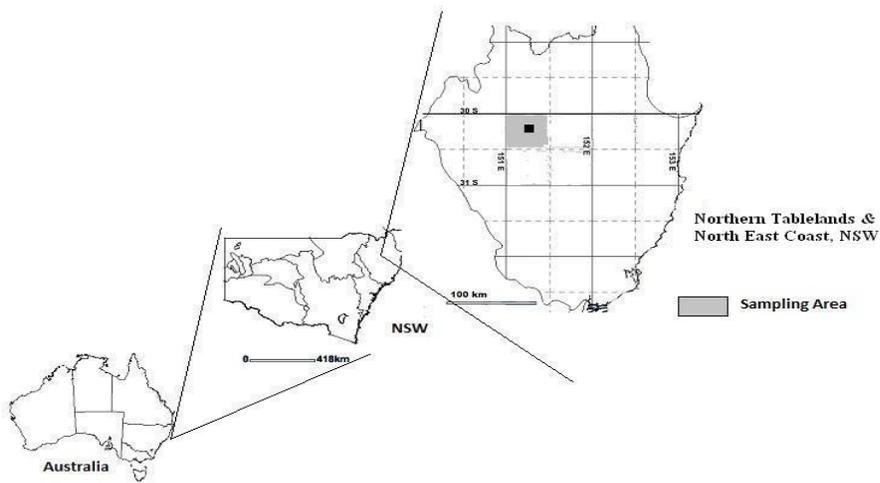


Figure 2. Size-density fractionation scheme of aggregate associated SOM. LF is light fraction and POM is particulate organic matter.

