Changes in soil carbon during the establishment of a hardwood plantation in
subtropical Australia

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Abstract

Soil carbon (C) pools are not only important to governing soil properties and nutrient cycling in forest ecosystems, but also play a critical role in global C cycling. Mulch and weed control treatments may alter the soil C pools by changing organic matter inputs to the forest ecosystem. We studied the 12-month mulch and weed control responses on the chemical composition of soil organic C and the seasonal dynamics of water extractable organic C (WEOC), hot water extractable organic C (HWEOC), chloroform-released organic C (CHCl₃-released C), and acid hydrolysed organic C (acid hydrolysable C) in a hardwood plantation of subtropical Australia. The results showed that compared with the non-mulch treatment, the mulch treatment significantly increased soil WEOC, HWEOC, and CHCl₃-released C over the four sampling months. The weed control treatment significantly reduced the amount of HWEOC and CHCl₃-released C compared with the no weed control treatment. Either the mulch or weed control treatment did not significantly affect soil acid hydrolysed organic C. There were no significant seasonal variations in soil WEOC, HWEOC, CHCl₃-released C,

and acid hydrolysed organic C in the hardwood plantation. Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy was used to study the structural chemistry of soil C pools in HF (hydrofluoric acid) treated soils collected 12 months after the mulch and weed control treatments were applied. Overall, O-alkyl C was the dominant C fraction, accounting for 33-43% of the total NMR signal intensity. The mulch treatment led to higher signal intensity in the alkyl C spectral region and A/O-A ratio (the ratio of alkyl C region intensity to O-alkyl C region intensity), but lower signal intensity in the aryl C and aromaticity. Compared with the no weed control treatment, the weed control treatment reduced signal intensity in the aryl C and aromaticity. Together, shifts in the amount and nature of soil C following the mulch and weed control treatments may be due to the changes in organic matter input and soil physical environments.

Keywords: ^{13}C CPMAS NMR; hardwood plantation; labile soil C pools; mulch; weed control with herbicide treatment

1. Introduction

Soil carbon (C) pools may affect soil chemical and biological properties that control nutrient cycling and consequently have important impacts on forest productivity and sustainability. Additionally, soil C pools form a large and dynamic reservoir of C, which is an important part of the global C cycle and a potential sink for atmospheric CO_2 . Due to the important role of soil C pools in nutrient cycling of forest ecosystems and global C balance, there has long been an interest in understanding the effect of forest soil management on soil C pools (Ussiri and Johnson, 2007).

In subtropical Australia, increasing land values have resulted in hardwood plantations being established in more westerly areas with below optimal rainfall and soil nutrients for

seedling survival. To overcome these problems, several management techniques, including residue mulch and weed control through herbicide treatment, are employed. A number of studies have reported the effects of residue mulch and weed control through herbicide treatment on soil C in forest ecosystems (Busse et al., 1996; Jiang et al., 2006). For example, Chen and Xu (2005) reported that retention of forest residues significantly enhanced accumulation of soil total C and N compared with residue removal in a 6-year-old slash pine plantation of subtropical Australia. In a long-term study examining the role of under-story weeds in ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws.), Busse et al. (1996) suggested a 33% decrease of total C content in surface soil after weed control through herbicide treatment. Furthermore, residue mulch and weed control treatment can also change the composition of soil organic C pools. For example, Mathers and Xu (2003) found that soil organic matter (SOM) under residue retention had greater proportion of O-alkyl C structures than that under residue removal in a 2-year-old pine plantation of subtropical Australia. Echeverria et al. (2004) suggested that weed control through herbicide treatment significantly decreased acid hydrolysable C compared with no herbicide treatment control.

Soil organic C pools consist of various fractions varying in degree of decomposition, recalcitrance, and turnover rate, and management practices may affect these fractions differently (Ghani et al., 2003). Numerous methods, including chemical, physical and biological fractionations, have been developed to characterize soil organic C pools and dynamics (Mathers et al., 2000; McLauchlan and Hobbie, 2004). For example, cold and hot water can extract soil organic C representing soluble, readily metabolizable C sources in soils (Chen et al., 2004; Nishiyama et al., 2001). Chloroform-released organic C was used to represent the microbial component of soil organic C. Paul et al. (2001) suggested that acid hydrolysis (e.g., using 6 M HCl) procedure may be an effective technique to extract labile

