Changes in carbon functional groups and their *in situ* microscale distribution under long-term continuous cropping

Zhe H. Weng[1,2], Ram C. Dalal[1], Brian J. Reid[3], Yong-Guan Zhu[4], Timothy I. McLaren[1], Brigid A. McKenna[1], Meghan Barnard[1], Casey L. Doolette[5], Enzo Lombi[5], Johannes Friedl[6,7], Peter

5   M. Kopittke[1]

[1]The University of Queensland, School of Agriculture and Food Sustainability, St. Lucia, Queensland 4072, Australia
[2]School of Agriculture, Food, and Wine, The University of Adelaide, Urrbrae, South Australia 5064, Australia
[3]University of East Anglia, School of Environmental Sciences, Norwich, NR4 7TJ, UK
10   [4]Institute of Urban Environment, Chinese Academy of Sciences (CAS), Xiamen, 361021, China
[5]Future Industries Institute, University of South Australia, Mawson Lakes, SA 5095, Australia
[6]Centre for Agriculture and the Bioeconomy, Queensland University of Technology, Brisbane, QLD 4000, Australia
[7]University of Natural Resources and Life Sciences Vienna, Department of Forest and Soil Sciences, Institute of
15   Soil Research, Vienna, Austria

Correspondence to: Zhe H. Weng (han.weng@adelaide.edu.au)

Abstract. Land use change is causing substantial loss of soil organic carbon (SOC). However, little is known regarding how this loss of SOC influences the composition of carbon (C) functional groups and their microscale distribution, with this being critical to the protection and storage of SOC. In this study, we examined the

20   mechanisms influencing preservation of C forms and their distribution by comparing soils under native vegetation and cropping at two Australian sites, Waco black Vertisol (70 y cropping) and Langlands-Logie grey Vertisol (10 y cropping). Land use change caused the loss of up to 23 % (5.3 mg C g^{-1} soil) of bulk SOC. Strikingly, the greatest loss occurred in the mineral-associated organic carbon (MAOC) fraction that accounted for 72-91 % of total SOC. Interestingly, despite losing up to 23 % of the bulk SOC, the C that remained after long-term cropping was similar

25   in speciation to that in the native soils revealed by near edge X-ray absorption fine structure spectroscopy. In a similar manner, the use of infrared microspectroscopy showed that the forms of C remained similar in the two land uses and that C was closely associated with clay minerals. This suggests that the SOC loss from mineral fraction was not due to preferential consumption of particular forms of carbon, but rather, an overall loss of SOC in the mineral fractions and an increase in overall lability. These observations provide critical evidence that

30   preservation of SOC is not driven by increasing compositional diversity or complexity in these Vertisols and that

physical protection of C is of importance. Potential exists to rebuild SOC in the mineral-associated fraction under cropping. Management practices should promote the building of organo-mineral associations.

1 Introduction

Protecting soil organic carbon (SOC) is vital for maintaining soil quality and ecosystem functions, supporting
35 agronomic productivity and environmental resilience, and is a key component of climate change mitigation and adaptation (Kopittke et al., 2024). However, land use change has significantly reduced SOC stocks in agricultural lands. As a result, total historical CO_2 emissions from soils due to agricultural intensification are estimated to be 116 Pg of carbon (C) (Sanderman et al., 2017), accounting for almost 17 % of cumulative CO_2 emissions by humanity since pre-industrial times. Under natural conditions, soils in tropical and subtropical regions are major
40 C sinks, retaining 36-60 % of ecosystem C in forests but are estimated to emit 0.2 Gt C y^{-1} following land use change for crop production (Don et al., 2011). Indeed, land use changes in the tropics and subtropics account for 12% of the human-induced greenhouse gas (GHG) emissions between 2007 and 2016 (Piabuo et al., 2024) and are projected to be the second largest source of GHG emissions (Pörtner et al., 2022).

Despite high SOC loss from (sub)tropical soils and ecosystems, the potential to protect and restore some of these
45 C remains unanswered. Therefore, there is an urgent need to better understand the mechanisms of how SOC behaves, with this being controlled by the composition of C functional groups (Gregory et al., 2016; Jones et al., 2016), their heterogeneous spatial distribution in relation to accessibility for decomposers (Poulton et al., 2018; Jensen et al., 2019) and temporal variability (Stevenson et al., 2016; Hirsch et al., 2017; Lehmann et al., 2020). It has been hypothesized that higher diversity of C functional groups can increase the metabolic investment for
50 microorganisms to assimilate complex and diverse SOC structures, with this resulting in preservation of SOC. Furthermore, a heterogenous spatial distribution of C functional groups limits the accessibility of decomposers to C substrates (Lehmann et al., 2020), thereby further preserving SOC. This can be further enhanced with physical occlusion and protection of SOC within the occluded particulate organic C (oPOC) and mineral associated organic C (MAOC) fraction (Willard et al., 2024). Therefore, understanding the changes in C content within various SOC
55 fractions is important for examining the preservation of SOC (Even and Cotrufo, 2024).

Previous studies have reported the impact of land use change on C functional groups based on soil extracts or hydrofluoric acid pre-treated soils (An et al., 2021). Synchrotron-based near edge X-ray absorption fine structure (NEXAFS) spectroscopy is particularly suited for the characterization of complex heterogeneous samples since absorption spectra depend directly on the local bonding environment of atoms (Lehmann and Solomon, 2010).

60 NEXAFS spectroscopy provided a way of probing this complex chemistry without the need for wet-chemical analysis techniques that profoundly change the nature of a sample whilst trying to measure it (Barnard et al., 2024). Whereas traditional wet chemistry studies require processes, as a consequence, spatial information of SOC distribution and organo-mineral associations was compromised (Weng et al., 2021). Infrared micro-spectroscopy (IRM) can provide *in situ* spatial information of organic C speciation in the soil. The higher brightness of 65 synchrotron sources (100-1000 times brighter than traditional IR light source) is especially useful when mapping C in soils due to the comparatively low C concentration in most soils (Weng et al., 2022). Such analyses provide microscale maps revealing the spatial distribution of specific C functional groups in soils that can be linked to their functions (Hondroudakis et al., 2024; Weng et al., 2024).

The objective of this present study was to examine the impact of land use change on C functional composition 70 and their distribution in microaggregates using high resolution and non-invasive approaches to better understand preservation of SOC forms. Two soils, a Waco black Vertisol and a Langlands-Logie grey Vertisol, were studied under native vegetation (grassland for Waco and forest for Langlands-Logie) and following transition to cropland (70 y for Waco and 10 y for Langlands-Logie). Cropped soils experience greater mechanical disturbance and larger fluctuations in water and plant inputs compared with the undisturbed sites. We hypothesised that (1) in 75 addition to the depletion of C content driven by land use change, subsequent cropping can affect functional composition of SOC with preservation of complex C forms after SOC loss and (2) strong correlations between the lateral distribution of C forms and clay minerals can provide physical protection of SOC, however, such correlation can be weakened following land use change.

2 Materials and methods

80 ### 2.1 Site description

The study sites, soil management, and agricultural practices are detailed in the Supplementary Information. Briefly, the study sites are located near Chinchilla, Queensland (Australia), being at 27°1'S, 151 14'E for Waco and 26°47'S, 150°53'E for Langlands-Logie. The field sites have a mean annual rainfall of 630 mm, with a mean annual temperature of 19.4°C at the time of sampling in 1986 (Dalal and Mayer, 1986). Cooler months (April- 85 September) receive 35 % of the annual rainfall, with the climate classified as subtropical. The mineralogical properties of the native and cropped sites were similar for both locations at Waco and Langlands-Logie. At Waco, the soil texture for the native site consists of 13 % sand, 14 % silt, and 72 % clay whilst the cropped site contains

14 % sand, 12 % silt, and 75 % clay. The Waco soil is described as deep black to greyish brown cracking clays, alkaline at depth, with dominant clay minerals being smectite and kaolinite. The parent material is clay alluvium

90   derived from basalt. At Langlands-Logie, the soil texture for the native site consists of 35 % sand, 16 % silt, and 49 % clay whilst the cropped site contains 32 % sand, 17 % silt, and 51 % clay. The Langland-Logie soil is dominated by quartz and kaolinite and is classified as deep dark greyish brown to dark brown cracking clay, alkaline at surface and acidic at depth. Parent material is alluvial clayey sediments formed from light grey shales, silt-stones, and fine to medium sandstones. Cropping increased bulk density compared with native vegetation

95   from 0.84 to 1.01 g cm^{-3} at Waco and 0.99 to 1.15 g cm^{-3} at Langlands-Logie (P < 0.01; Dalal and Mayer, 1986a). For the cropping site at both locations, monocropping was practiced every year with winter crops consisting of 90 % wheat, 9 % barley, and 1 % oat, and summer crops consisting of 92 % sorghum and 8 % sunflower (Supplementary Information).

