Spectroscopic assessment of three ecologically distinct organic carbon fractions of mineral soils

Lewis Walden[1], Farid Sepanta[1], and Raphael Viscarra Rossel[1]

[1]Soil and Landscape Science, School of Molecular and Life Sciences, Faculty of Science and Engineering, Curtin University, G.P.O. Box U1987, Perth, WA 6845, Australia

Correspondence: R.A. Viscarra Rossel (r.viscarra-rossel@curtin.edu.au)

Abstract.

Soil carbon (C) is heterogeneous and exists in various forms along a decomposition continuum from labile fast-cycling compounds to more persistent forms of C, which can reside in the soil for centuries to millennia. Methods for soil organic C fractionation aim to account for this complexity by separating soil organic C into distinct groups that exhibit similar turnover. Our aims were to (a) fractionate three mineral soils with small C concentrations (<2.5% C), different textures and mineralogy using a granulometric method to derive the particulate organic C in macroaggregates (POC_{mac}), the particulate organic C in microaggregates (POC_{mic}), and the mineral-associated organic carbon (MAOC), (b) test if mid-infrared (MIR) spectra can discriminate the distinct organic C fractions and characterise the critical organic and mineral functional groups, and c) explore the interactions between the dominant mineral and organic functional groups to elucidate C stabilisation. With a multivariate analyses we found that the MIR spectra use information from mineral and organic frequencies to discriminate the organic C fractions. Closer investigation on specific regions of the MIR spectrum showed that absorptions relating to silicates were more pronounced in the POC_{mac} and POC_{mic} fractions and clay mineral absorptions were stronger in the MAOC fraction. There was little organic C in the POC_{mic} and POC_{mac} fractions, respectively, and their spectra showed mostly mineralogical features. Most of the organic C in the soils was present as MAOC. The stretching vibration of the bonds in the alkyl CH_2 molecule was most prominent. However, absorptions from C=C and C=O stretching vibrations, due to alkenes and amides were also present. These molecules are known to form MAOC. We found that the wavenumbers associated with the absorption of alkyl CH_2 were positively correlated with the absorption of clay minerals, which may be used to infer the mineral association of organic C. Our results show that MIR spectroscopy can characterise the compositional differences between the C fractions and that the spectra could potentially infer C stabilisation in mineral soils.

1 Introduction

Soil organic matter and its composition are key to the accumulation and storage of soil carbon (C). Soil C is the largest component of soil organic matter which also includes other elements vital for soil health and productivity (nitrogen, phosphorus, and sulphur). The C stored in soil, its maintenance and potential to sequester atmospheric C are critical components to buffer carbon dioxide (CO_2) emissions and mitigate the effects of climate change. Soil C is heterogeneous and exist as a continuum

25 of decomposability from labile fast cycling compounds with a turnover of decades, to pyrogenic and recalcitrant C, that potentially resides in the soil for centuries (Lützow et al., 2006; Lehmann and Kleber, 2015; Cotrufo et al., 2019). Particulate organic carbon (POC) is more easily degradable and accessible to soil microorganisms reducing its permanence in the soil (Dungait et al., 2012). While the more stable C adsorbed on the mineral surfaces is biologically stabilised, leading to longer residence times in the soil (Poeplau et al., 2018; Six et al., 2002; Dungait et al., 2012). The interaction between stable and

30 labile C fractions, microbes, and soil mineralogy is the basis for our understanding of C dynamics.

Fractionation is a method used to separate C into more homogeneous fractions with a similar formation, chemical composition, and turnover in the soil (von Lützow et al., 2007). Methods of fractionation vary depending on the technique used to isolate the organic C fractions. These methods include chemical, thermal, and physical fractionation. Chemical fractionation methods use extractions, hydrolysis and oxidations to characterise the organic C compounds. The methods are a measure of

35 chemical recalcitrance that mimic enzymatic decay, whereby the oxidation resistant fraction contains C compounds which may be linked to a greater stability and reduced loss through mineralisation (Islam et al., 2022; Lützow et al., 2006). Thermal fractionation uses combustion to determine the C fractions. This method slowly heats a soil and the energy required to combust organic C is quantified, which then relates to the energy required for enzymatic decay (Sanderman and Grandy, 2020). Physical fractionation involves density or granulometric separations (von Lützow et al., 2007). This latter approach highlights

40 the importance of interactions between organic matter and soil mineralogy and our understanding that soil C protection occurs through organomineral associations and aggregation (Poeplau et al., 2018).

