1 Changes in soil carbon during the establishment of a hardwood plantation in

2 subtropical Australia

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10 Abstract

11 Soil carbon (C) pools are not only important to governing soil properties and nutrient

12 cycling in forest ecosystems, but also play a critical role in global C cycling. Mulch and weed

13 control treatments may alter the soil C pools by changing organic matter inputs to the forest

14 ecosystem. We studied the 12-month mulch and weed control responses on the chemical

15 composition of soil organic C and the seasonal dynamics of water extractable organic C

16 (WEOC), hot water extractable organic C (HWEOC), chloroform-released organic C ($CHCl_3$-

17 released C), and acid hydrolysed organic C (acid hydrolysable C) in a hardwood plantation of

18 subtropical Australia. The results showed that compared with the non-mulch treatment, the

19 mulch treatment significantly increased soil WEOC, HWEOC, and $CHCl_3$-released C over the

20 four sampling months. The weed control treatment significantly reduced the amount of

21 HWEOC and $CHCl_3$-released C compared with the no weed control treatment. Either the

22 mulch or weed control treatment did not significantly affect soil acid hydrolysed organic C.

23 There were no significant seasonal variations in soil WEOC, HWEOC, $CHCl_3$-released C,

24 and acid hydrolysed organic C in the hardwood plantation. Solid-state ^{13}C nuclear magnetic

25 resonance (NMR) spectroscopy was used to study the structural chemistry of soil C pools in

26 HF (hydrofluoric acid) treated soils collected 12 months after the mulch and weed control

27 treatments were applied. Overall, O-alkyl C was the dominant C fraction, accounting for 33-

28 43% of the total NMR signal intensity. The mulch treatment led to higher signal intensity in

29 the alkyl C spectral region and A/O-A ratio (the ratio of alkyl C region intensity to O-alkyl C

30 region intensity), but lower signal intensity in the aryl C and aromaticity. Compared with the

31 no weed control treatment, the weed control treatment reduced signal intensity in the aryl C

32 and aromaticity. Together, shifts in the amount and nature of soil C following the mulch and

33 weed control treatments may be due to the changes in organic matter input and soil physical

34 environments.

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

36 with herbicide treatment

37

1. Introduction

39   Soil carbon (C) pools may affect soil chemical and biological properties that control

40 nutrient cycling and consequently have important impacts on forest productivity and

41 sustainability. Additionally, soil C pools form a large and dynamic reservoir of C, which is an

42 important part of the global C cycle and a potential sink for atmospheric CO_2. Due to the

43 important role of soil C pools in nutrient cycling of forest ecosystems and global C balance,

44 there has long been an interest in understanding the effect of forest soil management on soil C

45 pools (Ussiri and Johnson, 2007).

46   In subtropical Australia, increasing land values have resulted in hardwood plantations

47 being established in more westerly areas with below optimal rainfall and soil nutrients for

48   seedling survival. To overcome these problems, several management techniques, including

49   residue mulch and weed control through herbicide treatment, are employed. A number of

50   studies have reported the effects of residue mulch and weed control through herbicide

51   treatment on soil C in forest ecosystems (Busse et al., 1996; Jiang et al., 2006). For example,

52   Chen and Xu (2005) reported that retention of forest residues significantly enhanced

53   accumulation of soil total C and N compared with residue removal in a 6-year-old slash pine

54   plantation of subtropical Australia. In a long-term study examining the role of under-story

55   weeds in ponderosa pine (*Pinus ponderosa* Dougl. Ex Laws.), Busse et al. (1996) suggested a

56   33% decrease of total C content in surface soil after weed control through herbicide treatment.

57   Furthermore, residue mulch and weed control treatment can also change the composition of

58   soil organic C pools. For example, Mathers and Xu (2003) found that soil organic matter

59   (SOM) under residue retention had greater proportion of O-alkyl C structures than that under

60   residue removal in a 2-year-old pine plantation of subtropical Australia. Echeverria et al.

61   (2004) suggested that weed control through herbicide treatment significantly decreased acid

62   hydrolysable C compared with no herbicide treatment control.

63   Soil organic C pools consist of various fractions varying in degree of decomposition,

64   recalcitrance, and turnover rate, and management practices may affect these fractions

65   differently (Ghani et al., 2003). Numerous methods, including chemical, physical and

66   biological fractionations, have been developed to characterize soil organic C pools and

67   dynamics (Mathers et al., 2000; McLauchlan and Hobbie, 2004). For example, cold and hot

68   water can extract soil organic C representing soluble, readily metabolizable C sources in soils

69   (Chen et al., 2004; Nishiyama et al., 2001). Chloroform-released organic C was used to

70   represent the microbial component of soil organic C. Paul et al. (2001) suggested that acid

71   hydrolysis (e.g., using 6 M HCl) procedure may be an effective technique to extract labile

72   constituents in the soil C pools. Both chloroform-released organic C and acid hydrolysable

73   organic C were reported to be a potential indicator of bioreactive soil organic C pools (Xu et

74   al., 1997). In recent years, the technique of solid-state ^{13}C nuclear magnetic resonance

75   spectroscopy with cross-polarization and magic-angle spinning (CPMAS-NMR) has been

76   increasingly used (Preston, 1996; Quideau et al., 2001). This technique allows one to obtain

77   information directly and non-destructively on C components of the entire soil sample without

78   any chemical or physical fractionation and is well suited to the characterization of natural soil

79   organic C pools (Schnitzer, 2001). In the present study, the aims were to investigate: (1) the

80   impacts of residue mulch and weed control through herbicide treatment on the amounts and

81   chemical composition of soil C; (2) the seasonal dynamics of the labile soil C pools; and (3)

82   the relationships among various C fractions measured.