A total of 25 soil cores (46-mm internal diameter) were sampled to 1.2 m depth at each field site on a 5 m × 8 m

100   grid over 0.1 ha to ensure representativeness. Soil samples were collected at 0-0.1 m depth sectioned from 0-1.2 m cores during April-July from both sites. Five of these soil cores were combined to yield a total of five replicate samples from each field site (five composite samples were obtained for each sampling depth). The soil samples were sealed in plastic bags in the field, transported to the laboratory and kept at 4 °C until further processing for analysis. The samples were dried at 25 °C, sieved to < 2 mm and stored in sealed plastic containers.

105   For background analyses, soil pH was measured using 1:5 soil:water suspension. Mineralogy was assessed for the native soils using X-ray diffraction. The total organic C and total N were determined through Dumas high-temperature combustion with soil pre-treated with 1 M sulfurous acid to remove inorganic C and measured using a CN elemental analyser (LECO Trumac CN analyzer, MI) (Rayment and Lyons, 2011).

2.2 Density fractionation

110   The density fraction method was conducted on above-mentioned soil samples as described by Kölbl and Kögel-Knabner (2004) and Steffens et al. (2009). The bulk soil was introduced to a sodium polytungstate (1.8 g cm^{-3}) and the floating free particulate organic matter was collected by aspiration. To separate the aggregate-occluded particulate organic matter from the remaining sample, the soil was subject to sonification in two waves of 200 J mL^{-1} (total of 400 J mL^{-1}) and then centrifuged and collected by aspiration. The free particulate organic matter

115   was manually washed, and the aggregate-occluded particulate organic matter washed using pressure filtration (5 bar) with ultrapure Milli-Q water until the filtrate for both fractions had an electrical conductivity (EC) < 5 µS cm^{-1}. Both fractions were freeze-dried at -60 °C. The remaining mineral-associated organic matter fraction was

washed using pressure filtration to an EC < 50 µS cm^{-1} and then sieved under gravity using a 53 µm steel sieve to separate the fine fraction of mineral-associated organic matter (< 53 µm) from the coarse fraction of mineral-associated organic matter (> 53 µm). Both mineral fractions were oven dried at 50 °C.

120   We have measured C contents for four fractions: free particulate organic matter ('fPOC'), aggregate-occluded organic matter ('oPOC'), organic matter associated with the coarse mineral fraction (> 53 µm; 'coarse MAOC') and the organic matter associated with the fine mineral fraction (< 53 µm; 'fine MAOC'). Isotope ratio mass spectrometry was used to measure fPOC and oPOC given that the sample mass for these fractions was not

125   sufficient for a CN elemental analyser. In addition, coarse and fine MAOC were analysed by a CN elemental analyser. Preliminary tests showed no difference in C contents between mineral fractions with and without acid pre-treatment. For N, the results giving the N contents of each fraction were termed the 'fPON', 'oPON', 'coarse MAON' and 'fine MAON'.

2.3 Near edge X-ray absorption fine structure (NEXAFS) spectroscopy

130   For near edge X-ray absorption fine structure (NEXAFS) analysis, five field replicates were composited from each of the two locations (Waco black Vertisol and Langlands-Logie grey Vertisol) and two land uses (native vegetation and cropped) because of the limited synchrotron beamtime. Three laboratory replicates of the composite bulk soils were analysed (n=3). The finely powdered soils without acid pre-treatment were loaded onto stainless-steel rulers and analysed as described below.

135   The synchrotron-based NEXAFS analyses were performed at the SXR Spectroscopy beamline (14ID) at the Australian Synchrotron (Clayton, Australia). The NEXAFS C and N K-edge spectra were collected using partial electron yield with the floodgun engaged to minimise charging from the samples. The C K-edge spectra were collected between 275-325 eV and N K-edge spectra between 380-420 eV at 0.1 eV step size and at an angle of 55° to the beam. A graphite standard was used for energy calibration by collecting the I$_0$ and sample spectra. The

140   double normalisation of the spectra was based on the I$_0$ and a photodiode measurement from the UHV analytical chamber (Stöhr, 2013). A pre- and post-edge linear subtraction was applied for the background normalisation using the Igor software.

Deconvolution and peak fitting of the double normalized spectra was carried out using MANTiS 2.3.02 (Lerotic et al., 2014). A non-linear least square fitting of multiple gaussians and one arctangent function were used to fit

145   all the NEXAFS data, following the procedure described in Solomon et al. (2007). Briefly, two arctangent

functions at 290.5 eV for aromatic/aliphatic C and 292.0 eV were set for hydroxylated aromatic C with full-width at half-maximum (FWHM) of 0.4 eV to generate a continuum of spectrum up to 294 eV. The FWHM of the Gaussian peaks was set at 0.4 eV and six Gaussian functions representing the main $1s–\pi^*$ transitions at 284.4, 285.2, 286.6, 287.6, 288.5, and 289.3 eV were resolved. Measures of the goodness of fitting using R^2 values of
150 better than 0.999 to reflect the proportion of variance explained.

2.4 Infrared microspectroscopy (IRM)

For IRM, bulk soil was separated into microaggregates (53-250 µm) by dry sieving using the method described by Weng et al. (2018). These microaggregates contained all C fractions and were different from the above-mentioned fractionation samples. Dry sieving can minimise the dissolution and translocation of various dissolved
155 organic C forms into different aggregate fraction that may be caused by wet sieving. We focused on the microaggregates (53–250 µm) because this size fraction has been reported to undergo rapid turnover and is less protected therefore prone for change (Kölbl and Kögel-Knabner, 2004; Steffens et al., 2009). Approximately 30 microaggregates were selected on glass-fibre filter papers and humidified gradually over 18 h (Lehmann et al., 2005). The aggregates were frozen at -20 °C before being sectioned (200 nm thick) using a diamond knife without
160 embedding media via a cryo-ultramicrotome at -55 °C. Thin sections were taken from the core of the aggregates after removing edges. A total of 8-10 sections per sample were directly collected on CaF_2 windows (IR transparent, 13 mm diameter, 0.5 mm thickness).

The sections taken from intact microaggregates were placed on CaF_2 windows and analysed at the IRM beamline at the Australian Synchrotron. Details of the system are described by Hernandez-Soriano et al. (2018). Briefly, a
165 Bruker Hyperion 3000 infrared microscope is coupled with a V80v Fourier transform infrared spectrometer. The measurements were conducted in the transmission mode (64 scans, 4 cm^{-1} resolution, and 5 µm step size). Background scans were repeated every 15 scans.

IRM maps were processed using the software OPUS 8.2 (Bruker Optik GmbH, Germany), featuring O-H groups of clays (3550-3740 cm^{-1}), aliphatic C (2800-3000 cm^{-1}), aromatic C (1500-1750 cm^{-1}), and polysaccharide C
170 (950-1170 cm^{-1}) (Hernandez-Soriano et al., 2018). Integration of the areas of these absorbance peaks was calculated. A linear regression between the integrals of C forms and clay mineral was conducted. Vertisols in this study are rich in smectites and illites (and kaolinites) with the Si-O band absorbing strongly and prominently at around 1020 cm^{-1} (Pentrák et al., 2012; Parikh et al., 2014). To minimise the interference of clay minerals with

the absorbance of polysaccharide, we can conduct second derivative for the range 950-1170 cm^{-1}. IRM data were
175   processed following the methods (Huang and Wang, 1995; Schindelin et al., 2015) as outlined in the
Supplementary Information.

2.5 Correlation analysis of NEXAFS and IRM

A hierarchical clustering dendrogram indicates the similarity relationships between C functional groups from
NEXAFS (first derivatives), their abundance from IRM, and total SOC content across land uses and soil types.
180   The heatmap shows the colour-coded correlation coefficient. The first derivative of the NEXAFS spectra (281-
296 eV) for each land use and soil type were divided into 30 bins and pairwise correlations between each energy
segment and distribution of aromatic C, aliphatic C, polysaccharide C, clay O-H from IRM and total SOC content
were assessed to explore relationships between C-speciation and C-distribution. This was achieved using functions
in the R packages "*prospectr*" (Stevens and Ramirez-Lopez, 2024) and "*gplots*" (Warnes et al., 2016).

2.6 Statistical analyses

All statistical analyses were run using R version 4.2.1 (Core, 2022). When F-tests were significant, means were
separated using *t*-tests to identify significant differences in SOC content between land uses for each fraction within
each soil type/ site. *p* values were provided in Figures. Confidence intervals were provided for NEXAFS spectra
($P = 0.05$).