Granulometric, or particle size fractionation separates soil into size fractions to gain an understanding of the turnover of the C in that soil (von Lützow et al., 2007). The separation reflects the accessibility of C in each fraction to decomposer microorganisms (Poeplau et al., 2018). The smaller fraction, characterised by silt and clay particles ($<50~\mu$m) are purported

45 to contain the stable mineral-associated organic carbon (MAOC), with C adsorbed onto clay mineral surfaces that make them relatively inaccessible for decomposition. The coarser fraction, representing coarse and fine sand, holds the particulate organic carbon (POC), which is thought to be more labile and with C that is more easily accessible to microbes. Aggregates reduce the accessibility of soil C to microbes for decomposition and therefore play a key role in the stabilisation and protection of C (Killham et al., 1993). Macroaggregates (POC_{mac}, $<250~\mu$m) provide the least physical protection of C (Beare et al., 1994).

50 Organic C in microaggregates (POC_{mic}, $250~\mu$m and $50~\mu$m) are a more stable store of POC (Balesdent et al., 2000; Six et al., 2002). Granulometric fractionation is used to determine the concentration of organic C present in each of the different fractions, which, when combined, equates to the total organic C content of a soil.

To improve our understanding of soil C stabilisation and dynamics, we need to characterise the chemical and mineralogical composition of the soil and its fractions. Solid-state ^{13}C Nuclear Magnetic Resonance (NMR) is commonly used to determine

55 the chemical composition of soil C (Baldock et al., 1997; Kögel-Knabner, 2000; Weng et al., 2022). However in soils with low C content and the presence of paramagnetic materials, such as iron, measurements can be slow (Mathers et al., 2002), limiting the number of samples that can be processed (Kinchesh et al., 1995; Baldock et al., 1989). Therefore, for mineral soils of Australia that are abundant with iron oxides (Viscarra Rossel et al., 2010; Hu et al., 2020), and when many samples need to be measured, NMR is impractical.

60   The mid-infrared (MIR) region of the electromagnetic spectrum, between 4000–400 cm^{-1}, contains information from funda-
mental vibrations and overtones of organic and inorganic functional groups in soil (Haberhauer and Gerzabek, 1999; Nguyen
et al., 1991). Thus, the MIR region is rich in information on the chemical and mineralogical composition of soil (Made-
jová et al., 2017). MIR spectroscopy is a rapid, cost-effective, and reproducible analytical method to characterise soils non-
destructively (McCarty et al., 2002). When combined with multivariate calibrations or machine learning, the technique can
65   accurately estimate the organic C content of soils (e.g. Arachchi et al., 2016; Gomez et al., 2020), and the organic C frac-
tions (Janik et al., 2007; Baldock et al., 2018). Wetterlind et al. (2022) used 2-dimensional correlations and support vector
machines to relate NMR-derived C functional groups to absorptions in the MIR spectrum. They found that key NMR organic
functional groups used to determine C stabilisation were strongly correlated with MIR features assigned to organic molecules.
This suggests that MIR spectroscopy could be used to determine the organic C composition of soil.

70   Here, we aimed to; a) Perform a granulometric fraction of typical Australian mineral soils with little organic carbon content to
derive the POC$_{mac}$, POC$_{mic}$ and MAOC fractions, b) test whether MIR spectra can discriminate the C fractions and characterise
the critical, distinct mineral and organic functional groups, and c) explore the relationship between the dominant mineral and
organic functional groups to elucidate C stabilisation.

2   Methods

2.1   Soil samples

We selected three soils with varying texture and mineralogy, a sandy soil, a sandy clay loam (SCL), and a sandy loam (SL).
The soil samples were oven dried at $60°C$, crushed and passed through a 2 mm sieve and analysed to determine their physico-
chemical properties (Table 1).