83

84   2. Materials and methods

85   *2.1. Site description, experimental design and soil sampling*

86   The experiment was established at Pechey, southeast Queensland (27°18'S, 152°3'E),

87   Australia. The site has a freely draining, well-textured Oxisol (Soil Survey Staff, 1999). Basic

88   soil properties are given in Table 1. The area lies within the sub-tropical zone with the

89   predominant weather pattern giving cool dry winters and warm wet summers. Rainfall

90   patterns are highly variable with an average of 851 mm (from 1995-2004).

91   The experiment, including four treatments, was set up in December 2004. Mulch and non-

92   mulch treatments were applied to six 24-m wide by 67.2-m long plots located at the west side

93   of a hill in the experimental site. This part of site was covered with woody weeds dominated

94   by *Acacia*, *Alphitonia* and *Eucalyptus* before the establishment of this experiment. The two

95   treatments were arranged in randomised block design with three replications. For the mulch

96 treatment, a tractor driven bush chopper was developed, which cut, chipped and spread all the

97 woody weeds as mulch over the plots before the plantation was established. On average, the

98 total weight of mulch in these plots was 1.57 kg m^{-2} (dry weight), with about 5- cm layer of

99 mulch covering over 80% of the soil (area). The C/N ratio of the mulch materials was 80.7.

100 For the non-mulch treatment, all the mulch was removed from the plots immediately

101 following site preparation. Weed control and no weed control treatments were applied to other

102 six 24-m wide by 67.2-m long plots located at the east side of the same hill in the experiment

103 site. This part of area was covered with grass weeds dominated by kikuyu (*Pennisetum*

104 *clandestinum* Chiov.) before the establishment of this experiment. The weed control and no

105 weed control treatments were also arranged in randomised block design with three

106 replications. Weed control treatment consisted of the use of roundup (glyphosate) before site

107 preparation and subsequent application of roundup and triclopyr, a pyridine, to eliminate all

108 competing vegetation. In the no weed control treatment, no herbicide was applied in these

109 plots. All the twelve plots were planted with *Eucalyptus pilularis* six months after the

110 treatments began at the plots. The planting space was 4 m (row space) by 2.4 m (tree space).

111   Soil samples were collected during 2005. The four sampling months include: (a) April;

112 (b) July; (c) October; and (d) December, which represent four seasons in Australia. At each

113 sampling time, five 0-10 cm soil cores (2 cm in diameter) were randomly taken from each

114 plot. To avoid the effect of tree seedlings, however, the soil cores were sampled two metres

115 away from tree lines. These five cores were then immediately bulked, placed in a cooler (ca. 4

116 ºC), and returned to the laboratory. The soil samples were sieved (< 2 mm) prior to physical,

117 chemical and biological analysis.

118

119 *2.2. Analysis of soil basic properties*

120   A soil sub-sample from each plot collected in December 2005 was oven-dried at 105ºC

121   to determine the soil moisture. Soil total C (TC) and total N (TN) were determined on an

122   Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser (Isoprime-

123   EuroEA 3000) using the sub-samples. Another sub-sample of moist soil was extracted with 2

124   M KCl and the concentrations of mineral N in KCl extractions were measured using a Lachat

125   Quickchem automated ion analyser (Quik Chem method 10-107-064-D for NH_4^+ and 10107-

126   04-1-H for NO_3^-). Soil pH was measured at a 1:2 soil/water (w/v) ratio. Soil cation exchange

127   capacity, conductivity, bulk density, and particle size were measured using the methods

128   described by Rayment and Higginson (1992).

129

130   *2.3. Water and hot water extraction of soil labile organic C pools*

131   Water extractable organic C (WEOC) was extracted by shaking 3 g (oven-dried

132   equivalent) moist soil at a soil/water (w/v) ratio of 1:10 on an end-to-end shaker at room

133   temperature for 30 min. The mixture was then spun at 3500 rpm for 20 min and filtered

134   through a 0.45 µm membrane filter into separate vials for C analysis (Ghani et al., 2003).

135   Hot water extractable organic C (HWEOC) was measured by the method described by

136   Sparkling et al. (1998). Moist soil samples (equivalent 3 g oven-dry weight) were incubated

137   with 30 ml distilled water in 50 ml polypropylene centrifuge tubes at 80°C (hot water

138   extraction) for 18 h, and the tubes were then shaken on an end-to-end shaker for 30 min. The

139   mixture in the tube was then spun at 3500 rpm for 20 min and filtered through a piece of

140   Whatman 42 paper (Whatman Ltd., Maidstone, UK), and then through a 0.45 µm filter

141   membrane. The organic C contents in cold water and hot water extracts were determined

142   using a SHIMADZU TOC- $_{VCPH/CPN}$ analyser.

143

144 *2.4. Chloroform-released organic C*

145   The chloroform-released organic C (CHCl$_3$-released C) was measured by the chloroform

146 fumigation-extraction method (Vance et al., 1987). In brief, duplicate field moist soil sub-

147 samples (equivalent 5 g oven-dry weight) were fumigated with chloroform for 24 h and then

148 extracted with 0.5 M K$_2$SO$_4$ for 2 h on an end-over-end shaker. The suspended samples were

149 centrifuged and filtered through Whatman 42 filter paper. Similar sets of unfumigated

150 samples were extracted in the same way. The amounts of organic C in the fumigated and un-

151 fumigated soil extracts were determined by the high temperature catalytic oxidation method

152 using a SHIMADZU TOC-$_{VCPH/CPN}$ analyser (Chen and Xu, 2005). CHCl$_3$-released C was

153 calculated as the difference in organic C between the fumigated and the unfumigated samples

154 and used to represent the microbial component of soil organic C.