3 Results

3.1 Changes in soil organic carbon in bulk soils and their aggregate size fractions

Long-term cropping significantly decreased the organic C concentration of bulk topsoils ($P < 0.01$): Waco black
Vertisol (1.54 *vs.* 1.27 %, being a 17 % decrease) and Langlands-Logie grey Vertisol (1.44 *vs.* 1.11 %, being a 23
% decrease) (Fig. 1).
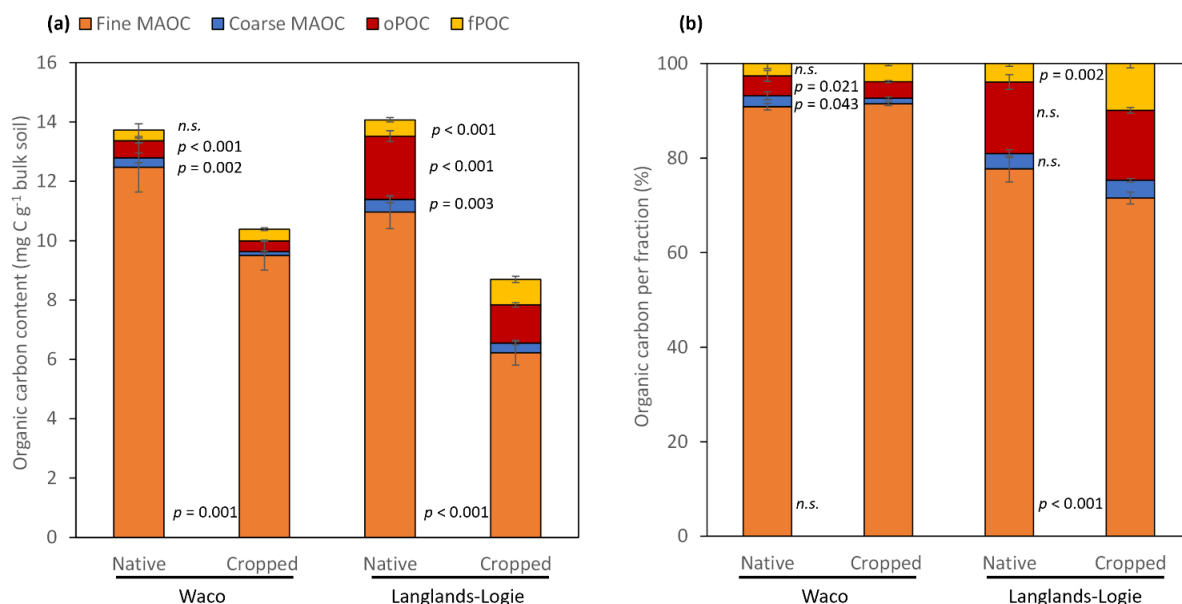
Figure 1: The organic carbon content (a) and the percentage of organic C (b) for each soil fraction within the bulk soil from topsoils (0-10 cm) collected from Waco and Langlands-Logie from two land uses. fPOC is free particulate organic C, oPOC is aggregate-occluded particulate organic C, coarse MAOC is coarse grained (> 53µm) mineral-associated organic C and fine MAOC is fine grained (< 53µm) mineral-associated organic C. Letters indicate least significant differences (95 % confidence level for each comparison) between the same fractions across land uses within each location. *p* values were given (n = 5). The mass recovery after fractionation for Waco and Langlands-Logie was over 91 %.

We then examined C fractions within the soil. The sum of four C fractions within the soil for Waco was 13.7 ± 2.3 mg C g^{-1} bulk soil (native) and 10.4 ± 3.7 mg C g^{-1} bulk soil (cropped) and Langlands-Logie grey Vertisol was 13.1 ± 1.1 mg C g^{-1} bulk soil (native) and 8.9 ± 2.9 mg C g^{-1} bulk soil (cropped). At both sites, for the native and cropped soils, the fine MAOC fraction was the dominant pool, accounting for 91 % (Waco) and 72-78 % (Langlands-Logie) of the total bulk C (Fig. 1). The oPOC fraction was the second largest pool of C, accounting for 4 % (Waco) and 15 % (Langlands-Logie) of the total bulk C. The fPOC accounted for 2.6-3.8 % of the total bulk C for Waco and 3.9-9.9 % for Langlands-Logie. The coarse MAOC was a comparatively minor proportion of total C in all soils (Fig. 1).

The impact of cropping on the C fractions varied depending on soils. In absolute terms, the largest decrease in C was for the MAOC fraction, with a loss of 3.0 mg MAOC g^{-1} bulk soil for Waco and 4.7 mg MAOC g^{-1} bulk soil

for Langlands-Logie (Fig. 1). The contribution of fPOC to the total bulk C for the Waco black Vertisol was similar for both native (0.36 mg C g^{-1} bulk soil) and cropped soil (0.39 mg C g^{-1} bulk soil) (Fig. 1a). For the Langlands-

215 Logie grey Vertisol, the fPOC fraction also accounted for only a small proportion of the total bulk C, being 0.55 mg C g^{-1} bulk soil for the native and 0.86 mg C g^{-1} bulk soil for cropped soil (Fig. 1a). In relative terms, in the Waco black Vertisol, the oPOC decreased from 4.2 (native) to 3.5 % (cropped), with a decrease in the coarse MAOC from 2.3 to 1.2 % and the fine MAOC remained at 91 %. In the Langlands-Logie grey Vertisol, oPOC varied between 14-15 % and 3.3-3.8 % in the coarse MAOC and a decrease in the fine MAOC from 78 to 72 %.

220 A similar pattern was observed for N in the various fractions as described for the C fractions (Supplementary Fig. S1). Indeed, for N in both the native and cropped soils, the fine MAON fraction was the dominant pool, accounting for 95-96 % (Waco) and 85-89 % (Langlands-Logie) of the total bulk N (Supplementary Fig. S1). Despite low N concentrations, cropping significantly decreased the total N concentration of bulk topsoils ($P < 0.05$): Waco black Vertisol (0.06 ± 0.01 *vs.* 0.04 ± 0.01 %, being a 33 % decrease) and Langlands-Logie grey Vertisol (0.09 ± 0.01

225 *vs.* 0.07 ± 0.01 %, being a 22 % decrease) (Supplementary Fig. S1).

3.2 Changes in C and N functional groups under long-term cropping examined using NEXAFS

Given the marked loss of SOC in bulk soils and their fractions with cropping (Fig. 1), we examined whether there was also a change in C speciation associated with this loss. For this, changes in C and N functional groups of bulk soils were examined using NEXAFS. In general, six C peaks dominated the NEXAFS spectra across two soil

230 types: (1) quinine (284.4 eV), (2) aromatic C (285.2 eV), (2) phenolic C–OH (286.6), (4) aliphatic C (287.6 eV), (5) carboxyl C–OOH (288.5 eV), and (6) O-alkyl-C (289.3 eV) (Fig. 2) (Lehmann and Solomon, 2010). In the Waco black Vertisol, the sharp resonance at 297.4 eV and 300 eV corresponded to L$_2$ and L$_3$ edges of potassium (Fig. 2a).
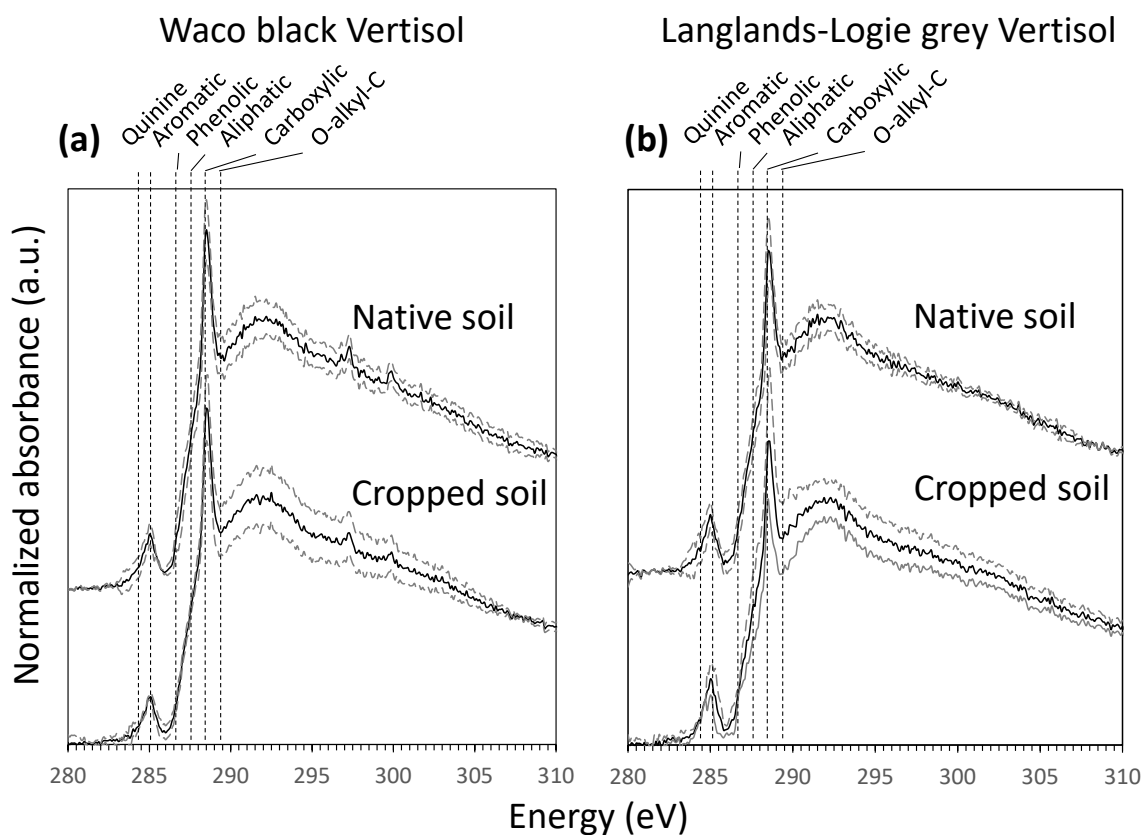
Figure 2. Synchrotron-based NEXAFS analyses of the Waco black Vertisol and Langlands-Logie grey Vertisol under the native vegetation and cropping. NEXAFS C K-edge spectra of bulk soil for the Waco soil (**a**) and the Langlands-Logie soil (**b**) (n=3, CV% < 3%), featuring: (1) quinine (284.4 eV), (2) aromatic C (285.2 eV), (3) phenolic C–OH (286.6 eV), (4) aliphatic C (287.6 eV), (5) carboxyl C–OOH (288.5 eV), and (6) O-alkyl C (289.3 eV). 95 % confidence intervals were plotted in dashed lines.

