Supplementary Information

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

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Materials and methods

Site description

The native vegetation and cropped sites for both the Waco Vertisol and the Langlands-Logie Vertisol were within 100 m of each other. For the Waco black Vertisol, the native vegetation was grass (dominant *Dichanthium sericeum*), whilst for the Langlands-Logie grey Vertisol the dominant native vegetation was brigalow (dominant *Acacia harpophylla* and associated *Casuarina cristata*). The land use change from native vegetation to cropping occurred 70 y ago for the Waco Vertisol and 10 y ago for the Langlands-Logie Vertisol. Average cropping intensity for the Waco soil was 0.6 winter crops per year and 0.3 summer crops per year, while 0.7 winter corps per year and 0.1 summer crops per year for the Langlands-Logie soil (Dalal and Mayer, 1986). Cropping intensity refers to the number of crops grown in a given agricultural year in the same field. There was a fallowing period for both sites, hence, cropping intensity is less than 1 and crop distribution is less than 100 %. For both sites, winter crops consisted of 90 % wheat, 9 % barley, and 1 % oat, and summer crops consisted of 92 % sorghum and 8 % sunflower. Mean stubble retention was 49 % of crop per year for the Waco soil and 77 % for the Langland-Logie soil. The mean rate of fertiliser application was 32.6 kg N ha^{-1} y^{-1} and 1 kg P ha^{-1} y^{-1} for the Waco soil and 7.5 kg N ha^{-1} y^{-1} for the Langland-Logie soil with no P fertiliser application. Average cultivation operations were five times per year for the Waco soil and four times per year for the Langland-Logie soil.

Density fractionation

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 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.

Image processing pipeline for IRM data

To account for different optical intensity in various parts of the image (based on heterogeneous distribution of organo-mineral compounds and potentially varying slice thickness), we normalised the optical proportions of the different classes of IRM data. For these, all four channels were summed. To exclude the influence of pixels in the background which are not part of the scanned soil aggregates, we used the histogram-based Huang thresholding algorithm (Huang and Wang, 1995) implemented in FIJI (Schindelin et al., 2015). All pixels comprising the sample were then divided by the sum image to compute the different local proportions of the channels. Based on the individual histogram of each IRM measurement, we derived the mean value of the normalised proportion of each channel and segmented all pixels higher than that mean value as high intensity region of a certain channel. After such a segmentation of each channel, the segmented images were combined to derive information about different local combinations of channels. These combinations were then simplified into a polysaccharide category including co-localised segments of polysaccharide and aromatic, a mixed category including the aromatic high intensity regions, an aliphatic category including various combinations with other channels, and a clay category including various combinations with other channels. According to the count of pixels in these four main categories, we computed their mean area proportion across the two replicate measurements (Weng et al., 2022).

References

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Huang, L.-K., Wang, M.-J.J., 1995. Image thresholding by minimizing the measures of fuzziness. Pattern recognition 28(1), 41-51.