155

156 *2.5. Acid hydrolysed soil organic C pools*

157   Acid hydrolysed soil organic C pool (acid hydrolysable C) was measured by the method

158 described by Sollins et al. (1999). 1 g air-dried soil sample was refluxed for 16 h in digestion

159 tubes with 10 ml of 6 M hydrochloric acid solution. The residue (unhydrolyzable soil organic

160 matter) was isolated, washed with 100 ml of deionized water, and dried overnight in an 80ºC

161 oven. The residue was then weighed and analysed for C content on an Isoprime isotope ratio

162 mass spectrometer with a Eurovector elemental analyser (Isoprime-EuroEA 3000). The

163 hydrolysable fraction was obtained by subtracting unhydrolyzable C from soil TC.

164

165 *2.6. Hydrofluoric acid pre-treatment of soil samples and solid-state ^{13}C NMR spectroscopy*

166   Soil samples were pre-treated with hydrofluoric acid (HF) prior to solid-state ^{13}C

167 CPMAS NMR analysis. The pre-treatment can remove a large amount of Fe^{3+} and Mn^{2+} in

168 soil, concentrate the organic matter of a whole soil sample and improve the signal/noise ratio.

169 After HF pre-treatment, solid-state ^{13}C CPMAS NMR analysis times can be decreased

170 without a reduction in spectral quality. In this study, soil samples collected in December 2005

171 were pre-treated with 2% HF according to the method described by Skjemstad et al. (1994)

172 and modified by Mathers et al. (2002) for NMR analysis.

173   Solid-state ^{13}C CPMAS NMR spectra of the HF-treated soils were obtained at a

174 frequency of 100.59 MHz on a Varian Unity Inova400 spectrometer (Varian Inc., Palo Alto,

175 CA). Soil samples were packed in a silicon nitride rotor (OD = 7 mm) and spun at 5 kHz at the

176 magic angle. Single contact times of 2 ms were applied, with an acquisition time of 14 ms,

177 and a recycle delay of 2.5 s. 6400 transients were collected for all samples and a Lorentzian

178 line broadening function of 20 Hz was applied to all spectra. Chemical shift values were

179 referenced externally to hexamethylbenzene at 132.1 ppm, which is equivalent to

180 tetramethylsilane at 0 ppm.

181   The solid-state ^{13}C CPMAS NMR spectra were divided into four regions representing

182 different chemical environments of a ^{13}C nucleus. These were alkyl C (0–50 ppm), O-alkyl C

183 (50–110 ppm), aromatic C (110–160 ppm) and carbonyl C (160–210 ppm). The relative

184 intensity of each functional group was measured by integration using the Varian NMR

185 software package (Version 6.1c, Varian Inc., Palo Alto, CA). In some instances, the chemical

186 shift regions were further divided, these were: O-alkyl C into methoxyl C (50–60 ppm),

187 carbohydrate C (60–95 ppm), and di-O-alkyl C (95–110 ppm); aromatic C into aryl C (110–

188 145 ppm) and phenolic C (145–160 ppm); and carbonyl C into carboxylic/amide/ester C (160–

189 190 ppm) and ketone/aldehyde C (190–210 ppm).

190   The A/O-A ratio, the ratio of alkyl C region intensity (0–50 ppm) to O-alkyl C region

191 intensity (50 –110 ppm), which has been recommended by Baldock and Preston (1995) as an

192   index of the extent of decomposition or of substrate quality for microbes, was also determined

193   in this study as an indicator of the quality of soil organic C. In addition, aromaticity has been

194   used to characterise the extent of humification of SOM, under the assumption that SOM

195   becomes aromatic during decomposition (Dai et al., 2001). The aromaticity was calculated as

196   the following equation:

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

198

199   *2.7. Statistical methods*

200   Two-way repeated measures ANOVA was carried out for soil WEOC, HWEOC, CHCl$_3$-

201   released C, and acid hydrolysable C over the four sampling seasons to test the significant

202   effects of the mulch treatment and sampling time or the weed control treatment and sampling

203   time using Statistix for Windows Version 8.0. T-test was used to examine the significant

204   differences in WEOC, HWEOC, CHCl$_3$-released C, and acid hydrolysable C between the

205   mulch and non-mulch treatments and between the weed control and no weed control

206   treatments at each sampling month. T-test was also conducted to test the significant

207   differences in basic soil properties (Table 1) and NMR data between the mulch and non-

208   mulch treatments and between the weed control and no weed control treatments. Pearson

209   linear correlations among WEOC, HWEOC, CHCl$_3$-released C, and acid hydrolysable C were

210   conducted using soil samples collected from four sampling months. In addition, we

211   determined the relationships between soil organic C functional groups and soil WEOC,

212   HWEOC, CHCl$_3$-released C, or acid hydrolysable C using the soil samples collected in

213   December 2005.

214

3. Results

215

216 Selected soil properties in 0-10 cm soil layer of the hardwood plantation are given in

217 Table 1. Mulched soils had significantly higher TC, TN, and soil moisture than the non-

218 mulched soils ($P < 0.05$). There were no significant differences in pH values, soil C/N ratio,

219 bulk density, sand or clay contents between the mulch and non-mulch treatments. The weed

220 control treatment significantly decreased soil TC ($P < 0.05$), but did not significantly affect

221 any other soil basic properties listed in Table 1.