240 Next, we assessed changes in the NEXAFS C K-edge spectra of soils with long-term cropping. The number of C forms observed using NEXAFS did not change with cropping, with spectra indicating the presence of aromatic C (285.2 eV), aliphatic C/phenolic C–OH (287.6 eV), and carboxyl C–OOH (288.5 eV). Importantly, we observed no pronounced differences in the intensities of these peaks with cropping compared to native vegetation. Indeed, in the Waco black Vertisol, when comparing cropping with native vegetation, carboxylic-C (45 to 35 %), aliphatic-C (10 to 7 %), and aromatic (12 to 17 %; Fig. 2a and

245 Supplementary Table S1) were all similar in the native soil and cropped soil. In a similar manner, in the Langlands-Logie grey Vertisol, O-alkyl C varied from 39 to 23% while the intensity of the rest of the C functional groups remained unchanged.

We also examined changes in the N K-edge spectra using NEXAFS (Supplementary Fig. S2), with two main features observed across all soils, being: (1) amide (protein, 401.2 eV), and (2) alkyl-N (amino acids, 406 eV). However, long-term cropping depleted both amide and alkyl N in the topsoils of the Waco black Vertisol and Langlands-Logie grey Vertisol (Supplementary

250 Fig. S2).

3.3 Distribution of C forms under long-term cropping examined using IRM

To complement the C and N K-edge NEXAFS analyses of bulk soils, the lateral (two dimensional) distribution of OC was analysed using IRM from the cross sections taken from intact microaggregates (53-250 µm) (Fig. 3 and 4). Firstly, across all soils, the distribution of C forms was highly heterogenous. Secondly, there was no apparent consistent pattern in the distribution

255 of C forms within the microaggregate sections, as might be expected if organic C had been occluded in a central core by clay minerals.

We examined microaggregate sections taken from soils under native vegetation to establish a baseline. Despite there being no apparent consistent pattern to the overall distribution of C forms within the microaggregate sections, the aliphatic-C appeared closely associated with clay, whilst polysaccharide-C was least correlated. This was confirmed by linear regression (Fig. 3b-d

260 and 4b-d), with aliphatic-C being strongly related with clay O-H minerals in the native Waco black Vertisol ($R^2 = 0.89$) and the native Langlands-Logie grey Vertisol ($R^2 = 0.88$) (Fig. 3b and 4b). The clay association with aromatic- and polysaccharide-C varied across soil types.
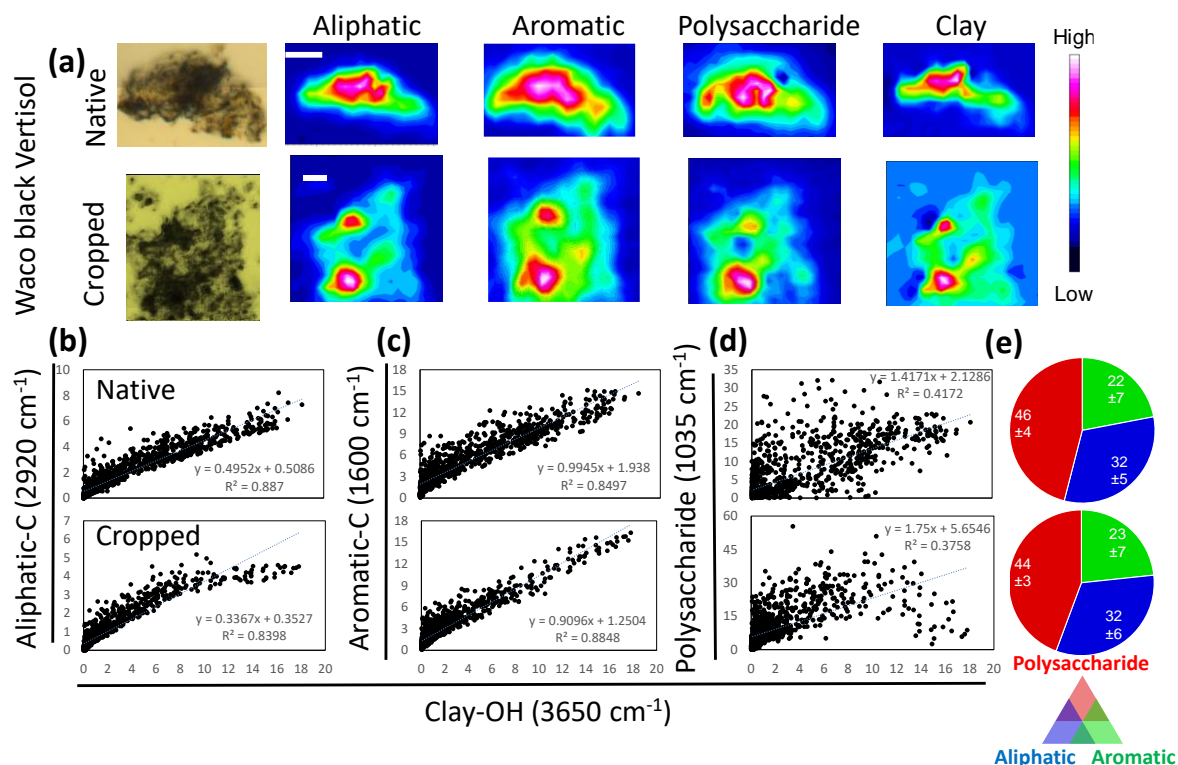
Figure 3. Synchrotron-based microspectroscopic analysis of microaggregates of the Waco black Vertisol under the native vegetation and cropping. Semi-thin (200 nm) sections of water-stable microaggregates (53-250 µm) isolated from two land uses analysed using synchrotron-based IR-microspectroscopy. **(a)** Combined RBG maps showing the distribution of polysaccharides-C (1035 cm^{-1}), aromatic-C (1600 cm^{-1}), and aliphatic-C (2920 cm^{-1}), were obtained from 64 coadded scans (4 cm^{-1} resolution), lateral resolution 5 µm. Two replicates are shown. White scale bars are 25 µm. Correlation between **(b)** aliphatic-C, **(c)** aromatic-C, and **(d)** polysaccharides-C with clay mineral. Regression analyses were performed using the collection of spectra obtained for both maps (*i.e.*, one spectrum per pixel). Only pixels where the selected absorption peak could be detected about baseline noise were included in the regressions. **(e)** Pie charts display normalized optical proportions of the four features analysed by an image processing pipeline.