constituents in the soil C pools. Both chloroform-released organic C and acid hydrolysable organic C were reported to be a potential indicator of bioreactive soil organic C pools (Xu et al., 1997). In recent years, the technique of solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization and magic-angle spinning (CPMAS-NMR) has been increasingly used (Preston, 1996; Quideau et al., 2001). This technique allows one to obtain information directly and non-destructively on C components of the entire soil sample without any chemical or physical fractionation and is well suited to the characterization of natural soil organic C pools (Schnitzer, 2001). In the present study, the aims were to investigate: (1) the impacts of residue mulch and weed control through herbicide treatment on the amounts and chemical composition of soil C; (2) the seasonal dynamics of the labile soil C pools; and (3) the relationships among various C fractions measured.

2. Materials and methods

2.1. Site description, experimental design and soil sampling

The experiment was established at Pechey, southeast Queensland (27°18'S, 152°3'E), Australia. The site has a freely draining, well-textured Oxisol (Soil Survey Staff, 1999). Basic soil properties are given in Table 1. The area lies within the sub-tropical zone with the predominant weather pattern giving cool dry winters and warm wet summers. Rainfall patterns are highly variable with an average of 851 mm (from 1995-2004).

The experiment, including four treatments, was set up in December 2004. Mulch and non-mulch treatments were applied to six 24-m wide by 67.2-m long plots located at the west side of a hill in the experimental site. This part of site was covered with woody weeds dominated by *Acacia*, *Alphitonia* and *Eucalyptus* before the establishment of this experiment. The two treatments were arranged in randomised block design with three replications. For the mulch

treatment, a tractor driven bush chopper was developed, which cut, chipped and spread all the woody weeds as mulch over the plots before the plantation was established. On average, the total weight of mulch in these plots was 1.57 kg m⁻² (dry weight), with about 5- cm layer of mulch covering over 80% of the soil (area). The C/N ratio of the mulch materials was 80.7. For the non-mulch treatment, all the mulch was removed from the plots immediately following site preparation. Weed control and no weed control treatments were applied to other six 24-m wide by 67.2-m long plots located at the east side of the same hill in the experiment site. This part of area was covered with grass weeds dominated by kikuyu (*Pennisetum clandestinum* Chiov.) before the establishment of this experiment. The weed control and no weed control treatments were also arranged in randomised block design with three replications. Weed control treatment consisted of the use of roundup (glyphosate) before site preparation and subsequent application of roundup and triclopyr, a pyridine, to eliminate all competing vegetation. In the no weed control treatment, no herbicide was applied in these plots. All the twelve plots were planted with *Eucalyptus pilularis* six months after the treatments began at the plots. The planting space was 4 m (row space) by 2.4 m (tree space).

Soil samples were collected during 2005. The four sampling months include: (a) April; (b) July; (c) October; and (d) December, which represent four seasons in Australia. At each sampling time, five 0-10 cm soil cores (2 cm in diameter) were randomly taken from each plot. To avoid the effect of tree seedlings, however, the soil cores were sampled two metres away from tree lines. These five cores were then immediately bulked, placed in a cooler (ca. 4 °C), and returned to the laboratory. The soil samples were sieved (< 2 mm) prior to physical, chemical and biological analysis.

2.2. Analysis of soil basic properties

A soil sub-sample from each plot collected in December 2005 was oven-dried at 105°C to determine the soil moisture. Soil total C (TC) and total N (TN) were determined on an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000) using the sub-samples. Another sub-sample of moist soil was extracted with 2 M KCl and the concentrations of mineral N in KCl extractions were measured using a Lachat Quickchem automated ion analyser (Quik Chem method 10-107-064-D for NH_4^+ and 10107-04-1-H for NO_3^-). Soil pH was measured at a 1:2 soil/water (w/v) ratio. Soil cation exchange capacity, conductivity, bulk density, and particle size were measured using the methods described by Rayment and Higginson (1992).

2.3. Water and hot water extraction of soil labile organic C pools

Water extractable organic C (WEOC) was extracted by shaking 3 g (oven-dried equivalent) moist soil at a soil/water (w/v) ratio of 1:10 on an end-to-end shaker at room temperature for 30 min. The mixture was then spun at 3500 rpm for 20 min and filtered through a 0.45 μm membrane filter into separate vials for C analysis (Ghani et al., 2003).