Table 1. Locations and physicochemical properties for the three soils used in this study.

Soil	Latitude	Longitude	TOC (%)	TN (%)	pH	Clay content (%)
Sand	-31.7491	116.6782	0.47	0.001	5.9	3.9
Sandy clay loam	-30.4065	117.1727	0.68	0.045	6.1	27.5
Sand loam	-34.3412	139.5082	2.07	0.127	7.3	18

2.2   Fractionation and organic C analysis

80   To separate the organic C fractions with different turnover times, we performed a physical granulometric separation (Fig. 1).
We used 30 g of the $\leq$2 mm soil in 135 ml of deionised water and an ultrasonic probe (Sonics VCX 500 Sonicator, Newtown,
Connecticut) with an energy output of 500 J ml^{-1} to disperse the samples (See Supplementary Information).

After dispersion, soils were fractionated using an automated wet sieving apparatus (Analysette 3 Pro, Fritsch GmbH, Idar-Oberstein, Germany) with two sieve sizes; 250 μm and 50 μm (Fig. 1). Thus the $\leq$2 mm soil samples separated into three distinct particle size fractions; 250–2000 μm, 50–250 μm, and <50 μm (Fig. 1).

To recover the <50 μm fraction from the water we flocculated the samples with 1 gL^{-1} gypsum (CaSO$_4$ · 2H$_2$O, solubility: 2 g L^{-1} at 20°C) to increase recovery and improve the timeliness of the procedure. The three fractions were oven dried overnight at 60°C before fine grinding to $\approx$<80 μ for the spectroscopy (Le Guillou et al., 2015) (described below), and soil C analyses (Fig. 1). We replicated the fractionation procedure three times for each soil.

The organic C content of the samples from each size fraction were determined using an elemental analyser (SoliTOC Cube, Elementar Analysensysteme, Hanau, Germany). The SL soil contained carbonates, making it critical to separate the organic C from the inorganic C. The elemental analyser uses a two step heating process to heat and hold the samples at two temperatures (400, 900°C) to determine the inorganic and organic C content of the sample. Samples are heated to 400°C at 70°C min^{-1} and held at that temperature for 230 seconds to determine the organic C content. Samples are then heated to 900°C in nitrogen pyrolysis to combust carbonate minerals and determine the inorganic C content of the sample. This resulted in the distinct C fractions, the particulate organic C in macroaggregates (POC$_{mac}$; 250 μm and 50 μm), the particulate organic C in microaggregates (POC$_{mic}$; 250–2000 μm) and the mineral-associated organic carbon (MAOC; <50 μm) (Fig. 1). To determine the relative C content of each fraction in the whole soil, C values were multiplied by the proportion weight of the fraction in the whole soil.
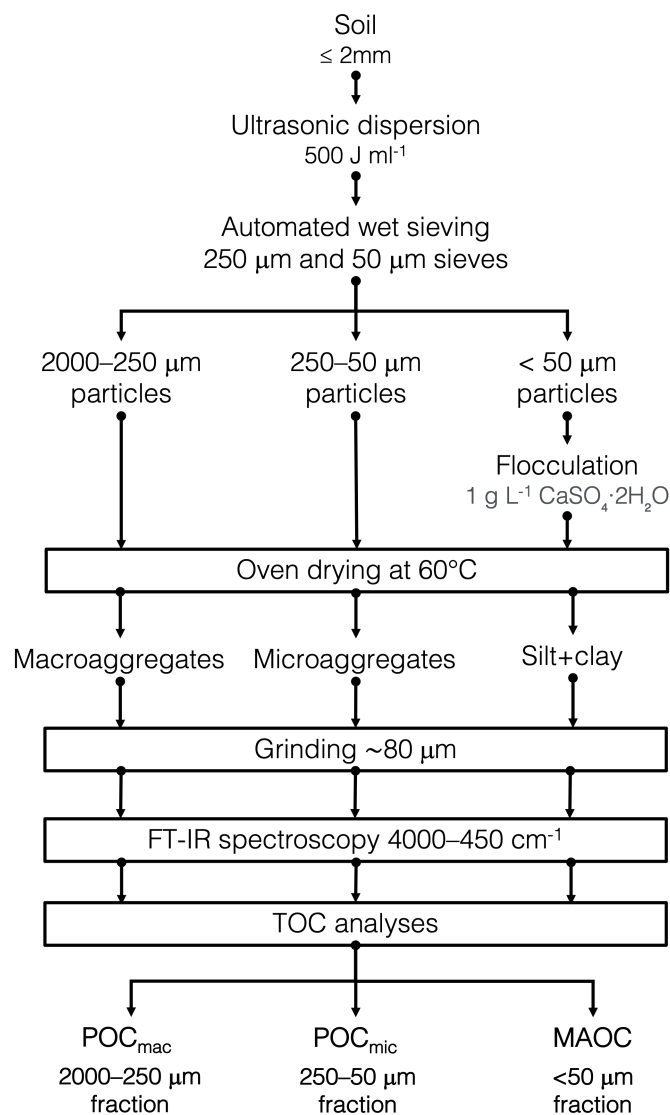
Figure 1. The soil fractionation process to isolate soil carbon fractions and the analytical methods used.