Next, we examined the impact of cropping on the distribution of various C forms within the microaggregate sections. From visual observation of the maps, there were no clear differences between the cropped and native soils, with this perhaps not unexpected given the somewhat subtle differences observed in bulk C speciation from NEXAFS. Linear regressions confirmed that, with long-term cropping, aliphatic C remained closely correlated with clay for the cropped Waco Black Vertisol whilst the aliphatic C-clay correlation weakened in the cropped Langlands-Logie grey Vertisol. The R^2 values were substantially greater for polysaccharide C under native vegetation than cropping in the Langlands-Logie grey Vertisol from 0.73 to 0.44, whilst the correlation remained unchanged in the topsoil of Waco black Vertisol (0.38-0.42).
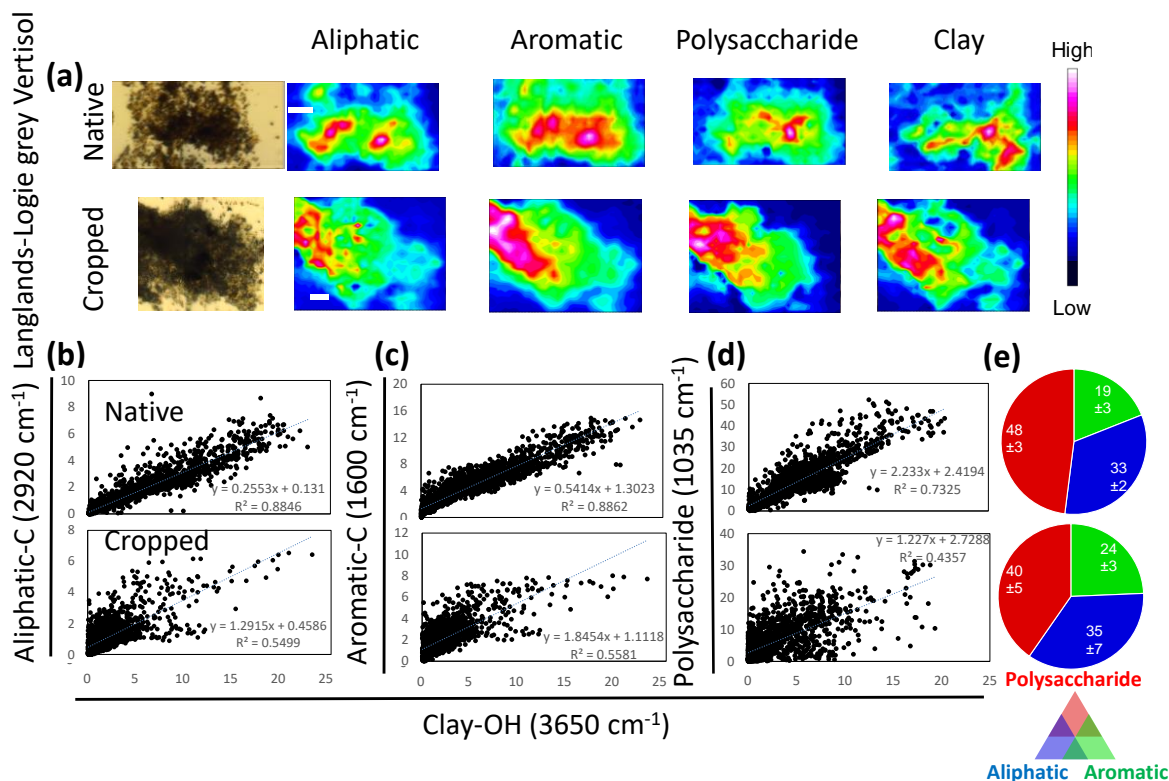
Figure 4. Synchrotron-based microspectroscopic analysis of microaggregates of the Langlands-Logie grey Vertisol under the native vegetation and cropping. Semi-thin (200 nm) sections of water-stable microaggregates (53-250 µm) isolated from two land uses analysed using synchrotron-based IR-microspectroscopy. **(a)** Combined RBG maps showing the distribution of polysaccharides-C (1035 cm⁻¹), aromatic-C (1600 cm⁻¹), and aliphatic-C (2920 cm⁻¹), were obtained from 64 coadded scans (4 cm⁻¹ resolution), lateral resolution 5 µm. Two replicates are shown. White scale bars are 25 µm. Correlation between **(b)** aliphatic-C, **(c)** aromatic-C, and **(d)** polysaccharides-C with clay mineral. Regression analyses were performed using the collection of spectra obtained for both maps (*i.e.*, one spectrum per pixel). Only pixels where the selected absorption peak could be detected about baseline noise were included in the regressions. **(e)** Pie charts display normalized optical proportions of the four features analysed by an image processing pipeline.

To quantify these observations from IRM, we developed an image processing pipeline to quantify the mean area proportion of polysaccharide-C, aliphatic-C, and aromatic-C (all pixels comprising the samples divided by the sum images across the intact section). For the Waco soil, the proportion of polysaccharide-C (44-48 %), aliphatic-C (27-33 %), and aromatic-C (20-29 %) was similar in the native vegetation and cropped soil (Fig. 3e). For the Langlands-Logie soil, long-term cropping slightly decreased polysaccharide-C (40 %) compared with the native vegetation (48 %) in the topsoil (Fig. 4e). Principal component analysis indicated that the distribution of C forms associated with clay mineral was less affected by cropping in the Waco soil

whereas the aliphatic C-clay association was weakened with cropping in the Langlands-Logie soil and the polysaccharide C-clay association was most vulnerable towards SOC loss (Supplementary Fig. S3 and S4).

A hierarchical clustering dendrogram (Fig. 5) indicated the similarity in relationships between the first derivatives of NEXAFS spectra (Fig. 2) and changes in C distribution (Fig. 3-4) and soil properties across land uses and soil types. Heavy fraction,
300 polysaccharide, clay- and silt-C were in the same cluster, which were positively correlated with aromatic-C, phenolic-C, and aliphatic-C. Total organic C, total N, light fraction, and sand-C were in the same cluster which were positively correlated with carboxyl C–OOH and O-alkyl C that were vulnerable to SOC loss.
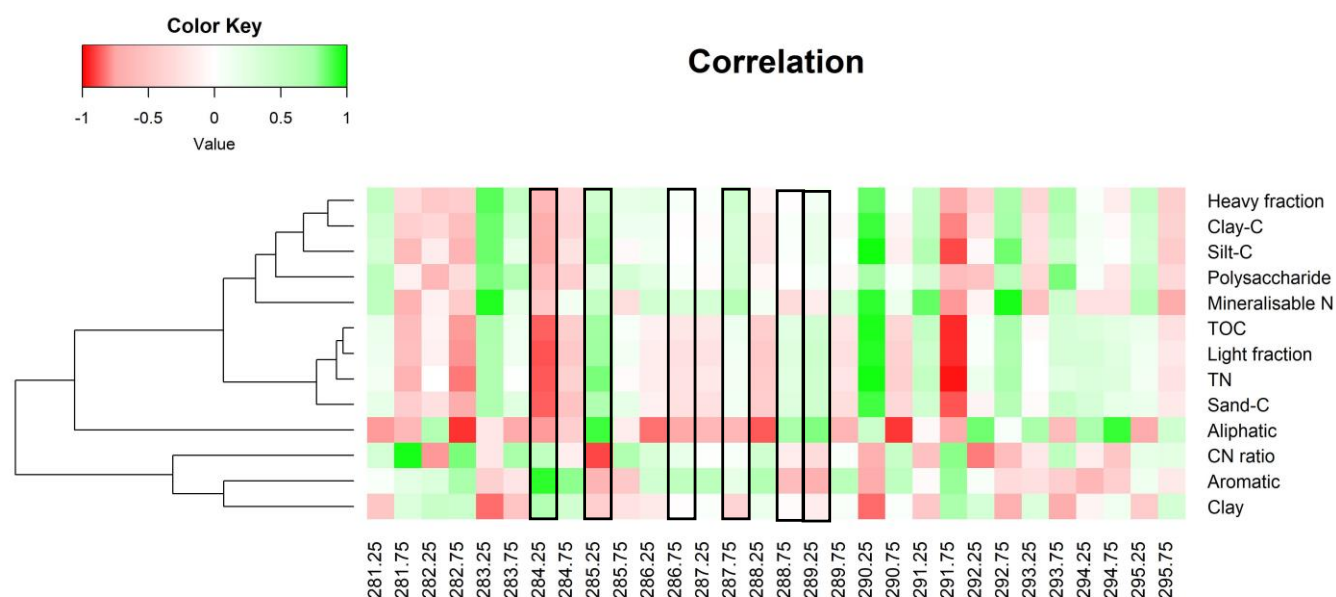
Figure 5. A hierarchical clustering dendrogram indicate the similarity relationships between the first derivatives of
305 **near edge X-ray absorption fine structure (NEXAFS) spectra (Fig. 2) and changes in soil properties across land uses**
and soil types. The first derivative of the NEXAFS spectra, 281-296 eV for C K-edge across all land uses and soil types were divided into 30 bins and pairwise correlations between each energy segment and distribution of aromatic C, aliphatic C, polysaccharide C, clay OH from IRM (Fig. 3 and 4) and other soil properties were assessed to explore relationships between C-speciation and C-distribution. The heatmap presents the colour-coded correlation co-efficient.

310 **4 Discussion**

4.1 Impact of long-term cropping on bulk C content and fractions

In the current study, land use change from native vegetation to cropping caused significant SOC loss in the bulk soils (17-23 %). This is consistent with values reported previously in global meta-analyses, such as the 20-60 % reduction reported after

long-term cropping by Kopittke et al. (2017) and the value of 42 % reported by Guo and Gifford (2002). In global tropical

315 systems, SOC decline due to conversion of primary forests to cropland and perennial crops has been reported to be between

25 and 30 % (Don et al., 2011). Indeed, land use change from native tropical vegetation to continuous cropping up to 82 years

was found to decrease SOC stocks by 43 % in subtropical Australia (Barnard et al., 2024). In the current study, the net losses

in SOC in cropped soils in comparison to their native counterparts, can be the result of that the formation of new SOC from

lower C inputs in cropped soils cannot counterbalance the loss of native SOC, and destruction of organo-mineral associations

320 with cultivation (Even and Cotrufo, 2024). The land use change from native vegetation to cropping may have altered the C

flows and inputs in both locations.