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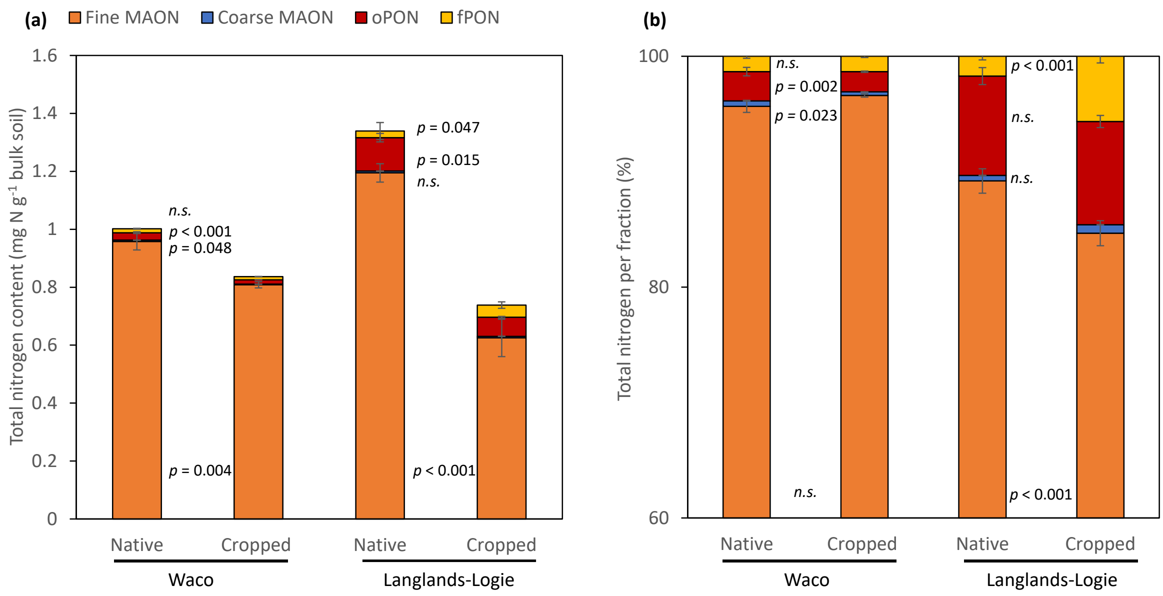
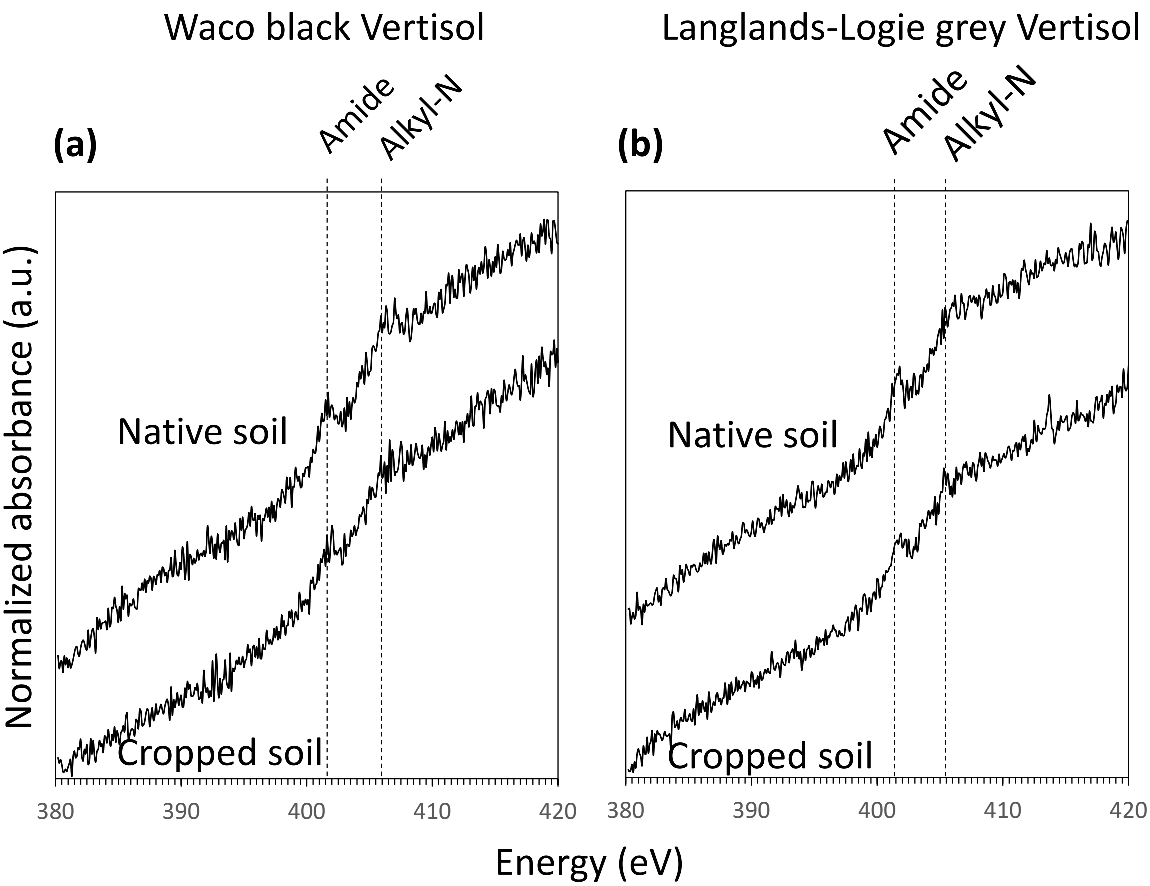
Supplementary Table 1. Relative proportion of organic C functional groups in the Waco and Langlands-Logie soils under different land uses identified by C (1s) near edge X-ray absorption fine structure (NEXAFS) spectroscopy. Measures of the goodness of fitting using R^2 errors of better than 0.999 were achieved for the data.