Hot water extractable organic C (HWEOC) was measured by the method described by Sparkling et al. (1998). Moist soil samples (equivalent 3 g oven-dry weight) were incubated with 30 ml distilled water in 50 ml polypropylene centrifuge tubes at 80°C (hot water extraction) for 18 h, and the tubes were then shaken on an end-to-end shaker for 30 min. The mixture in the tube was then spun at 3500 rpm for 20 min and filtered through a piece of Whatman 42 paper (Whatman Ltd., Maidstone, UK), and then through a 0.45 μm filter membrane. The organic C contents in cold water and hot water extracts were determined using a SHIMADZU TOC- VCPH/CPN analyser.

2.4. Chloroform-released organic C

The chloroform-released organic C (CHCl_3 -released C) was measured by the chloroform fumigation-extraction method (Vance et al., 1987). In brief, duplicate field moist soil sub-samples (equivalent 5 g oven-dry weight) were fumigated with chloroform for 24 h and then extracted with 0.5 M K_2SO_4 for 2 h on an end-over-end shaker. The suspended samples were centrifuged and filtered through Whatman 42 filter paper. Similar sets of unfumigated samples were extracted in the same way. The amounts of organic C in the fumigated and unfumigated soil extracts were determined by the high temperature catalytic oxidation method using a SHIMADZU TOC- VCPH/CPN analyser (Chen and Xu, 2005). CHCl_3 -released C was calculated as the difference in organic C between the fumigated and the unfumigated samples and used to represent the microbial component of soil organic C.

2.5. Acid hydrolysed soil organic C pools

Acid hydrolysed soil organic C pool (acid hydrolysable C) was measured by the method described by Sollins et al. (1999). 1 g air-dried soil sample was refluxed for 16 h in digestion tubes with 10 ml of 6 M hydrochloric acid solution. The residue (unhydrolyzable soil organic matter) was isolated, washed with 100 ml of deionized water, and dried overnight in an 80°C oven. The residue was then weighed and analysed for C content on an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000). The hydrolysable fraction was obtained by subtracting unhydrolyzable C from soil TC.

2.6. Hydrofluoric acid pre-treatment of soil samples and solid-state ^{13}C NMR spectroscopy

Soil samples were pre-treated with hydrofluoric acid (HF) prior to solid-state ^{13}C CP MAS NMR analysis. The pre-treatment can remove a large amount of Fe^{3+} and Mn^{2+} in

soil, concentrate the organic matter of a whole soil sample and improve the signal/noise ratio. After HF pre-treatment, solid-state ^{13}C CPMAS NMR analysis times can be decreased without a reduction in spectral quality. In this study, soil samples collected in December 2005 were pre-treated with 2% HF according to the method described by Skjemstad et al. (1994) and modified by Mathers et al. (2002) for NMR analysis.

Solid-state ^{13}C CPMAS NMR spectra of the HF-treated soils were obtained at a frequency of 100.59 MHz on a Varian Unity Inova400 spectrometer (Varian Inc., Palo Alto, CA). Soil samples were packed in a silicon nitride rotor (OD = 7 mm) and spun at 5 kHz at the magic angle. Single contact times of 2 ms were applied, with an acquisition time of 14 ms, and a recycle delay of 2.5 s. 6400 transients were collected for all samples and a Lorentzian line broadening function of 20 Hz was applied to all spectra. Chemical shift values were referenced externally to hexamethylbenzene at 132.1 ppm, which is equivalent to tetramethylsilane at 0 ppm.

The solid-state ^{13}C CPMAS NMR spectra were divided into four regions representing different chemical environments of a ^{13}C nucleus. These were alkyl C (0–50 ppm), O-alkyl C (50–110 ppm), aromatic C (110–160 ppm) and carbonyl C (160–210 ppm). The relative intensity of each functional group was measured by integration using the Varian NMR software package (Version 6.1c, Varian Inc., Palo Alto, CA). In some instances, the chemical shift regions were further divided, these were: O-alkyl C into methoxyl C (50–60 ppm), carbohydrate C (60–95 ppm), and di-O-alkyl C (95–110 ppm); aromatic C into aryl C (110–145 ppm) and phenolic C (145–160 ppm); and carbonyl C into carboxylic/amide/ester C (160–190 ppm) and ketone/aldehyde C (190–210 ppm).