When further examining the SOC fractions, our findings that the MAOC is the dominant fraction in heavy clay soils are

consistent with previous studies showing that fine MAOC contributes the majority of C with oPOC making a smaller but still

important contribution (Traore et al., 2020; Kleber et al., 2015). These results highlight the importance of organo-mineral

325 interactions (*i.e.,* fine MAOC) and physical occlusion of particulate organic matter through aggregation (*i.e.,* oPOC) in building

SOC stocks (Angst et al., 2017). Silt- and clay-rich Vertisols in the current study, consist of montmorillonites with a high

permanent negative change and surface area, can further enhance OC sorption (Traore et al., 2020).

Overall, SOC loss as a result of long-term cropping was predominantly from the two largest fractions, being the fine MAOC

and oPOC fractions (Fig. 1). Although the fine MAOC fraction is generally considered the most persistent fraction (Dungait

330 et al., 2012), we showed that losses were greatest from this fraction given that it accounted for 72-91% of the total SOC.

Indeed, decreases in fine MAOC accounted for 88 % of the total decrease in SOC. These observations are in accordance with

previous findings that have shown that the mineral-associated fractions can be sensitive to land use changes (Yeasmin et al.,

2023; Hondroudakis et al., 2024; Barnard et al., 2024). The factors that influencing MAOC following land use changes can be

driven by tillage, root exudates, and microbial processes (Even and Cotrufo, 2024).

335 We also examined the impact of cropping on other fractions. The fPOC fraction is often more accessible to biodegradation

hence rapid turnover can occur even in the native vegetation systems. Although the fPOC fraction is often more sensitive to

land use changes (Hondroudakis et al., 2024; Barnard et al., 2024), we found this fraction made only small contributions to

the total bulk C for these subtropical semi-arid Vertisols and was not subject to land use change (Fig. 1a and b). In a similar

manner, the coarse MAOC contributed the smallest proportion of total C in all soils (Fig. 1).

340 **4.2 Impact of long-term cropping on preservation of C forms**

Given the marked loss of SOC with cropping (Fig. 1), we examined whether there was also a change in C speciation associated

with this loss. NEXAFS analyses revealed that conversion to cropping did not cause a marked change in the C forms (Fig. 2

and Supplementary Table S1). Therefore, our first hypothesis that subsequent cropping can affect functional composition of

SOC with preservation of complex C forms after SOC loss is not supported.

345   These findings provide important information regarding the underlying mechanisms driving SOC preservation. Specifically, we have shown that the SOC loss (Fig. 1) was not due to preferential consumption of particular forms of C and the concomitant accumulation of complex or highly diverse forms of C, but rather, an overall depletion of all C forms. These observations are important in understanding the mechanisms that higher complexity and diversity could result in preservation of complex C forms (De Nobili et al., 2020). Rather, we show here in a long-term cropping system that the most persistent SOC (*i.e.,* the

350   SOC remaining after long-term land use change and disruption) was similar in form to that from the undisturbed native soil (Fig. 2). Thus, our data do not support the hypothesis that increasing SOC storage is driven by preservation of complex C forms. Our findings here are somewhat similar to those of Jones et al. (2023) who reported that persistent soil organic matter in a podzol chronosequence was not compositionally complex or diverse. In a similar manner, the study of Leifeld and Leifeld and Kögel-Knabner (2005) used ^{13}C nuclear magnetic resonance (NMR) analyses and showed that there was no change in the

355   composition in response to land use change.

Closer examination of the NEXAFS analyses revealed carboxylic-C functional group as the most prominent feature in the two agroecosystems, followed by O-alkyl-C and aromatic-C (Fig. 2; Supplementary Table S1). The phenolic-C represents a tracer for lignin in the form of O-substituted aryl-C and potentially ketonic-C and phenyl-C attached to amide group (Urquhart and Ade, 2002). The O-alkyl-C component represents mostly polysaccharides and fractions of alcohol and ether-C (Lehmann and

360   Solomon, 2010). These results support the previous findings of the impact of land use change on C functional groups in (sub)tropical forest soils dominated by carboxylic-C (34-40 %), O-alkyl C (22-24 %), and aromatic C (17-19 %) (Solomon et al. 2007).

Nevertheless, previous studies using NEXAFS reported that the proportion of aromatic C increased, and the phenolic-C and O-alkyl C decreased over time when converted native tropical forest and subtropical grassland to agriculture (Solomon et al.

365   2007). The changes in C functional groups indicated that mineralisation and depletion of O-alkyl structures, in the form of cellulose, or carbohydrates, or short chain oxidation of phenylpropane units of lignin, with continuous cropping (Solomon et al. 2007). This increased aromatic-C functional group may be resulted from accumulation of H- and C-substituted aryl-C structures and aliphatic components such as (poly)methylene during mineralisation with cropping. However, such increased aromaticity was not evident in this current study. It is likely that reduced return of organic matter to the soil (fPOC in Fig. 1)

370   and rapid organic matter mineralisation in both cropped and native vegetated systems depleted carboxylic-C group in these two agroecosystems. This is supported by NEXAFS analyses that showed lack of response in the composition of fPOC, oPOC, and MAOC to land use change (Barnard et al., 2024; Hondroudakis et al., 2024).

4.3 Impact of long-term cropping on lateral heterogeneity of C forms

We further investigated the *in-situ* distribution of C functional groups in sections taken from intact microaggregates using high

375   resolution microspectroscopic techniques. The IRM mapping supports the fractionation results that binding of C to the mineral fraction contributes to the majority of SOC as MAOC. We showed that strong association between aliphatic- and aromatic-C

with clay OH minerals whereas polysaccharide-C was less correlated to clay. IRM analyses revealed high heterogeneity of SOC distribution as well as the consequences of land use change. Our data suggest that preservation of SOC in these Vertisols is likely regulated by the physical protection of SOC with clay minerals.

380 Several overall observations from these IRM analyses can be made to advance the understanding of how organic C behaves at the microscale that are critical to preservation of SOC. Firstly, we found that land use change did not result in a marked change in C forms within these sections from intact microaggregates. Indeed, for the Waco soil, the proportion of polysaccharide-C (44-48 %), aliphatic-C (27-33 %), and aromatic-C (20-29 %) was similar in the native vegetation and cropped soil (Fig. 3e), whilst for the Langlands-Logie soil, the proportion of polysaccharide-C (40-48 %), aliphatic-C (32-35 %), and aromatic-C

385 (19-25 %) was also similar in the native vegetation and cropped soil (Fig. 4e). Related to this observation, it was also noted that the IRM mapping supported the results of the fractionation, with strong correlations between both the aliphatic-C and the clay OH minerals and between the aromatic-C and the clay OH minerals, with this contributing to the MAOC fraction being the dominant C pool (Fig. 3b & c and 4b & c). In contrast, polysaccharide-C was less correlated to clay (Fig. 3d and 4d). Therefore, such findings supported our second hypothesis that strong correlations between the lateral distribution of C forms

390 and clay minerals can provide physical protection of SOC. Indeed, the largest proportion of organic C was found in the clay-size fractions of the native Waco black Vertisol (52 %) and the Langlands-Logie grey Vertisol (44 %) (Barnard et al., 2024). Despite most of SOC loss being MAOC upon land use change, the distribution of C forms in association with clay minerals remained less affected. Thus, these two observations (*i.e.,* the close correlation between SOC forms and clay minerals, plus the similarity of SOC forms in native soils and cropping soils) further supported the NEXAFS results that preservation of SOC

395 was not due to the formation of complex C compounds, rather the physical protection of SOC. The surfaces of clay minerals can adsorb organic molecules which is an important mechanism for preservation of SOC (Kleber et al., 2021). Soil minerals also contribute to the formation and destruction of aggregates that regulate protection of organic matter against microbial mineralisation (Lehmann et al., 2020). Indeed, a hierarchical clustering dendrogram (Fig. 5) indicated the similarity in relationships between the first derivatives of NEXAFS spectra (Fig. 2) and changes in C distribution (Fig. 3-4) and soil

400 properties across land uses and soil types. Heavy fraction, polysaccharide, clay- and silt-C were in the same cluster, which were positively correlated with aromatic-C, phenolic-C, and aliphatic-C. Total organic C, total N, light fraction, and sand-C were in the same cluster which were positively correlated with carboxyl C–OOH and O-alkyl C that were vulnerable to SOC loss.