2.3 Mid infrared spectroscopy

We recorded the MIR spectra of the finely ground samples of the ≤ 2 mm whole soil samples and of the the the POC_{mac}, POC_{mic} and MAOC fractions using a diffuse reflectance infrared Fourier transform (DRIFT) spectrometer (Bruker-Invenio HTS-XT, Massachusetts, United States). The finely ground samples were loaded into a 24-well sample plates and spectra were recorded from 4000–450 cm^{-1} with a spectral resolution of 4 cm^{-1} and measuring 64 scans per sample. The spectrometer was calibrated before each sample plate with a gold standard (Bruker, Massachusetts, United States). We recorded four spectral replicates per

sample. The replicates were averaged to produce one spectrum per sample. Reflectance spectra were transformed to log 1/R (or apparent absorbance), and were offset corrected prior to analyses.

2.3.1 Canonical variate analyses

To determine whether the spectra could distinguish the POC_{mac}, POC_{mic} and MAOC fractions and identify the MIR frequencies that effectively discriminate between them, we used a canonical variate analysis (CVA)(Campbell, 1984). Before the CVA we baselined the spectra with a quartic polynomial (Liland et al., 2010), normalised with the standard normal variate (Barnes et al., 1989) to reduce multiplicative effects, filtered the spectra to remove random noise using the Savitzky-Golay filter with a window size of seven and a quadratic polynomial (Zimmermann and Kohler, 2013), and mean centred the spectra. We performed the CVA as follows. Our data formed a matrix with 27 observations and 426 wavenumbers. The variance-covariance matrix of the data is partitioned into a pooled within-classes variance–covariance matrix, which represents the variability within each fraction, and a between-classes variance–covariance matrix, which captures the differences between the fractions. By partitioning the variance-covariance matrix into within- and between-class components, CVA allows for the identification of linear combinations of variables (canonical variates) that maximize the separation between the groups. Here we have three soil fractions, giving two eigenvalues and eigenvectors. We multiplied the eigenvectors with the mean centred spectra to derive the canonical scores. We plotted the first two CVA scores to assess the discrimination of the C fractions by the spectra. To identify the wavenumbers that contribute most to the discrimination we calculated the variance attributed to the differences between classes (Ertlen et al., 2010). The total variance for each wavelength can be partitioned into two components, the pooled within-class variance and the variance between classes. When standardising the total variance to 1, the between-class variance is the proportion of the total variance resulting from the difference between classes (Viscarra Rossel and Webster, 2011). Thus, to identify the wavenumbers that contribute the most to the discrimination of the C fractions, we plotted this between-class variance against wavenumber.

2.3.2 Spectroscopic analyses and investigation of spectral regions

Following the CVA, we investigated specific regions of the spectra to determine whether there were unique features in each of the C fractions. We isolated six separate regions of the spectra (Table 2) that contain frequencies with absorptions that represent mineralogical and organic functional groups. We selected the regions 3730–3650 cm^{-1}, 3000–2820 cm^{-1}, 1925–1825 cm^{-1}, 1660–1550 cm^{-1}, 1545–1510 cm^{-1}, and 1070–1020 cm^{-1} which correspond to the specific absorptions of mineral and organic functional groups (Table 2). Each of the regions was isolated from the whole spectra of each C fraction and then baselined with a linear polynomial to isolate the specific absorption.