The A/O-A ratio, the ratio of alkyl C region intensity (0–50 ppm) to O-alkyl C region intensity (50–110 ppm), which has been recommended by Baldock and Preston (1995) as an

index of the extent of decomposition or of substrate quality for microbes, was also determined in this study as an indicator of the quality of soil organic C. In addition, aromaticity has been used to characterise the extent of humification of SOM, under the assumption that SOM becomes aromatic during decomposition (Dai et al., 2001). The aromaticity was calculated as the following equation:

$$Aromaticity(\%) = \left[\frac{AromaticC(110 - 160 ppm)}{AromaticC + alkylC + O - alkylC} \right] \times 100$$

2.7. Statistical methods

Two-way repeated measures ANOVA was carried out for soil WEOC, HWEOC, CHCl₃-released C, and acid hydrolysable C over the four sampling seasons to test the significant effects of the mulch treatment and sampling time or the weed control treatment and sampling time using Statistix for Windows Version 8.0. T-test was used to examine the significant differences in WEOC, HWEOC, CHCl₃-released C, and acid hydrolysable C between the mulch and non-mulch treatments and between the weed control and no weed control treatments at each sampling month. T-test was also conducted to test the significant differences in basic soil properties (Table 1) and NMR data between the mulch and non-mulch treatments and between the weed control and no weed control treatments. Pearson linear correlations among WEOC, HWEOC, CHCl₃-released C, and acid hydrolysable C were conducted using soil samples collected from four sampling months. In addition, we determined the relationships between soil organic C functional groups and soil WEOC, HWEOC, CHCl₃-released C, or acid hydrolysable C using the soil samples collected in December 2005.

3. Results

Selected soil properties in 0-10 cm soil layer of the hardwood plantation are given in Table 1. Mulched soils had significantly higher TC, TN, and soil moisture than the non-mulched soils ($P < 0.05$). There were no significant differences in pH values, soil C/N ratio, bulk density, sand or clay contents between the mulch and non-mulch treatments. The weed control treatment significantly decreased soil TC ($P < 0.05$), but did not significantly affect any other soil basic properties listed in Table 1.

3.1. Effects of mulch

The mulch treatment significantly influenced soil WEOC ($F = 4.42$; $df = 1$; $P < 0.05$), HWEOC ($F = 4.58$; $df = 1$; $P < 0.05$), and CHCl_3 -released C ($F = 5.74$; $df = 1$; $P < 0.05$) over the four sampling months (Fig. 1A, B, and C). Average values from four sampling months for WEOC, HWEOC, and CHCl_3 -released C in the mulched soils were 77, 1609, and 1118 mg kg^{-1} soil, respectively, higher than those in the non-mulched soils (Table 2). However, the mulch treatment had no significant effect on soil acid hydrolysable C ($F = 3.01$; $df = 1$; $P > 0.05$) (Fig. 1D). There were no significant seasonal variations in soil WEOC, HWEOC, CHCl_3 -released C, and acid hydrolysable C across the two treatments ($P > 0.05$, Fig. 1). The ^{13}C CPMAS NMR spectra of soil organic C in the mulch and non-mulch treatments are shown in Fig. 2 A and B. The relative intensities of C functional groups in the HF-treated soils demonstrate that O-alkyl C spectral region was highest (36.6% for the non-mulched soil and 36.3% for the mulched soil), followed by alkyl C (23.7% for the mulched soil) or aromatic C (25.9% for the non-mulched soil), and the lowest intensity was found in the carbonyl C region (17.0% for the mulched soil and 16.8% for the non-mulched soil) (Table 3). Compared with the non-mulch treatment, the mulch treatment significantly increased alkyl C

intensity and A/O-A ratio, but significantly decreased aryl C intensity and aromaticity (Table 3, Fig. 2 A and B).

3.2. Effects of weed control

In comparison to the weed control soils, the no weed control soils exhibited significantly greater amount of HWEOC ($F = 5.21$; $df = 1$; $P < 0.05$) and CHCl_3 -released C ($F = 4.35$; $df = 1$; $P < 0.05$) (Fig. 3B and C). However, the weed control treatment did not significantly affect WEOC ($F = 4.11$; $df = 1$; $P > 0.05$) and acid hydrolysable C ($F = 2.07$; $df = 1$; $P > 0.05$) (Fig. 3A and D). There were no significant seasonal variations in any of the above measurements across the two treatments ($P > 0.05$, Fig. 3). Table 2 shows the mean values and the ranges of each of the measurements across the four sampling months and the two treatments. The results show considerable variation in the ranges of each of the measurements between minimum and maximum values. These may reflect variations between the four sampling months and between the blocks. Similar to the soil under the mulch and non-mulch treatments, ^{13}C CPMAS NMR spectra of soil organic C in the weed control and no weed control treatments show that O-alkyl C spectral region was highest among the four C functional regions (Table 3). The weed control treatment significantly decreased aryl C intensity, but did not affect the intensity of any other C groups (Table 3, Fig. 2 C and D). A/O-A ratio was not affected by the weed control treatment. However, soil organic C in the weed control treatment had smaller aromaticity than that in the no weed control treatment.