Secondly, in this study we showed that long-term continuous cropping can weaken the correlations between the distribution

405 of C forms and clay minerals. This was particularly evident in the Langlands-Logie soil with quartz (51 %) as the dominant mineral and less impacted in the smectite-dominant (60 %) Waco soil. This further explained that the majority of SOC loss occurred in the MAOC fraction. From visual observation of the IRM maps, there were no clear differences between the cropped and native soils. Linear regressions confirmed that, with long-term cropping, aliphatic C remained closely correlated with clay for the cropped Waco Black Vertisol whilst the aliphatic C-clay correlation weakened in the cropped Langlands-Logie grey

410 Vertisol. Ligand exchange may explain the strong resilience of aliphatic-clay associations against cropping-driven

mineralisation. Such organo-mineral interactions can be strengthened in the case of Waco clay through anion exchange and/or sorption between carboxylic and phenolic/aliphatic C functional groups and clay mineral-OH groups such as phyllosilicate edges and hydroxyl groups of Fe, Mn, and Al (Adhikari and Yang, 2015; Newcomb et al., 2017). Further, slower turnover of SOC in clay and fine silt fractions than sand fractions has also been reported (Marschner et al., 2008). However, the R^2 values

415   were substantially greater for polysaccharide C under native vegetation than cropping in the Langlands-Logie grey Vertisol (0.73 to 0.44) whilst the correlation remained unchanged in the Waco black Vertisol (0.38-0.42). The strong correlations between polysaccharide-C and clay in the native Langlands-Logie soil can be explained by the high organic input from native forest whereas weaker correlations were observed in the grassland-derived native Waco soil. Furthermore, principal component analysis indicated that the distribution of C forms associated with clay mineral was less affected by cropping in the Waco soil

420   whereas the aliphatic C-clay association was weakened with cropping in the Langlands-Logie soil and the polysaccharide C-clay association was most vulnerable towards SOC loss (Supplementary Fig. S4).

Thirdly, across all soils (both native and cropped), the distribution of C forms was highly heterogenous, with no apparent consistent pattern in the distribution of C forms within the core of microaggregates (Fig. 3-4). These findings support the growing body of microscopic evidence (Weng et al., 2022; Weng et al., 2024; Hondroudakis et al., 2024) against the hypothesis

425   that the formation of microaggregates occurs via the occlusion of organic matter by mineral particles (Six et al., 1998; Tisdall and Oades, 1982). Indeed, our findings align with the observations from previous studies. For example, there was no gradient in the C concentration from aggregate surface to the interior for C forms for a Vertisol and a Oxisol after conversion from native vegetation to cropping (Hernandez-Soriano et al., 2018). In a similar manner, organic C was heterogeneously distributed within microaggregates with no clear pattern from three forest-derived soils (Lehmann et al., 2008). Instead, preferential

430   localisation of organic matter in fine-scale discrete areas has been observed by numerous studies (Vogel et al., 2014; Steffens et al., 2017; Kopittke et al., 2018). It is noted that such organo-mineral associations often occur at submicron scales whereas the resolution of synchrotron-based IRM of 5 µm may limit the observations.

5 Conclusions

We have provided, for the first time, two-dimensional microscale information of C forms and their distribution in the

435   microaggregates under long-term land use change from native forest/grassland to cropping. Long term cropping of subtropical semi-arid Vertisols depleted SOC by up to 23 %. Despite being persistent fractions, the largest C losses with cropping were in the two largest pools (fine MAOC and oPOC). This highlights the importance of organo-mineral interactions and occlusion of particulate organic matter through aggregation in maintaining SOC stocks. This was confirmed by IRM and NEXAFS analyses showing that despite the loss of fine MAOC and oPOC, there were only modest changes in C forms and distribution, with this

440   supporting the importance of 'spatial heterogeneity' as an important controller of preservation of SOC as opposed to being related to the formation of recalcitrant and highly complex C compounds. Although highly heterogenous distribution of C forms within intact microaggregates, strong associations between C forms and clay were maintained under cropping in the

Waco black Vertisol but weakened in the Langlands-Logie grey Vertisol. The findings of this study provide new insight into protection and storage of SOC. The in-situ analyses supported observations from the fractionation analysis, demonstrating that
445 the binding of organic C to the mineral fraction is critical in determining preservation of SOC under land use change.

Code and data availability.

The data and analyses that support these findings will be made available in response to a reasonable request but are not hosted in an online repository at this time in order to protect the privacy of growers.

Author contributions

450 ZHW, PMK, and RCD designed the experiments, and ZHW carried them out. BAM, MB, CLD, EL, and conducted analyses. BJR, YGZ, and TIM contributed to writing and revising of the manuscript. ZHW drafted the manuscript with contributions from all authors.

Competing interests

The contact author has declared that none of the authors has any competing interests.

455 **Acknowledgements**

The authors thank the Grains Research and Development Corporation (GRDC) for funding this research (UOQ1910-003RTX). Part of this research was undertaken on the Soft X-ray spectroscopy beamline and the Infrared microscopy beamline at the Australian Synchrotron, part of ANSTO (grant numbers AS213/SXR/17477 and AS213/IRM/17479). Dr Andrew Biggs and Tony King from the Department of Resources (Queensland Government) are also gratefully acknowledged for their
460 custodianship of the archived Vertosol soil series.

465

References

Adhikari, D. and Yang, Y.: Selective stabilization of aliphatic organic carbon by iron oxide, Scientific reports, 5, 1-7, 2015.

An, Z., Bernard, G. M., Ma, Z., Plante, A. F., Michaelis, V. K., Bork, E. W., Carlyle, C. N., Baah-Acheamfour, M., and Chang, S. X.: Forest land-use increases soil organic carbon quality but not its structural or thermal stability in a hedgerow system, Agriculture, Ecosystems & Environment, 321, 107617, 2021.

Angst, G., Mueller, K. E., Kögel-Knabner, I., Freeman, K. H., and Mueller, C. W.: Aggregation controls the stability of lignin and lipids in clay-sized particulate and mineral associated organic matter, Biogeochemistry, 132, 307-324, 2017.

Barnard, M., Dalal, R. C., Weng, Z. H., Schweizer, S. A., and Kopittke, P. M.: Physicochemical protection is more important than chemical functional composition in controlling soil organic carbon retention following long-term land-use change, Geoderma, 452, 117098, 2024.

Core, R.: Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, Online: https://www. r-project. org, 2022.

Dalal, R. and Mayer, R. J.: Long term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. I. Overall changes in soil properties and trends in winter cereal yields, Soil Research, 24, 265-279, 1986.

De Nobili, M., Bravo, C., and Chen, Y.: The spontaneous secondary synthesis of soil organic matter components: A critical examination of the soil continuum model theory, Applied Soil Ecology, 154, 103655, 2020.

Don, A., Schumacher, J., and Freibauer, A.: Impact of tropical land-use change on soil organic carbon stocks–a meta-analysis, Global Change Biology, 17, 1658-1670, 2011.

Dungait, J. A., Hopkins, D. W., Gregory, A. S., and Whitmore, A. P.: Soil organic matter turnover is governed by accessibility not recalcitrance, Global Change Biology, 18, 1781-1796, 2012.

Even, R. J. and Cotrufo, M. F.: The ability of soils to aggregate, more than the state of aggregation, promotes protected soil organic matter formation, Geoderma, 442, 116760, 2024.

Gregory, A., Dungait, J., Watts, C., Bol, R., Dixon, E., White, R., and Whitmore, A.: Long-term management changes topsoil and subsoil organic carbon and nitrogen dynamics in a temperate agricultural system, European Journal of Soil Science, 67, 421-430, 2016.

Guo, L. B. and Gifford, R.: Soil carbon stocks and land use change: a meta analysis, Global Change Biology, 8, 345-360, 2002.

Hernandez-Soriano, M. C., Dalal, R. C., Warren, F. J., Wang, P., Green, K., Tobin, M. J., Menzies, N. W., and Kopittke, P. M.: Soil organic carbon stabilization: mapping carbon speciation from intact microaggregates, Environmental science & technology, 52, 12275-12284, 2018.

Hirsch, P. R., Jhurreea, D., Williams, J. K., Murray, P. J., Scott, T., Misselbrook, T. H., Goulding, K. W., and Clark, I. M.: Soil resilience and recovery: rapid community responses to management changes, Plant and Soil, 412, 283-297, 2017.

Hondroudakis, L., Kopittke, P. M., Dalal, R. C., Barnard, M., and Weng, Z. H.: The influence of land use and management on the behaviour and persistence of soil organic carbon in a subtropical Ferralsol, Soil, 10, 451-465, 2024.

Huang, L.-K. and Wang, M.-J. J.: Image thresholding by minimizing the measures of fuzziness, Pattern recognition, 28, 41-51, 1995.

Jensen, J. L., Schjønning, P., Watts, C. W., Christensen, B. T., Peltre, C., and Munkholm, L. J.: Relating soil C and organic matter fractions to soil structural stability, Geoderma, 337, 834-843, 2019.

Jones, A. R., Orton, T. G., and Dalal, R. C.: The legacy of cropping history reduces the recovery of soil carbon and nitrogen after conversion from continuous cropping to permanent pasture, Agriculture, Ecosystems & Environment, 216, 166-176, 2016.

Jones, A. R., Dalal, R. C., Gupta, V. V., Schmidt, S., Allen, D. E., Jacobsen, G. E., Bird, M., Grandy, A. S., and Sanderman, J.: Molecular complexity and diversity of persistent soil organic matter, Soil Biology and Biochemistry, 109061, 2023.