Table 2. Features used to investigate the mineral and organic composition of the POC_{mac}, POC_{mic} and MAOC fractions, their spectral range, absorption, and possible assignments.

Feature	Spectral range (cm^{-1})	Absorption (cm^{-1})	Assignment	Reference
1	3730–3650	3696	Clay minerals	Nguyen et al. (1991)
2	3000–2820	2930, 2860	Alkyl CH_2	Haberhauer and Gerzabek (1999)
3	1925–1825	1875	Quartz minerals	Nguyen et al. (1991)
4	1660–1550	1630, 1610	Amide I, Aromatic, phenyl	Niemeyer et al. (1992); Verchot et al. (2011)
5	1545–1510	1525	Amide II, Aromatic rings	Movasaghi et al. (2008)
6	1070–1020	1045	Carbohydrate	Pietikäinen et al. (2000)

2.3.3 Mineral association of organic compounds

135 To determine if there was any potential mineral association of organic compounds, we tested the relationship between clay minerals smectite and kaolinite, and alkyl CH_2 absorptions of the C fractions. To gain a better understanding of the features of the C fraction in relation to the whole soil, we multiplied the absorption values at each wavenumber by the proportion of each fraction in the whole soil. We then isolated regions assigned to clay minerals kaolinite (3696 cm^{-1}) and smectite (3622 cm^{-1}), and the asymmetric stretching of C–H bonds vibrations (2930 cm^{-1}) in alkyl CH_2 molecules. We subsetted the whole MIR
140 spectra to include the peak, upper, and lower limits of each feature. The subset spectra were then baseline corrected with a linear polynomial and peak height was calculated using the R library PAVO (Maia et al., 2013). We fitted a linear model to the peak height data, and report the coefficients, R^2, and F-test results. Linear models separately for each clay mineral.

3 Results

The organic C concentration of the soils was <2% (Table 3). The SL soil had more organic C than the sandy soil. The MAOC
145 fraction consistently contained more organic C than the POC_{mic} and POC_{mac} fractions, respectively (Table 3). The fractionation procedure performed well with an average recovery of > 98 % C for all three soils tested (Table 3). The greatest recovery was in the sandy soil sample followed by SL and SCL soils, respectively.

Table 3. Organic carbon content (%) of the soils and the POC_{mac}, POC_{mic} and MAOC fractions.

	Organic C (%)			
	POC_{mac}	POC_{mic}	MAOC	TOC
Sand	0.04	0.02	0.04	0.1
Sand clay loam	0.02	0.06	0.59	0.68
Sand loam	0.31	0.5	1.29	2.07