222

223 *3.1. Effects of mulch*

224 The mulch treatment significantly influenced soil WEOC ($F = 4.42$; $df = 1$; $P < 0.05$),

225 HWEOC ($F = 4.58$; $df = 1$; $P < 0.05$), and $CHCl_3$-released C ($F = 5.74$; $df = 1$; $P < 0.05$) over

226 the four sampling months (Fig. 1A, B, and C). Average values from four sampling months

227 for WEOC, HWEOC, and $CHCl_3$-released C in the mulched soils were 77, 1609, and 1118

228 mg kg^{-1} soil, respectively, higher than those in the non-mulched soils (Table 2). However, the

229 mulch treatment had no significant effect on soil acid hydrolysable C ($F = 3.01$; $df = 1$; $P >$

230 0.05) (Fig. 1D). There were no significant seasonal variations in soil WEOC, HWEOC,

231 $CHCl_3$-released C, and acid hydrolysable C across the two treatments ($P > 0.05$, Fig. 1). The

232 ^{13}C CPMAS NMR spectra of soil organic C in the mulch and non-mulch treatments are

233 shown in Fig. 2 A and B. The relative intensities of C functional groups in the HF-treated

234 soils demonstrate that O-alkyl C spectral region was highest (36.6% for the non-mulched soil

235 and 36.3% for the mulched soil), followed by alkyl C (23.7% for the mulched soil) or

236 aromatic C (25.9% for the non-mulched soil), and the lowest intensity was found in the

237 carbonyl C region (17.0% for the mulched soil and 16.8% for the non-mulched soil) (Table 3).

238 Compared with the non-mulch treatment, the mulch treatment significantly increased alkyl C

239   intensity and A/O-A ratio, but significantly decreased aryl C intensity and aromaticity (Table

240   3, Fig. 2 A and B).

241

242   *3.2. Effects of weed control*

243   In comparison to the weed control soils, the no weed control soils exhibited significantly

244   greater amount of HWEOC ($F = 5.21$; $df = 1$; $P < 0.05$) and $CHCl_3$-released C ($F = 4.35$; $df =$

245   1; $P < 0.05$) (Fig. 3B and C). However, the weed control treatment did not significantly affect

246   WEOC ($F = 4.11$; $df = 1$; $P > 0.05$) and acid hydrolysable C ($F = 2.07$; $df = 1$; $P > 0.05$) (Fig.

247   3A and D). There were no significant seasonal variations in any of the above measurements

248   across the two treatments ($P > 0.05$, Fig. 3). Table 2 shows the mean values and the ranges of

249   each of the measurements across the four sampling months and the two treatments. The

250   results show considerable variation in the ranges of each of the measurements between

251   minimum and maximum values. These may reflect variations between the four sampling

252   months and between the blocks. Similar to the soil under the mulch and non-mulch

253   treatments, ^{13}C CPMAS NMR spectra of soil organic C in the weed control and no weed

254   control treatments show that O-alkyl C spectral region was highest among the four C

255   functional regions (Table 3). The weed control treatment significantly decreased aryl C

256   intensity, but did not affect the intensity of any other C groups (Table 3, Fig. 2 C and D).

257   A/O-A ratio was not affected by the weed control treatment. However, soil organic C in the

258   weed control treatment had smaller aromaticity than that in the no weed control treatment.

259

260   *3.3. Relationships among the soil labile C pools and between organic C functional groups*

261   *and labile C pools*

262   All the data collected from four treatments and from four sampling months were pooled

263   together to examine the correlations among soil WEOC, HWEOC, $CHCl_3$-released C, and

264   acid hydrolysable C. The four soil C pools were significantly correlated with each other. The

265   correlation between HWEOC and WEOC was the highest ($r = 0.72$, $n = 48$, $P < 0.01$),

266   followed by the correlation between HWEOC and $CHCl_3$-released C ($r = 0.67$, $n = 48$, $P <$

267   0.01). The lowest, but significant relationship was found between WEOC and acid

268   hydrolysable C ($r = 0.35$, $n = 48$, $P < 0.05$). Pearson correlation analysis between organic C

269   functional groups and labile C pools showed that *O*-alkyl C was significantly correlated with

270   acid hydrolysable C (Fig. 4A), and aromaticity was negatively related to HWEOC (Fig. 4B).

271

272   **4. Discussion**

273   Our results indicate that management practices, such as mulch and weed control with

274   herbicide treatment, had important impacts on soil C pools during the establishment of the

275   hardwood plantation in subtropical Australia. However, soil C pools determined by various

276   techniques responded differently to the management practices.

277

278   *4.1. Effects of mulch on soil C pools*

279   Significant increase in soil WEOC content after mulched with plant residues has been

280   found in many studies (Franchini et al., 2001; Jensen et al., 1997). These studies generally

281   reported immediate increases in soil WEOC content upon amendment with plant residues due

282   to the input of soluble materials and soil WEOC content may return rapidly to background

283   level because of the rapid decomposition of these soluble materials in soil (Franchini et al.,

284   2001). In our present study, soil WEOC content was consistently higher in the mulched soil

285   than in the non-mulched soil throughout the whole year (Fig. 1A), which may indicate that the

286 soluble materials in the plant material should not be the only sources of WEOC in the

287 mulched soil. Schiff et al. (1997) reported that besides leaching from organic matter, WEOC

288 was released from microbial activity and root exudation. In this study, significantly higher

289 $CHCl_3$-released C (Fig. 1C) in the mulched soil than in the non-mulched soil, and significant

290 relationship between WEOC and $CHCl_3$-released C (r = 0.53, P < 0.05) may suggest that

291 microorganisms are important sources of WEOC in these plantation soils. $CHCl_3$-released C,

292 which represents the microbial biomass C, was significantly greater in the mulched soil than

293 in the non-mulched soil, which agrees with other studies (Jensen et al., 1997; Wardle et al.,

294 1999). For example, Mendham et al. (2002) suggested that residue amendment increased