Kleber, M., Bourg, I. C., Coward, E. K., Hansel, C. M., Myneni, S. C., and Nunan, N.: Dynamic interactions at the mineral–organic matter interface, Nature Reviews Earth & Environment, 2, 402-421, 2021.

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., and Nico, P. S.: Mineral-Organic Associations: Formation, Properties, and Relevance in Soil Environments, 10.1016/bs.agron.2014.10.005, 2015.

Kölbl, A. and Kögel-Knabner, I.: Content and composition of free and occluded particulate organic matter in a differently textured arable Cambisol as revealed by solid-state 13C NMR spectroscopy, Journal of Plant Nutrition and Soil Science, 167, 45-53, 2004.

515   Kopittke, P. M., Dalal, R. C., Finn, D., and Menzies, N. W.: Global changes in soil stocks of carbon, nitrogen, phosphorus, and sulphur as influenced by long-term agricultural production, Global change biology, 23, 2509-2519, 2017.

Kopittke, P. M., Hernandez-Soriano, M. C., Dalal, R. C., Finn, D., Menzies, N. W., Hoeschen, C., and Mueller, C. W.: Nitrogen-rich microbial products provide new organo-mineral associations for the stabilization of soil organic matter, Global Change Biology, 24, 1762-1770, 2018.

520   Kopittke, P. M., Dalal, R. C., McKenna, B. A., Smith, P., Wang, P., Weng, Z., van der Bom, F. J., and Menzies, N. W.: Soil is a major contributor to global greenhouse gas emissions and climate change, Soil, 10, 873-885, 2024.

Lehmann, J. and Solomon, D.: Organic carbon chemistry in soils observed by synchrotron-based spectroscopy, in: Developments in soil science, Elsevier, 289-312, 2010.

Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S., and Jacobsen, C.: Spatial complexity of soil organic matter forms at nanometre scales, Nature Geoscience, 1, 238-242, 2008.

525   Lehmann, J., Liang, B., Solomon, D., Lerotic, M., Luizão, F., Kinyangi, J., Schäfer, T., Wirick, S., and Jacobsen, C.: Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy for mapping nano-scale distribution of organic carbon forms in soil: Application to black carbon particles, Global Biogeochemical Cycles, 19, 2005.

Lehmann, J., Hansel, C. M., Kaiser, C., Kleber, M., Maher, K., Manzoni, S., Nunan, N., Reichstein, M., Schimel, J. P., and Torn, M. S.: Persistence of soil organic carbon caused by functional complexity, Nature Geoscience, 13, 529-534, 2020.

530   Leifeld, J. and Kögel-Knabner, I.: Soil organic matter fractions as early indicators for carbon stock changes under different land-use?, Geoderma, 124, 143-155, 2005.

Lerotic, M., Mak, R., Wirick, S., Meirer, F., and Jacobsen, C.: MANTiS: a program for the analysis of X-ray spectromicroscopy data, Journal of synchrotron radiation, 21, 1206-1212, 2014.

535   Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P. M., Hamer, U., Heim, A., Jandl, G., and Ji, R.: How relevant is recalcitrance for the stabilization of organic matter in soils?, Journal of plant nutrition and soil science, 171, 91-110, 2008.

Newcomb, C. J., Qafoku, N. P., Grate, J. W., Bailey, V. L., and De Yoreo, J. J.: Developing a molecular picture of soil organic matter–mineral interactions by quantifying organo–mineral binding, Nature communications, 8, 1-8, 2017.

540   Parikh, S. J., Goyne, K. W., Margenot, A. J., Mukome, F. N., and Calderón, F. J.: Soil chemical insights provided through vibrational spectroscopy, Advances in agronomy, 126, 1-148, 2014.

Pentrák, M., Czímerová, A., Madejová, J., and Komadel, P.: Changes in layer charge of clay minerals upon acid treatment as obtained from their interactions with methylene blue, Applied Clay Science, 55, 100-107, 2012.

Piabuo, S. M., Puatwoe, J. T., Eckebil, P. P. T., Nghogekeh, T. R., and Foundjem-Tita, D.: Foreign direct investment and carbon emissions from land use, land-use change, and forestry (LULUCF): empirical evidence from tropical forest countries, Environment, Development and Sustainability, 26, 15639-15665, 2024.

545

Pörtner, H.-O., Roberts, D. C., Poloczanska, E., Mintenbeck, K., Tignor, M., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., and Möller, V.: IPCC, 2022: Summary for policymakers, 2022.

Poulton, P., Johnston, J., Macdonald, A., White, R., and Powlson, D.: Major limitations to achieving "4 per 1000" increases in soil organic carbon stock in temperate regions: Evidence from long-term experiments at Rothamsted Research, United Kingdom, Global Change Biology, 24, 2563-2584, 2018.

550

Rayment, G. E. and Lyons, D. J.: Soil chemical methods: Australasia, CSIRO publishing2011.

Sanderman, J., Hengl, T., and Fiske, G. J.: Soil carbon debt of 12,000 years of human land use, Proceedings of the National Academy of Sciences, 114, 9575-9580, 2017.

555   Schindelin, J., Rueden, C. T., Hiner, M. C., and Eliceiri, K. W.: The ImageJ ecosystem: An open platform for biomedical image analysis, Molecular reproduction and development, 82, 518-529, 2015.

Six, J., Elliott, E., Paustian, K., and Doran, J.: Aggregation and soil organic matter accumulation in cultivated and native grassland soils, Soil Science Society of America Journal, 62, 1367-1377, 1998.

Solomon, D., Lehmann, J., Kinyangi, J., Amelung, W., Lobe, I., Pell, A., Riha, S., Ngoze, S., Verchot, L., and Mbugua, D.: Long-term impacts of anthropogenic perturbations on dynamics and speciation of organic carbon in tropical forest and subtropical grassland ecosystems, Global Change Biology, 13, 511-530, 2007.

560

Steffens, M., Kölbl, A., and Kögel-Knabner, I.: Alteration of soil organic matter pools and aggregation in semi-arid steppe topsoils as driven by organic matter input, European journal of soil science, 60, 198-212, 2009.

Steffens, M., Rogge, D. M., Mueller, C. W., Höschen, C., Lugmeier, J., Kölbl, A., and Kögel-Knabner, I.: Identification of
565 distinct functional microstructural domains controlling C storage in soil, Environmental science & technology, 51, 12182-
12189, 2017.

Stevenson, B. A., Sarmah, A. K., Smernik, R., Hunter, D. W., and Fraser, S.: Soil carbon characterization and nutrient ratios
across land uses on two contrasting soils: Their relationships to microbial biomass and function, Soil Biology and
Biochemistry, 97, 50-62, 2016.

570 Stöhr, J.: NEXAFS spectroscopy, Springer Science & Business Media2013.

Tisdall, J. and Oades, J. M.: Organic matter and water-stable aggregates in soils, Journal of soil science, 33, 141-163, 1982.

Traore, S., Thiombiano, L., Bationo, B. A., Koegel-Knabner, I., and Wiesmeier, M.: Organic carbon fractional distribution
and saturation in tropical soils of West African savannas with contrasting mineral composition, Catena, 190, 104550, 2020.

Vogel, C., Mueller, C. W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M., and Kögel-Knabner, I.: Submicron
575 structures provide preferential spots for carbon and nitrogen sequestration in soils, Nature Communications, 5, 1-7, 2014.

Weng, Z., Lehmann, J., Van Zwieten, L., Joseph, S., Archanjo, B. S., Cowie, B., Thomsen, L., Tobin, M. J., Vongsvivut, J.,
and Klein, A.: Probing the nature of soil organic matter, Critical Reviews in Environmental Science and Technology, 1-22,
2021.

Weng, Z., Van Zwieten, L., Tavakkoli, E., Rose, M. T., Singh, B. P., Joseph, S., Macdonald, L. M., Kimber, S., Morris, S.,
580 and Rose, T. J.: Microspectroscopic visualization of how biochar lifts the soil organic carbon ceiling, Nature Communications,
13, 5177, 2022.

Weng, Z. H., Kopittke, P. M., Schweizer, S., Jin, J., Armstrong, R., Rose, M., Zheng, Y., Franks, A., and Tang, C.: Shining a
Light on How Soil Organic Carbon Behaves at Fine Scales under Long-Term Elevated CO2: An 8 Year Free-Air Carbon
Dioxide Enrichment Study, Environmental Science & Technology, 2024.

585 Willard, S. J., Liang, G., Adkins, S., Foley, K., Murray, J., and Waring, B.: Land use drives the distribution of free, physically
protected, and chemically protected soil organic carbon storage at a global scale, Global Change Biology, 30, e17507, 2024.

Yeasmin, S., Singh, B., Johnston, C. T., Quan, H., and Sparks, D. L.: Changes in particulate and mineral-associated organic
carbon with land use in contrasting soils, Pedosphere, 33, 421-435, 2023.