The sandy soil spectra show strong absorption at around 1990–1790 cm^{-1} (Fig 2a), which are characteristic of Si–O bonds in quartz Hofmeister and Bowey (2006). The SCL soil had very strong absorption at 3696–3500 cm^{-1} (Fig 2b) where the
150 bending vibration of O–H hydroxyl occurs (Nguyen et al., 1991). The shape and intensity of these absorptions are characteristic of kaolinite (Madejová et al., 2017; Nguyen et al., 1991).The SL soil contained carbonates (2500 cm^{-1},Nguyen et al. (1991)), and was smectitic with characteristic absorptions in the 3700–3500 cm^{-1} (Fig 2c) region with the shape and intensity of smectite (Madejová et al., 2017). The SL also had the most prominent organic absorptions at 3000–2820 cm^{-1} which are characteristic of the asymmetric and symmetric stretching of C–H vibrations in alkyl CH_2 molecules. These same absorptions
155 were less prevalent in the SCL soil (Fig 2b), and not visible in the whole soils spectra of the sand soil (Fig 2a).
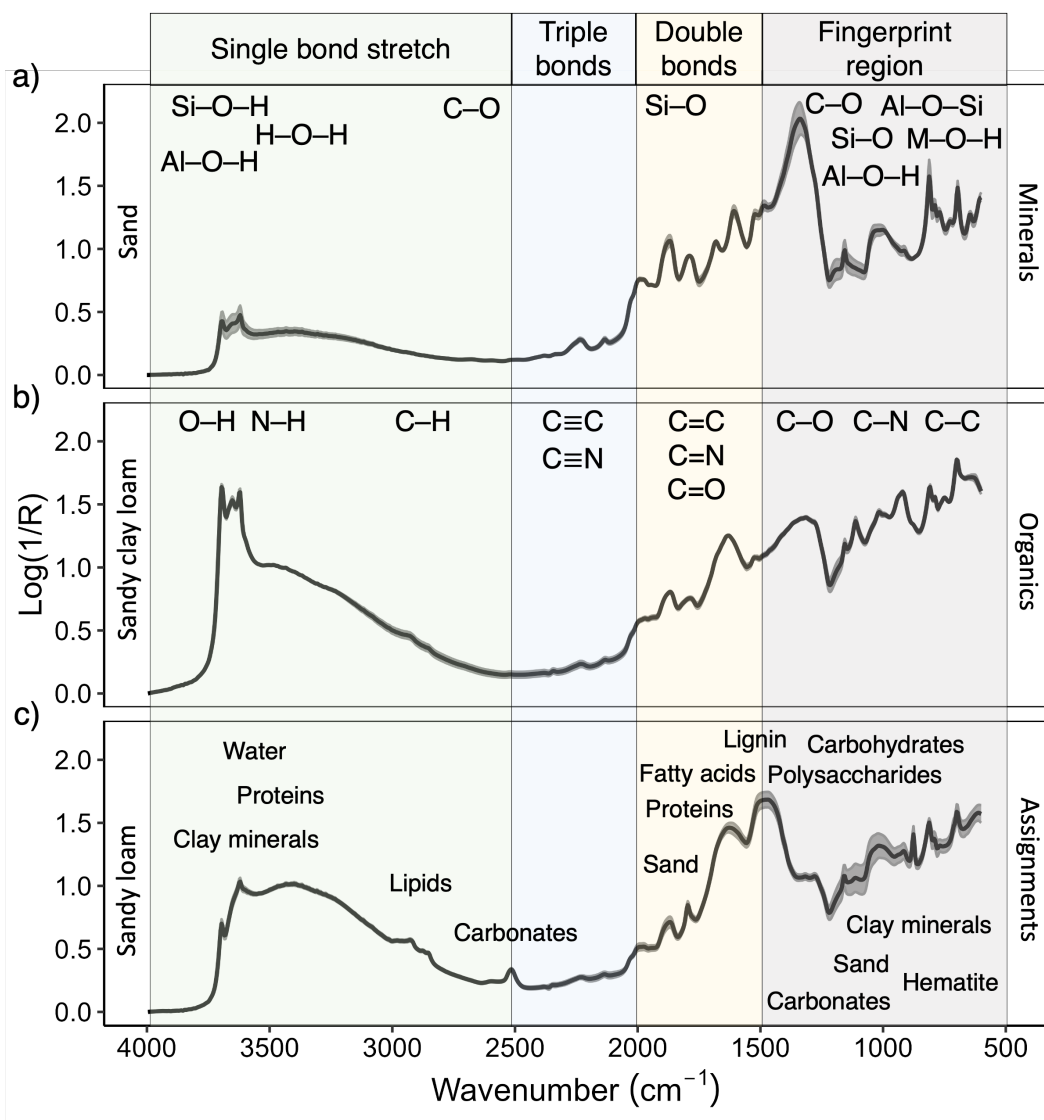
Figure 2. Mean whole soil spectra of the three soils used in this study with key regions of the MIR spectrum highlighted throughout, a) Sand whole soil spectra with key mineral abosorptions found in the MIR spectrum present across the single, double, triple bond and fingerprint region, b) Sand clay loam whole soil spectra with key organic abosorptions found in the MIR spectrum present across the single, double, triple bond and fingerprint region, and b) Sandy loam whole soil spectra with the functional assignments of mineralogical and organic compounds present in the MIR spectrum. Grey shaded areas around the spectra indicate the 95% confidence interval of the mean spectra plotted as the black solid line.