3.3. Relationships among the soil labile C pools and between organic C functional groups and labile C pools

All the data collected from four treatments and from four sampling months were pooled together to examine the correlations among soil WEOC, HWEOC, CHCl₃-released C, and acid hydrolysable C. The four soil C pools were significantly correlated with each other. The correlation between HWEOC and WEOC was the highest ($r = 0.72$, $n = 48$, $P < 0.01$), followed by the correlation between HWEOC and CHCl₃-released C ($r = 0.67$, $n = 48$, $P < 0.01$). The lowest, but significant relationship was found between WEOC and acid hydrolysable C ($r = 0.35$, $n = 48$, $P < 0.05$). Pearson correlation analysis between organic C functional groups and labile C pools showed that *O*-alkyl C was significantly correlated with acid hydrolysable C (Fig. 4A), and aromaticity was negatively related to HWEOC (Fig. 4B).

4. Discussion

Our results indicate that management practices, such as mulch and weed control with herbicide treatment, had important impacts on soil C pools during the establishment of the hardwood plantation in subtropical Australia. However, soil C pools determined by various techniques responded differently to the management practices.

4.1. Effects of mulch on soil C pools

Significant increase in soil WEOC content after mulched with plant residues has been found in many studies (Franchini et al., 2001; Jensen et al., 1997). These studies generally reported immediate increases in soil WEOC content upon amendment with plant residues due to the input of soluble materials and soil WEOC content may return rapidly to background level because of the rapid decomposition of these soluble materials in soil (Franchini et al., 2001). In our present study, soil WEOC content was consistently higher in the mulched soil than in the non-mulched soil throughout the whole year (Fig. 1A), which may indicate that the

soluble materials in the plant material should not be the only sources of WEOC in the mulched soil. Schiff et al. (1997) reported that besides leaching from organic matter, WEOC was released from microbial activity and root exudation. In this study, significantly higher CHCl_3 -released C (Fig. 1C) in the mulched soil than in the non-mulched soil, and significant relationship between WEOC and CHCl_3 -released C ($r = 0.53$, $P < 0.05$) may suggest that microorganisms are important sources of WEOC in these plantation soils. CHCl_3 -released C, which represents the microbial biomass C, was significantly greater in the mulched soil than in the non-mulched soil, which agrees with other studies (Jensen et al., 1997; Wardle et al., 1999). For example, Mendham et al. (2002) suggested that residue amendment increased microbial biomass C in surface soils (0-5 cm) 1 and 5 years after treatment in an eucalypt plantation. The increased CHCl_3 -released C (microbial biomass C) in the mulched soil may be due to the increased supply of available organic C and nutrients by mulching plant residue on the soil surface. Another explanation for the increased CHCl_3 -released C is that the mulch treatment reduced soil temperature variation and the evaporation rate, which may favour the growth of soil microbes (Athy et al., 2006). HWEOC is considered to be mobilizable, labile and easily decomposable soil C pools (Ghani et al., 2003). Significantly higher HWEOC in the mulched soil than in the non-mulched soil throughout the whole year was found in this study (Fig. 1B). This is plausible since the hot water method may not only extract part of soil microbial biomass C but also some soluble soil C and amines (Ghani et al., 2003). The mulch treatment increased WEOC and CHCl_3 -released C, therefore would also increase HWEOC. However, there were some reports in which a declining trend in soil HWEOC after amendment with plant residues was found (Tirol-Padrea et al., 2007). Although most labile C pools were increased by the mulch treatment in the 0-10 cm soil layer in this plantation, the size of the acid hydrolysable C was not significantly affected. Lack of statistically significant

differences in acid hydrolysable C between the mulch and non-mulch treatments might be due to a large spatial variability between the blocks at this experimental site (Table 2). In addition, acid hydrolysable C includes more slow-turnover materials than the other three soil C pools, and these slow-turnover materials in the soils are less sensitive to the forest managements (McLauchlan and Hobbie, 2004).