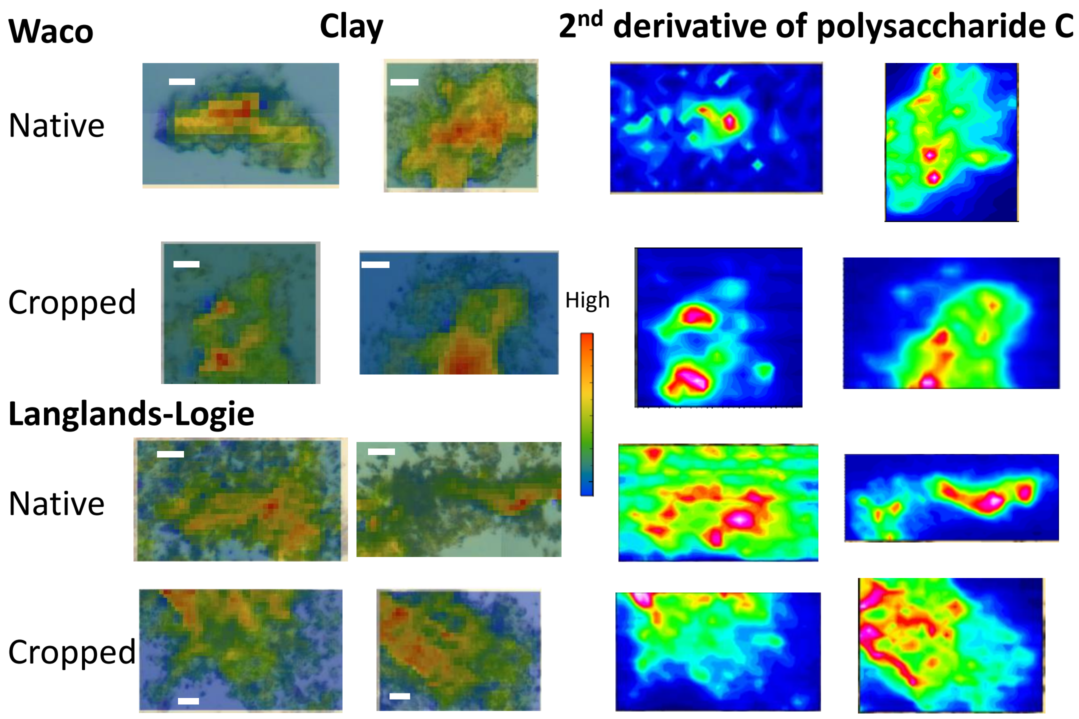
Site	Land use	Quinine	Aromatic	Phenolic	Aliphatic	Carboxylic	O-alkyl-C
		------------------------------------- % -------------------------------------					
Waco	Native	6.4 ± 2.1	12 ± 2.3	11 ± 2.3	9.6 ± 1.3	45 ± 4.3	17 ± 3.1
	Cropped	5.6 ± 1.7	17 ± 1.7	9.5 ± 1.9	7.1 ± 2.4	35 ± 3.5	26 ± 2.7
Langlands-Logie	Native	1.9 ± 1.1	4.7 ± 1.2	14 ± 1.5	2.0 ± 0.5	39 ± 3.5	39 ± 2.9
	Cropped	2.4 ± 0.7	8.6 ± 2.3	14 ± 2.3	8.8 ± 3.1	44 ± 4.1	23 ± 4.1