3.1 Multivariate analysis

There was no overlap of the organic C fractions on the canonical plane; the three fractions were clearly distinguished (Fig. 3a). The spectra contain the mineral and organic information to discriminate the C fractions of the soil regardless of the type of soil. The first canonical variate (CV) was most useful for discriminating between the fractions of POC_{mac}, POC_{mic}, and MAOC

160 (Fig. 3a). Calculating the between-group variance showed the contribution of individual wavenumbers to the discrimination of the C fractions.

The wavenumbers most useful in the discrimination of the soil C fractions were those related to kaolinite (3696 cm^{-1}, 930 cm^{-1}), and quartz (1990 cm^{-1}, 1875 cm^{-1}, 1790 cm^{-1}, 815 cm^{-1}). Other key wavenumbers included those related to amines (1610 cm^{-1}), alkyl CH$_2$ groups (2930 cm^{-1}, 2860 cm^{-1}) (Figure 3b, Table 4). Absorptions at 3522 cm^{-1} and

165 3400 cm^{-1} were due to the addition of gypsum to flocculate the MAOC (Fig 3b).
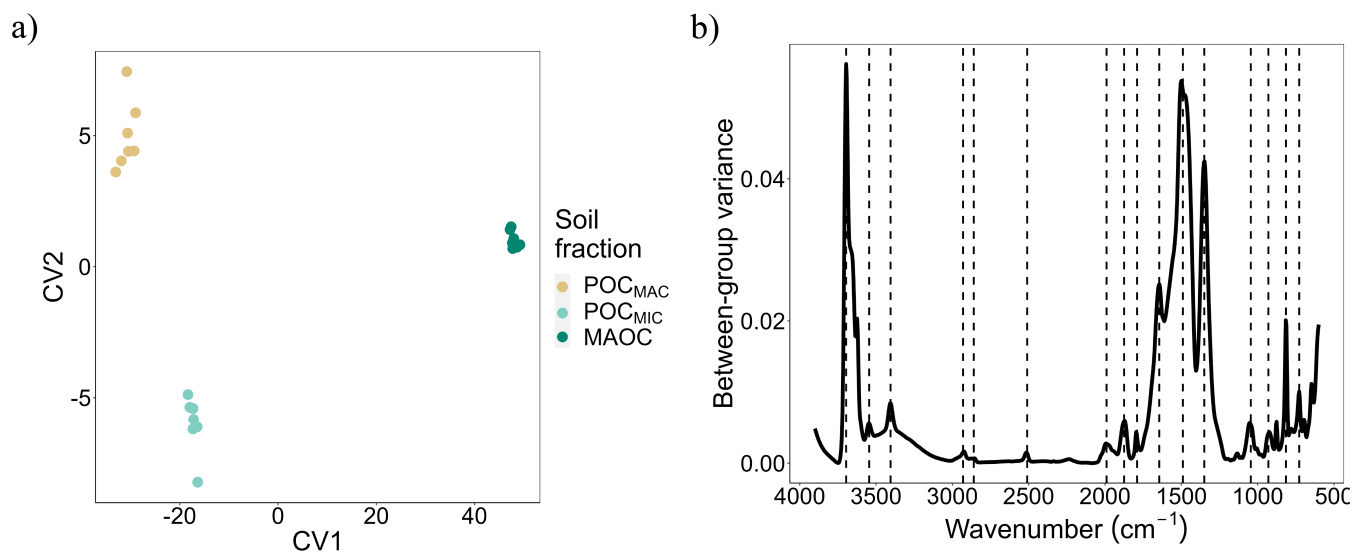
Figure 3. Canonical variate analysis of soil C fractions, a) scatter plot of canonical variate 2 plotted against canonical variate 1 coloured by soil fraction, b) between-classes variance as a proportion of the total variance plotted against wavenumber for the soil C fractions, with important wavenumbers used in their discrimination as dashed vertical lines.