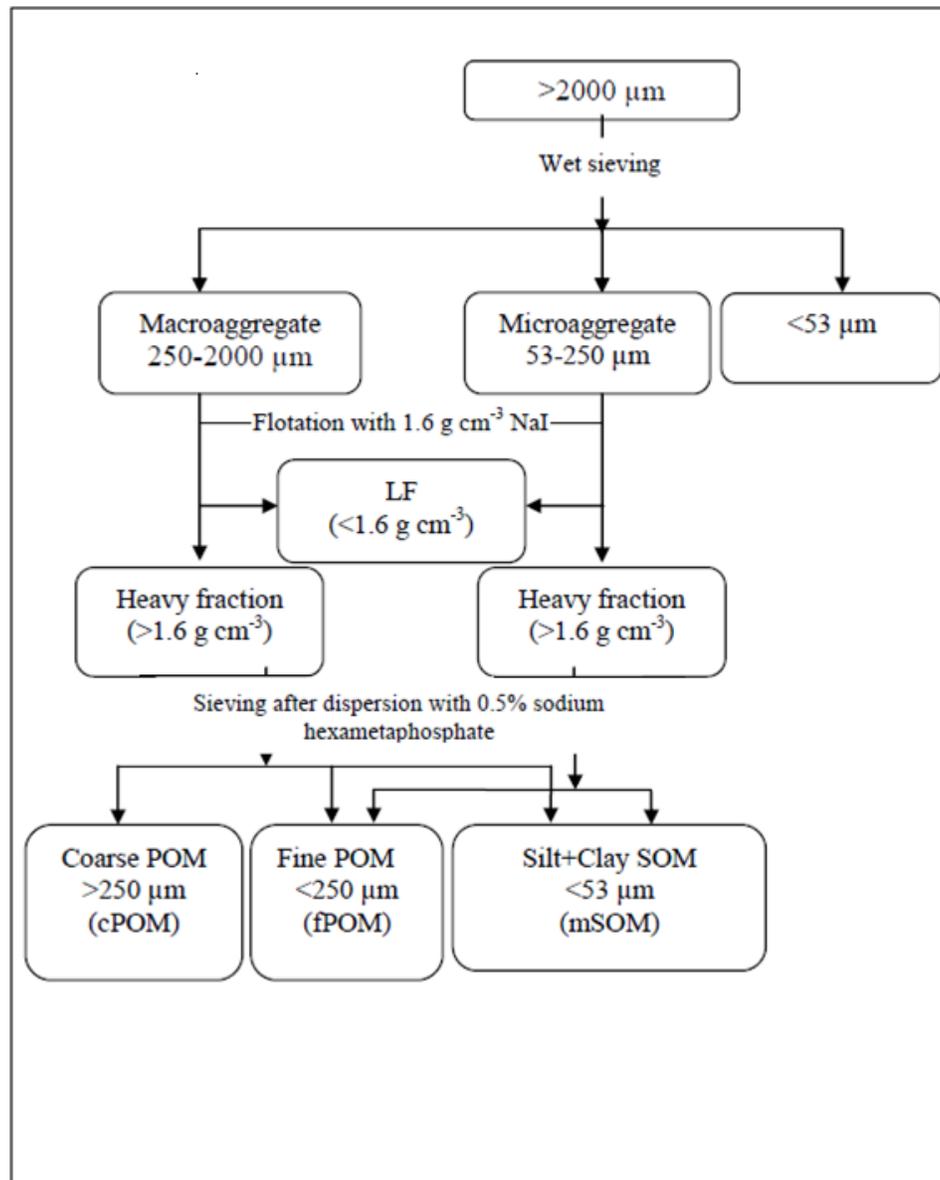


Figure 3. ^{13}C CPMAS NMR signals of macroaggregates ($>250\ \mu\text{m}$), microaggregates ($<250\ \mu\text{m}$) and $<53\ \mu\text{m}$ fraction in native pasture (NP), crop-pasture rotation (CP) and woodland (WL).