295 microbial biomass C in surface soils (0-5 cm) 1 and 5 years after treatment in an eucalypt

296 plantation. The increased $CHCl_3$-released C (microbial biomass C) in the mulched soil may

297 be due to the increased supply of available organic C and nutrients by mulching plant residue

298 on the soil surface. Another explanation for the increased $CHCl_3$-released C is that the mulch

299 treatment reduced soil temperature variation and the evaporation rate, which may favour the

300 growth of soil microbes (Athy et al., 2006). HWEOC is considered to be mobilizable, labile

301 and easily decomposable soil C pools (Ghani et al., 2003). Significantly higher HWEOC in

302 the mulched soil than in the non-mulched soil throughout the whole year was found in this

303 study (Fig. 1B). This is plausible since the hot water method may not only extract part of soil

304 microbial biomass C but also some soluble soil C and amines (Ghani et al., 2003). The mulch

305 treatment increased WEOC and $CHCl_3$-released C, therefore would also increase HWEOC.

306 However, there were some reports in which a declining trend in soil HWEOC after

307 amendment with plant residues was found (Tirol-Padrea et al., 2007). Although most labile C

308 pools were increased by the mulch treatment in the 0-10 cm soil layer in this plantation, the

309 size of the acid hydrolysable C was not significantly affected. Lack of statistically significant

310   differences in acid hydrolysable C between the mulch and non-mulch treatments might be due

311   to a large spatial variability between the blocks at this experimental site (Table 2). In addition,

312   acid hydrolysable C includes more slow-turnover materials than the other three soil C pools,

313   and these slow-turnover materials in the soils are less sensitive to the forest managements

314   (McLauchlan and Hobbie, 2004).

315   ^{13}C CPMAS NMR represents one of the most powerful tools to investigate soil organic

316   matter and it can provide a semi-quantitative evaluation of C distribution in soil samples. O-

317   alkyl C had highest intensity among the four C functional groups. This was consistent with

318   the findings of Mathers and Xu (2003) in a 2-year-old pine plantation of subtropical Australia,

319   while Chen et al. (2004) and Oades et al. (1988) found that alkyl C region was predominant in

320   the Australian soils. The significantly greater alkyl C structures and A/O-A ratio in the

321   mulched soils compared with those in the non-mulched soils were not expected in this study

322   (Table 3). Generally, the result indicated that soil organic matter (SOM) in the mulched soils

323   was at an increased extent of decomposition and had poorer substrate quality than SOM taken

324   from the non-mulched plots. Similar data were reported by Mathers et al. (2003) who

325   assessed the SOM under double harvest residues or no harvest residues in two second-rotation

326   eucalyptus plantations of southwest Australia and found that alkyl C structures and A/O-A

327   ratio were increased by the retention of double harvest residues. However, in another study on

328   a second-rotation pine plantation of southeast Queensland, Australia, Mathers and Xu (2003)

329   demonstrated that amending soil with harvest residues would result in greater proportion of

330   O-alkyl C structures, but lower A/O-A ratio as a result of increased O-alkyl C structures. The

331   different responses of SOM to residue amending may be due to the residue types. The harvest

332   residues used by Mathers et al. (2003) and in this present study were dominant by Eucalyptus,

333   while those in the study of Mathers and Xu (2003) mainly consisted of pine materials. Pine

334   litter is typically dominated by O-alkyl C, which consists of more than 70% of organic C

335   (Almendros et al., 2000), while Eucalyptus litter comprises a greater proportion of alkyl C

336   than pine litter (Skene et al., 1997). The aromaticities were lower in the mulch treatment than

337   in the non-mulch treatment in this study, contradicting the A/O-A ratio to indicate that SOM

338   from the non-mulched soils was more decomposed and had poorer substrate quality than

339   SOM taken from the mulched plots. Mathers et al. (2003) and Baldock et al. (1997) suggested

340   that the use of A/O-A ratio might be limited since it can be affected by the nature of the

341   original C input into the soil. The proportion of lignin is also a decisive factor of substrate

342   quality for heterotrophic microbes, and the presence of a large amount of lignin will protect

343   some of the polysaccharide C (which appears in the O-alkyl C region) in SOM from microbial

344   decomposition. The main substitutes of lignin contribute to both the O-alkyl and aromatic C

345   regions (Hatcher, 1987). The O-alkyl C region may, therefore, overrate the amount of readily

346   decomposable C when significant amounts of lignin are present.

347

348   *4.2. Effects of weed control with herbicide application on soil C pools*

349   Weed control treatment significantly decreased HWEOC throughout the whole

350   experimental year, but did not significantly affect WEOC (Fig. 3 A and B). This is consistent

351   with the findings of Chen and Xu (2005). The result is not surprising as root mass and

352   exudates greatly influence C turnover (Kuzyakov et al., 2001) which would affect the

353   depletion of organic matter and HWEOC in SOM (Ghani et al., 2003). In this study, we did

354   find a significantly lower root biomass in the weed control plots (Data not shown). The

355   effects of the weed control with herbicide treatment on soil $CHCl_3$-released C (microbial

356   biomass C) reported in the literature are contradictory. For example, Roslycky (1982)

357   reported that herbicide application increased soil $CHCl_3$-released C. On the other hand, Busse

358 et al. (2006) found that weed control with herbicide treatment resulted in significant decline in

359 soil $CHCl_3$-released C in a young conifer plantation of north Carolina, USA. In the present

360 study, soil $CHCl_3$-released C was significantly lower in the weed control plots than in the no

361 weed control plots (Fig. 3C). Generally, weed control through herbicide treatment can alter

362 microbial biomass by either direct herbicide toxicity or reduced soil C input from root

363 turnover, litter-fall, and root exudates by the elimination of under-story plants. Since

364 herbicides exert few direct effects on soil microflora at realistic field concentrations (Vitousek

365 et al., 1992), it is likely that adverse effects of the weed control with herbicide treatment are

366 due largely to reduced input of C substrates from root turnover, litter-fall, and rhizosphere

367 exudates by the elimination of under-story vegetation. The information about the impacts of

368 the weed control treatment on soil acid hydrolysable C and chemical compositions of soil

369 organic C is scant. The result from this study showed the weed control treatment tended to

370 lower soil acid hydrolysable C although this was not statistically significant (Fig. 3D). It has

371 been suggested that soil acid hydrolysable C is affected by organic inputs from both above-

372 ground litters and below-ground root turnover and exudates (McLauchlan and Hobbie, 2004).