¹³C CPMAS NMR represents one of the most powerful tools to investigate soil organic matter and it can provide a semi-quantitative evaluation of C distribution in soil samples. O-alkyl C had highest intensity among the four C functional groups. This was consistent with the findings of Mathers and Xu (2003) in a 2-year-old pine plantation of subtropical Australia, while Chen et al. (2004) and Oades et al. (1988) found that alkyl C region was predominant in the Australian soils. The significantly greater alkyl C structures and A/O-A ratio in the mulched soils compared with those in the non-mulched soils were not expected in this study (Table 3). Generally, the result indicated that soil organic matter (SOM) in the mulched soils was at an increased extent of decomposition and had poorer substrate quality than SOM taken from the non-mulched plots. Similar data were reported by Mathers et al. (2003) who assessed the SOM under double harvest residues or no harvest residues in two second-rotation eucalyptus plantations of southwest Australia and found that alkyl C structures and A/O-A ratio were increased by the retention of double harvest residues. However, in another study on a second-rotation pine plantation of southeast Queensland, Australia, Mathers and Xu (2003) demonstrated that amending soil with harvest residues would result in greater proportion of O-alkyl C structures, but lower A/O-A ratio as a result of increased O-alkyl C structures. The different responses of SOM to residue amending may be due to the residue types. The harvest residues used by Mathers et al. (2003) and in this present study were dominant by Eucalyptus, while those in the study of Mathers and Xu (2003) mainly consisted of pine materials. Pine

litter is typically dominated by O-alkyl C, which consists of more than 70% of organic C (Almendros et al., 2000), while Eucalyptus litter comprises a greater proportion of alkyl C than pine litter (Skene et al., 1997). The aromaticities were lower in the mulch treatment than in the non-mulch treatment in this study, contradicting the A/O-A ratio to indicate that SOM from the non-mulched soils was more decomposed and had poorer substrate quality than SOM taken from the mulched plots. Mathers et al. (2003) and Baldock et al. (1997) suggested that the use of A/O-A ratio might be limited since it can be affected by the nature of the original C input into the soil. The proportion of lignin is also a decisive factor of substrate quality for heterotrophic microbes, and the presence of a large amount of lignin will protect some of the polysaccharide C (which appears in the O-alkyl C region) in SOM from microbial decomposition. The main substitutes of lignin contribute to both the O-alkyl and aromatic C regions (Hatcher, 1987). The O-alkyl C region may, therefore, overrate the amount of readily decomposable C when significant amounts of lignin are present.

4.2. Effects of weed control with herbicide application on soil C pools

Weed control treatment significantly decreased HWEOC throughout the whole experimental year, but did not significantly affect WEOC (Fig. 3 A and B). This is consistent with the findings of Chen and Xu (2005). The result is not surprising as root mass and exudates greatly influence C turnover (Kuzyakov et al., 2001) which would affect the depletion of organic matter and HWEOC in SOM (Ghani et al., 2003). In this study, we did find a significantly lower root biomass in the weed control plots (Data not shown). The effects of the weed control with herbicide treatment on soil CHCl₃-released C (microbial biomass C) reported in the literature are contradictory. For example, Roslycky (1982) reported that herbicide application increased soil CHCl₃-released C. On the other hand, Busse

et al. (2006) found that weed control with herbicide treatment resulted in significant decline in soil CHCl_3 -released C in a young conifer plantation of north Carolina, USA. In the present study, soil CHCl_3 -released C was significantly lower in the weed control plots than in the no weed control plots (Fig. 3C). Generally, weed control through herbicide treatment can alter microbial biomass by either direct herbicide toxicity or reduced soil C input from root turnover, litter-fall, and root exudates by the elimination of under-story plants. Since herbicides exert few direct effects on soil microflora at realistic field concentrations (Vitousek et al., 1992), it is likely that adverse effects of the weed control with herbicide treatment are due largely to reduced input of C substrates from root turnover, litter-fall, and rhizosphere exudates by the elimination of under-story vegetation. The information about the impacts of the weed control treatment on soil acid hydrolysable C and chemical compositions of soil organic C is scant. The result from this study showed the weed control treatment tended to lower soil acid hydrolysable C although this was not statistically significant (Fig. 3D). It has been suggested that soil acid hydrolysable C is affected by organic inputs from both above-ground litters and below-ground root turnover and exudates (McLauchlan and Hobbie, 2004). Thus, the potential lower acid hydrolysable C in the weed control plots was probably attributed to less C substrate input. The impact of the weed control treatment on chemical compositions of soil organic C is shown in Table 3, Fig. 2 C and D. The decreased aryl C and aromaticity may suggest that the C substrates in soil input from the decomposition of weed residue and root exudates in this plantation are mainly aromatic chemicals, which is consistent with the study of Pomilio et al. (2000), who found aromatic chemicals are main components of plant root exudates.