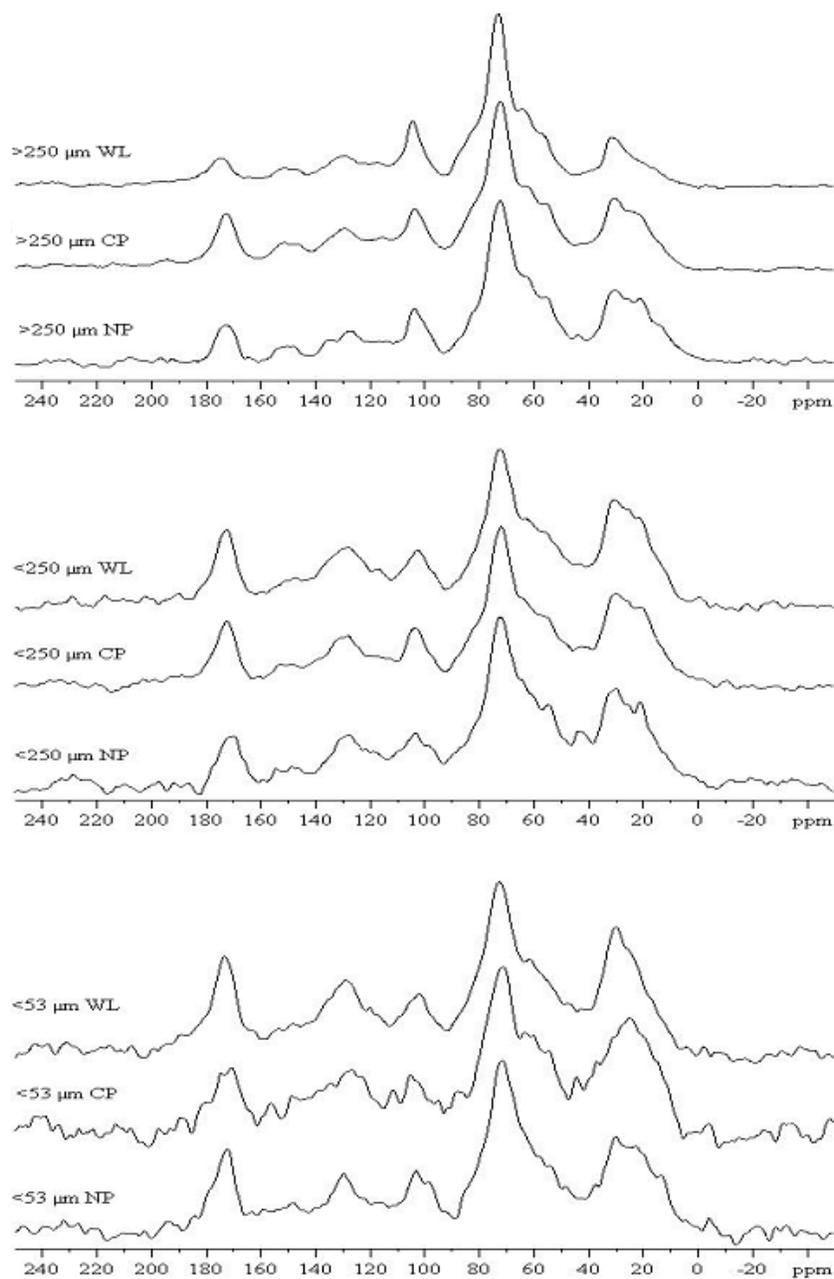


Figure 4. Percent O-alkyl carbon reduction, alkyl carbon and aromatic carbon accumulation between macro- and microaggregates (>250 – <250 μm); microaggregates and <53 μm fraction (<250 – <53 μm). The negative accumulation values indicate reduction of alkyl and aromatic carbon.

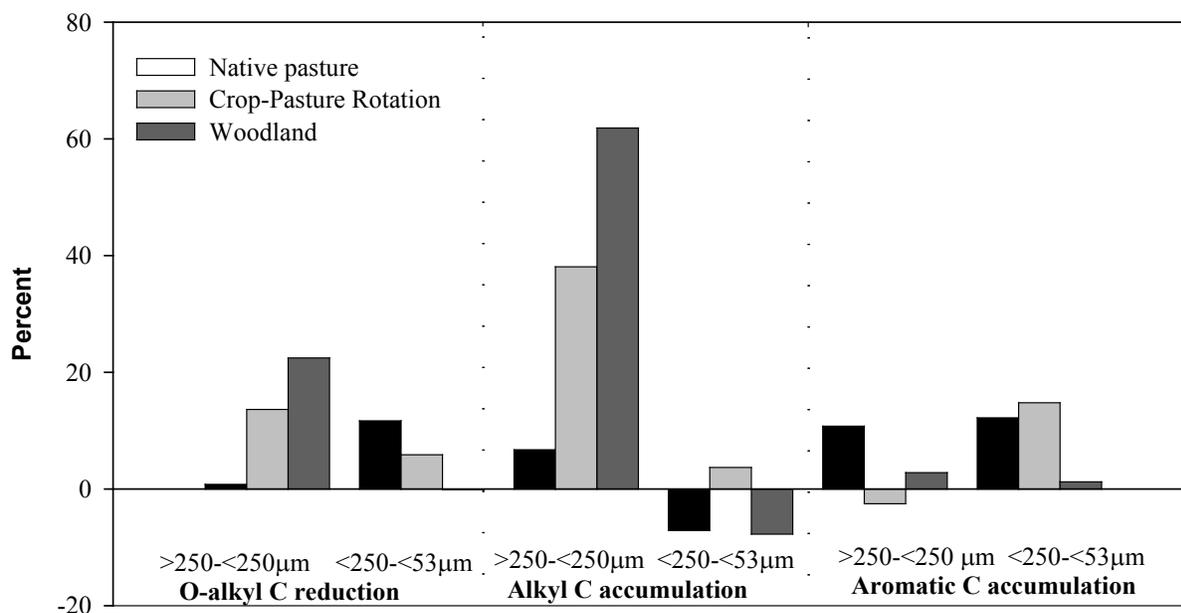


Figure 5. Percent O-alkyl carbon reduction, alkyl carbon and aromatic carbon accumulation between Light fraction and Coarse particulate organic matter (LF-cPOM); Coarse particulate organic matter and Fine particulate organic matter in macroaggregates (cPOM-fPOM_{Ma}); Fine particulate organic matter between macro- and microaggregates (fPOM_{Ma}-fPOM_{Mi}); Fine particulate and Mineral associated soil organic matter in macroaggregates (fPOM_{Ma}-mSOM_{Ma}); Fine particulate and Mineral associated soil organic matter in microaggregates (fPOM_{Mi}-mSOM_{Mi}). The negative reduction values indicate accumulation of O-alkyl carbon and negative accumulation indicated reduction of alkyl and aromatic carbon.