373 Thus, the potential lower acid hydrolysable C in the weed control plots was probably

374 attributed to less C substrate input. The impact of the weed control treatment on chemical

375 compositions of soil organic C is shown in Table 3, Fig. 2 C and D. The decreased aryl C and

376 aromaticity may suggest that the C substrates in soil input from the decomposition of weed

377 residue and root exudates in this plantation are mainly aromatic chemicals, which is

378 consistent with the study of Pomilio et al. (2000), who found aromatic chemicals are main

379 components of plant root exudates.

380

381 *4.3. Seasonal dynamics of soil labile C pools and relationships among the soil C fractions*

382   Soil labile C pools are not static but dynamic entities over time (Jiang et al., 2006;

383   Wardle, 1998). However, little seasonal variations in either soil labile C pools were found in

384   this study. Lack of significance of temporal changes in soil labile C pools may be due to the

385   high degree of within-treatment variability (Table 2).

386   Significant correlations between the labile organic C pools measured by four extraction

387   techniques (water, hot water, $CHCl_3$-released and acid hydrolysable), indicated that they

388   partly represented similar C pools in soil. However, the quantity was very different among

389   these four pools. For example, WSOC was about 4.9-6.7% of HWEOC, but HWEOC was

390   about 14.3-16.1% of acid hydrolysable C (Table 2). In this case, acid hydrolysable C was

391   much greater than the labile soil C pools measured with other techniques, supporting that acid

392   hydrolysable C includes more slow-turnover material than the other techniques (McLauchlan

393   and Hobbie, 2004).

394   The relationships between C functional groups revealed by ^{13}C CPMAS NMR and labile

395   C pools measured by four extraction techniques were determined using Pearson linear

396   correlation analysis. Significant positive relationship between soil acid hydrolysable C and O-

397   alkyl C (Fig. 4A) suggests that O-alkyl C may be the main components of acid hydrolysable

398   C. This finding was consistent with Wilson et al. (1983), who reported that O-alkyl C and

399   acetal C are two major types of C present in soil 6 M HCl hydrolysable C. In a number of

400   studies, O-alkyl C has been shown to be the first region to lose intensity during

401   decomposition and therefore labile components among the four C functional groups (Mather

402   and Xu, 2003; Skjemstad et al., 1994). However, we did not find any significant relationships

403   between O-alkyl C and WEOC, HWEOC, or $CHCl_3$-released C. This may be due to the semi-

404   quantity method used for calculating O-alkyl C. The proportion of O-alkyl C in SOM could

405   decrease even if the labile C components increased as a result of a larger amount of other C

406 functional groups input (e.g. recalcitrant C components) from plant residues or exudates.

407 Another explanation for the no significant relationships between O-alkyl C and WEOC,

408 HWEOC, or $CHCl_3$-released C could be attributed to the HF treatment of SOM. The pre-

409 treatment with HF could result in the loss of part organic C, especially water soluble C in soil

410 (Mathers et al., 2002). HWEOC was reported as one of the most sensitive indicators among

411 the soil biochemical measurements and considered to reflect the changes in soil organic C

412 caused by different soil management practices (Ghani et al., 2003). The negative relationships

413 between aromaticity and HWEOC (Fig. 4B) confirmed that the aromaticity index is a better

414 estimate of the degree of decomposition or humification and better index of SOM quality or

415 soil fertility in these soils than A/O-A ratio.

416

417 5. Conclusions

418   Mulch and weed control treatments in the hardwood plantation of subtropical Australia

419 induced the changes in quantity and chemical composition of soil C pools. Results from this

420 study suggest that both HWEOC and $CHCl_3$-released C are sensitive measurements for

421 determining impacts of the weed control and mulch treatments, and acid hydrolysable C is

422 less sensitive to the treatments. However, significant correlations among WEOC, HWEOC,

423 $CHCl_3$-released C and acid hydrolysable C indicated that they partly represented similar C

424 pools in soil. The use of solid-state ^{13}C CPMAS NMR enabled the detection of changes in

425 chemical composition of soil C pools 12 months after the mulch and weed control treatments

426 were applied. The results showed that the mulch treatment led to higher signal intensity in the

427 alkyl C spectral region and A/O-A ratio, but lower signal intensity in the aryl C and

428 aromaticity. The weed control treatment, compared with the no weed control treatment,

429 reduced signal intensity in the aryl C and aromaticity. This study highlighted that the

430 aromaticity index is a better estimate of the degree of decomposition or humification and

431 better index of SOM quality or soil fertility in these soils than A/O-A ratio in these plantation

432 soils.

433

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438

439 **Reference**

440 Almendros, G., Dorado, J., Gonzalez-Vila, F.J., Blanco, M.J., Lankes, U., 2000. [13]C

441   NMR assessment of decomposition patterns during composting of forest and

442   shrub biomass. Soil Biol. Biochem. 32, 793-804.