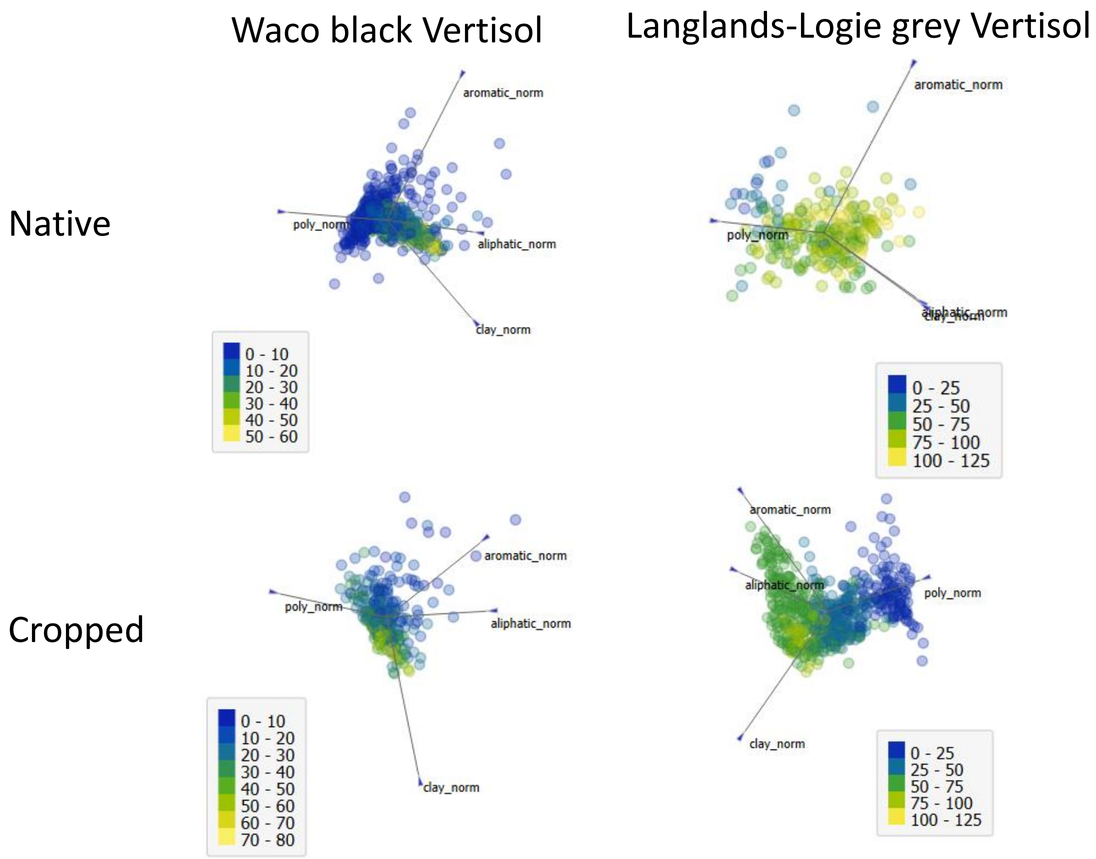
Supplementary Figure S1. The organic carbon content (a) and the percentage of organic N (b) for each soil fraction within the bulk soil from topsoils (0-10 cm) collected from Waco and Langlands-Logie from two land uses. fPOC is free particulate organic C, oPOC is aggregate-occluded particulate organic 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 for Waco and Langlands-Logie was over 91 %.



Supplementary Figure S2. Synchrotron-based NEXAFS analyses of the Waco black Vertisol and Langlands-Logie grey Vertisol under the native vegetation and cropping. NEXAFS N K-edge spectra of bulk soil for the Waco soil (a) and the Langlands-Logie soil (b) (n=3, CV% < 3%), featuring: (1) amide (protein) at 401.2 eV and (2) alkyl-N at 406 eV.



Supplementary Figure S3. Distribution of clay mineral for the Waco black Vertisol and Langlands-Logie grey Vertisol converted from native grassland to cropping. Spectral maps showing the distribution of clay-OH (3650 cm^{-1}) as in false-colour heatmaps and 2^{nd} derivative of polysaccharide-C. The optical micrographs of the semi-thin sections are in the background for the clay channel.



Supplementary Figure S4. Principal component analysis of the distribution of C forms associated with clay mineral for the Waco black Vertisol and Langlands-Logie grey Vertisol under the native vegetation and cropping. To account for different optical intensity in various parts of the image (based on heterogeneous distribution of organo-mineral compounds and potentially varying slice thickness), the optical proportions of the different classes were normalised.