4.3. Seasonal dynamics of soil labile C pools and relationships among the soil C fractions

Soil labile C pools are not static but dynamic entities over time (Jiang et al., 2006; Wardle, 1998). However, little seasonal variations in either soil labile C pools were found in this study. Lack of significance of temporal changes in soil labile C pools may be due to the high degree of within-treatment variability (Table 2).

Significant correlations between the labile organic C pools measured by four extraction techniques (water, hot water, CHCl_3 -released and acid hydrolysable), indicated that they partly represented similar C pools in soil. However, the quantity was very different among these four pools. For example, WSOC was about 4.9-6.7% of HWEOC, but HWEOC was about 14.3-16.1% of acid hydrolysable C (Table 2). In this case, acid hydrolysable C was much greater than the labile soil C pools measured with other techniques, supporting that acid hydrolysable C includes more slow-turnover material than the other techniques (McLauchlan and Hobbie, 2004).

The relationships between C functional groups revealed by ^{13}C CPMAS NMR and labile C pools measured by four extraction techniques were determined using Pearson linear correlation analysis. Significant positive relationship between soil acid hydrolysable C and O-alkyl C (Fig. 4A) suggests that O-alkyl C may be the main components of acid hydrolysable C. This finding was consistent with Wilson et al. (1983), who reported that O-alkyl C and acetal C are two major types of C present in soil 6 M HCl hydrolysable C. In a number of studies, O-alkyl C has been shown to be the first region to lose intensity during decomposition and therefore labile components among the four C functional groups (Mather and Xu, 2003; Skjemstad et al., 1994). However, we did not find any significant relationships between O-alkyl C and WEOC, HWEOC, or CHCl_3 -released C. This may be due to the semi-quantity method used for calculating O-alkyl C. The proportion of O-alkyl C in SOM could decrease even if the labile C components increased as a result of a larger amount of other C

functional groups input (e.g. recalcitrant C components) from plant residues or exudates. Another explanation for the no significant relationships between O-alkyl C and WEOC, HWEOC, or CHCl₃-released C could be attributed to the HF treatment of SOM. The pre-treatment with HF could result in the loss of part organic C, especially water soluble C in soil (Mathers et al., 2002). HWEOC was reported as one of the most sensitive indicators among the soil biochemical measurements and considered to reflect the changes in soil organic C caused by different soil management practices (Ghani et al., 2003). The negative relationships between aromaticity and HWEOC (Fig. 4B) confirmed that the aromaticity index is a better estimate of the degree of decomposition or humification and better index of SOM quality or soil fertility in these soils than A/O-A ratio.

5. Conclusions

Mulch and weed control treatments in the hardwood plantation of subtropical Australia induced the changes in quantity and chemical composition of soil C pools. Results from this study suggest that both HWEOC and CHCl₃-released C are sensitive measurements for determining impacts of the weed control and mulch treatments, and acid hydrolysable C is less sensitive to the treatments. However, significant correlations among WEOC, HWEOC, CHCl₃-released C and acid hydrolysable C indicated that they partly represented similar C pools in soil. The use of solid-state ¹³C CPMAS NMR enabled the detection of changes in chemical composition of soil C pools 12 months after the mulch and weed control treatments were applied. The results showed that the mulch treatment led to higher signal intensity in the alkyl C spectral region and A/O-A ratio, but lower signal intensity in the aryl C and aromaticity. The weed control treatment, compared with the no weed control treatment, reduced signal intensity in the aryl C and aromaticity. This study highlighted that the

aromaticity index is a better estimate of the degree of decomposition or humification and better index of SOM quality or soil fertility in these soils than A/O-A ratio in these plantation soils.

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