443 Athy, E.R., Keiffer, C.H., Stevens, M.H., 2006. Effects of mulch on seedlings and soil

444   on a closed landfill. Restor. Ecol. 14, 233-241.

445 Baldock, J.A., Oades, J.M., Nelson, P.N., Skene, T.M., Golchin, A., Clarke, P., 1997.

446   Assessing the extent of decomposition of natural organic materials using solid-state

447   ^{13}C NMR spectroscopy. Aust. J. Soil Res. 35, 1061–1083.

448 Baldock, J.A., Preston, C.M., 1995. Chemistry of carbon decomposition processes in forests

449   as revealed by solid-state carbon-13 nuclear magnetic resonance. In: McFee, W.W.,

450   Kelly, J. M. (Eds.), Carbon Forms and Functions in Forest Soils. Soil Science Society

451   of America, Madison, WI. 89-117 p.

452 Busse, M.D., Beattie, S.E., Powers, R.F., Sanchez, F.G., Tiarks, A.E., 2006. Microbial
453   community responses in forest mineral soil to compaction, organic matter removal,
454   and vegetation control. Can. J. For. Res. 36,577-588.

455 Busse, M.D., Cochran, P.H. and Barrett, J.W. 1996. Changes in ponderosa pine site
456   productivity following removal of understory vegetation. Soil Sci. Soc. Am. J. 60,
457   1614-1621.

458 Chen, C.R., Xu, Z.H., 2005. Soil carbon and nitrogen pools and microbial properties in a 6-
459   year-old slash pine plantation of subtropical Australia: impacts of harvest residue
460   management. For. Ecol. Manage. 206, 237-247.

461 Chen, C.R., Xu, Z.H., Mathers, N.J., 2004. Soil carbon pools in adjacent natural and
462   plantation forests of subtropical Australia. Soil Sci. Soc. Am. J. 68, 282-291.

463 Dai, K.H., Johnson, C.E., Driscoll, C.T., 2001. Organic matter chemistry and dynamics in
464   clear-cut and unmanaged hardwood forest ecosystems. Biogeochem. 54, 51-83.

465 Echeverria, M.E., Markewitz, D., Morris, L.A., Hendrick, R.L., 2004. Soil organic matter
466   fractions under managed pine plantations of the South-eastern USA. Soil Sci. Soc.
467   Am. J. 68, 950-958.

468 Franchini, J.C., Gonzalez-Vila, F.J., Cabrera, F., Miyazawa, M., Pava, M.A., 2001. Rapid
469   transformations of plant water-soluble organic compounds in relation to cation
470   mobilization in an acid Oxisol. Plant Soil 231, 55-63.

471 Ghani, A., Dexter, M., Perrott, K.W., 2003. Hot-water extractable carbon in soils: a sensitive
472   measurement for determining impacts of fertilisation, grazing and cultivation. Soil
473   Biol. Biochem. 35, 1231-1243.

474 Hatcher, P.G., 1987. Chemical structural studies of natural lignin by dipolar rephrasing solid-
475   state ^{13}C nuclear magnetic resonance. Org. Geochem. 11, 31-39.

476   Jensen, L.S., Mueller, T., Magid, J., Nielsen, N.E., 1997. Temporal variation of C and N

477   mineralization, microbial biomass and extractable organic pools in soil after oilseed

478   rape straw incorporation in the field. Soil Biol. Biochem. 29, 1043-1055.

479   Jiang, P.K., Xu, Q.F., Xu, Z.H., Cao, Z.H., 2006. Seasonal changes in soil labile organic

480   carbon pools within a Phyllostachys praecox stand under high rate fertilization and

481   winter mulch in subtropical China. For. Ecol. Manage. 236, 30-36.

482   Kuzyakov, Y., Ehrensberger, H., Stahr, K., 2001. Carbon partitioning and belowground

483   translocation by Lolium perenne. Soil Biol. Biochem. 33, 61-74.

484   Mathers, N.J., Mao, X.A., Xu, Z.H., Saffigna, P.G., Berners-Price, S.J., Perera, M.C.S., 2000.

485   Recent advances in applications of ^{13}C and ^{15}N NMR spectroscopy to soil organic

486   matter studies. Aust. J. Soil Res. 38, 769-787.

487   Mathers, N.J., Mendham, D.S., O'Connell, A.M., Grove, T.S., Xu, Z.H., Saffigna, P.G., 2003.

488   How does residue management impact soil organic matter composition and quality

489   under Eucalyptus globulus plantations in south-western Australia? For. Ecol. Manage.

490   179, 253-267.

491   Mathers, N.J., Xu, Z.H., 2003. Solid-state ^{13}C NMR spectroscopy, characterization of soil

492   organic matter under two contrasting residue management regimes in a 2-year-old

493   pine plantation of subtropical Australia. Geoderma 114, 19-31.

494   Mathers, N.J., Xu, Z.H., Berners-Price, S.J., Perera, M.C.S, Saffigna, P.G., 2002.

495   Hydrofluoric acid pre-treatment for improving ^{13}C CPMAS NMR spectral quality of

496   forest soils in southeast Queensland, Australia. Aust. J. Soil Res. 40, 655-674.

497   McLauchlan, K.K., Hobbie, S.E., 2004. Comparison of labile soil organic matter fractionation

498   techniques. Soil Sci. Soc. Am. J. 68, 1616-1625.

499   Mendham, D.S., Sankaran, K.V., O'Connell, A.M., Grove, T.S., 2002. Eucalyptus globulus

500   harvest residue management effects on soil carbon and microbial biomass at 1 and 5

501   years after plantation establishment. Soil Biol. Biochem. 34, 1903-1912.

502   Nishiyama, M., Sumikawa, Y., Guan, G., Marumoto, T., 2001. Relationship between

503   microbial biomass and extractable organic carbon content in volcanic and non-

504   volcanic ash soil. Appl. Soil Ecol. 17, 183-187.

505   Oades, J.M., Waters, A.G., Vassallo, A.M., Wilson, M.A., Jones, G.P., 1988. Influence of

506   management on the composition of organic matter in a red-brown earth as shown by

507   ^{13}C nuclear magnetic resonance. Aust. J. Soil Res. 26, 289-299.

508   Paul, E.A., Morris, S.J., Bohm, S., 2001. Determination of soil C pool sizes and turnover

509   rates: Biophysical fractionation and tracers. In: Lal, R. (Eds). Assessment Methods for

510   Soil Carbon. Lewis Publ., Boca Raton, FL. 139-206 p.

511   Pomilio, A.B., Leicach, S.R., Grass, M.Y., Ghersa, C.M., Santoro, M., Vitale, A.A., 2000.

512   Constitutes of the root exudate of Avena fatua grown under far-infrared-enriched light.

513   Phytochem. Anal. 11,304-308.

514   Preston, C.M., 1996. Application of NMR to soil organic matter analysis: History and

515   prospects. Soil Sci. 161, 144-165.

516   Quideau, S.A., Chadwick, O.A., Trumbore, S.E., Johnson-Maynard, J.L., Graham, R.C.,

517   Anderson, M.A., 2001. Vegetation control on soil organic matter dynamics. Org.

518   Geochem. 32, 247-252.

519   Rayment, G.E., Higginson, F.R., 1992. Australian Laboratory Handbook of Soil and Water

520   Chemical Methods. Inkata Press, Melbourne, Australia.

521   Roslycky, E.B., 1982. Glyphosate and the response of the soil microflora. Soil Biol. Biochem.

522   14, 87-92.

523   Schiff, S., Aravena, R., Trumbore, S.E., Hinton, M.J., Elgood, R., Dillon, P.J., 1997. Export

524   of DOC from forested catchments on the Precambrian Shield of Central Ontario:

525   Clues from ^{13}C and ^{14}C. Biogeochem. 36,43-65.

526   Schnitzer, M., 2001. The in situ analysis of organic matter in soils. Can. J. Soil Sci. 81, 249-

527   254.

528   Skene, T.M., Skjemstad, J.O., Oades, J.M., Clarke, P.J., 1997. The influence of inorganic

529   matrices on the decomposition of Eucalyptus litter. Aust. J. Soil Res. 35, 73-87.

530   Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., Newman, R.H., 1994. The removal of

531   magnetic materials from surface soils: a solid state ^{13}C CP/MAS NMR study. Aust. J.

532   Soil Res. 32, 1215-1229.

533   Soil Survey Staff, 1999. Soil Taxonomy: A Basic System of Soil Classification for Making

534   and Interpreting Soil Survey. US Department of Agriculture Soil Conservation

535   Service, Washington.

536   Sollins, P., Glassman, C., Paul, E.A., Swanston, C., Lajtha, K., Heil, J.W., Elliott, E.T., 1999.

537   Soil carbon and nitrogen: Pools and fractions. In: Robertson, G. P. (Eds.), Standard

538   Soil Methods for Long-term Ecological Research. Oxford Univ., New York. 89-105 p.

539   Sparling, G., Vojvodic-Vukovic, M., Schipper, L.A., 1998. Hot-water-soluble C as a simple

540   measure of labile soil organic matter: the relationship with microbial biomass C. Soil

541   Biol. Biochem. 30, 1469-1472.

542   Tirol-Padrea, A., Ladhab, J.K., Regmic, A.P., Bhandarid, A.L., Inubushie, K., 2007. Organic

543   amendments affect soil parameters in two long-term rice-wheat experiments. Soil Sci.

544   Soc. Am. J. 71, 442-452.

545 Ussiri, D.A.N., Johnson, C.E., 2007. Organic matter composition and dynamics in a northern

546   hardwood forest ecosystem 15 years after clear-cutting. For. Ecol. Manage. 240, 131-

547   142.

548 Vance, E.D., Brookes, P.C., Jenkinsen, D.S., 1987. An extraction method for measuring soil

549   microbial biomass C. Soil Biol. Biochem. 19, 703-707.

550 Vitousek, P.M., Andariese, S.W., Matson, P.A., Morris, L., Sanford, R.L., 1992. Effects of

551   harvest intensity, site preparation, and herbicide use on soil nitrogen transformations

552   in a young loblolly pine plantation. For. Ecol. Manage. 49, 277-292.

553 Wardle, D.A., 1998. Controls of temporal variability of the soil microbial biomass: A global-

554   scale synthesis. Soil Biol. Biochem. 30, 1627-1637.

555 Wardle, D.A., Yeates, G.W., Nicholson, K.S., Bonner, K.I., Watson, R.N., 1999. Response of

556   soil microbial biomass dynamics, activity and plant litter decomposition to agricultural

557   intensification over a seven-year period. Soil Biol. Biochem. 31, 1707-1720.

558 Wilson, M.A., Heng, S., Goh, K.M., Pugmire, R.J., Grant, D.M., 1983. Studies of litter and

559   acid insoluble soil organic matter fraction using ^{13}C-cross polarization nuclear

560   magnetic resonance spectroscopy with magic angle scanning. J. Soil Sci. 34, 83-97.

561 Xu, J.M., Cheng, H.H., Koskinen, W.C., Molina, J.A.E., 1997. Characterization of potentially

562   bioreactive soil organic carbon and nitrogen by acid hydrolysis. Nutr. Cycl.

563   Agroecosys. 49, 267-271.

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565