Table 4. Discriminating wavenumbers (cm^{-1}) used separate soil C fractions, their vibrational modes, and possible assignments

Wavenumber (cm^{-1})	Vibrational mode	Assignment	Reference
3696	O–H stretching	Clay minerals	Nguyen et al. (1991)
3520, 3400	O–H stretching	Gypsum	Palacio et al. (2014)
2930	asymmetric stretching C–H	Alkyl CH$_2$	Haberhauer and Gerzabek (1999)
2860	symmetric stretching C–H	Alkyl CH$_2$	Haberhauer and Gerzabek (1999)
2500	Overtone band	Carbonate	Tatzber et al. (2007)
1990	Si–O vibration	Quartz minerals	Nguyen et al. (1991)
1875	Si–O vibration	Quartz minerals	Nguyen et al. (1991)
1790	Si–O vibration	Quartz minerals	Nguyen et al. (1991)
1645	C=O stretching,	Amide I	Movasaghi et al. (2008)
1490	C–N bending, CH phenyl rings bending	Amide II	Haberhauer and Gerzabek (1999)
1370	Si–O overtone	Quartz	Volkov et al. (2021); Hofmeister and Bowey (2006)
1045	C–O bending	Carbohydrate	Movasaghi et al. (2008); Pietikäinen et al. (2000)
930	Al–OH deformation	Clay minerals	Nguyen et al. (1991)
815	Si–O–Si symmetric stretching	Quartz minerals	Koike et al. (2013)
728	CO$_3$ asymmetric bending	Calcite	Nguyen et al. (1991)

3.2 Spectral characterisation of organic and mineral components of the C fractions

The C fractions of the soils we tested showed unique and shared features. For example, the stretching of O–H bonds vibrations in kaolinite and smectite (3696 cm^{-1}, 930 cm^{-1}) were more intense in the MAOC fraction of all three soils (Fig. 4–6, panel 1). However, these absorptions still exist in the POC$_{mac}$ and POC$_{mic}$ fraction, which suggests that there may be clay particles

170 present in the macro and microaggregates. Absorptions characteristic of Si–O bonds in quartz (1990 cm^{-1}, 1875 cm^{-1}, 1790 cm^{-1}) were more pronounced in the spectra of the (POC$_{mac}$, POC$_{mic}$) fractions and were not present in the MAOC spectra (Fig. 4, 5). As we use a granulometric fractionation, the absorptions of coarser minerals (i.e. quartz) are likely to be greater in the POC$_{mac}$ fraction.

For the sand and SCL soils there were no absorptions from the stretching C–H vibrations of the alkyl CH$_2$ molecule in the

175 POC$_{mac}$ and only small absorptions in the POC$_{mic}$. However, these absorptions were strongly present in the MAOC fraction. In the POC$_{mac}$, POC$_{mic}$ there were frequencies relating to C=O bond in amide I (Fig. 4, 5, panel 4), C–N vibrations in Amide II (Fig. 4, 5, panel 5), and C–O bonds in carbohydrates (Fig. 4, 5, panel 6) present. However, given the very low C content of the POC$_{mac}$ and POC$_{mic}$ fractions of the sand and SCL soils, these absoprtions are more likely to be due to Si–O combination bands (Tan, 2003)(Fig 4–6, panel 4–6).

180 The POC$_{mic}$ fraction of all three soils had smaller absorptions for frequencies relating to Si–O vibrations in quartz and greater absorptions for those assigned to O–H bonds clay minerals compared to the POC$_{mac}$. The POC$_{mic}$ fraction may contain microaggregates with some clay minerals (Fig 4–6, panel 1). This C fraction also had more C (Table 3) and a stronger alkyl CH$_2$ absorption than the POC$_{mac}$ fraction in all three soils.

The MAOC fraction had the strongest absoprtions for the stretching of C–H bonds in alkyl CH$_2$ (Fig 4–6-2) in all three
185   soils. In the MAOC of the SL soil there were strong absorptions for the C=C bonds of alkenes and aromatic molecules (Fig 6,
panel 5), which was not present in the sandy and SCL soil. There were prominent absorptions for the C=O bonds of amine I
molecules of the MAOC in sandy soil, with a small absorption present in the SL and SCL soil (Fig 4–6, panel 4).
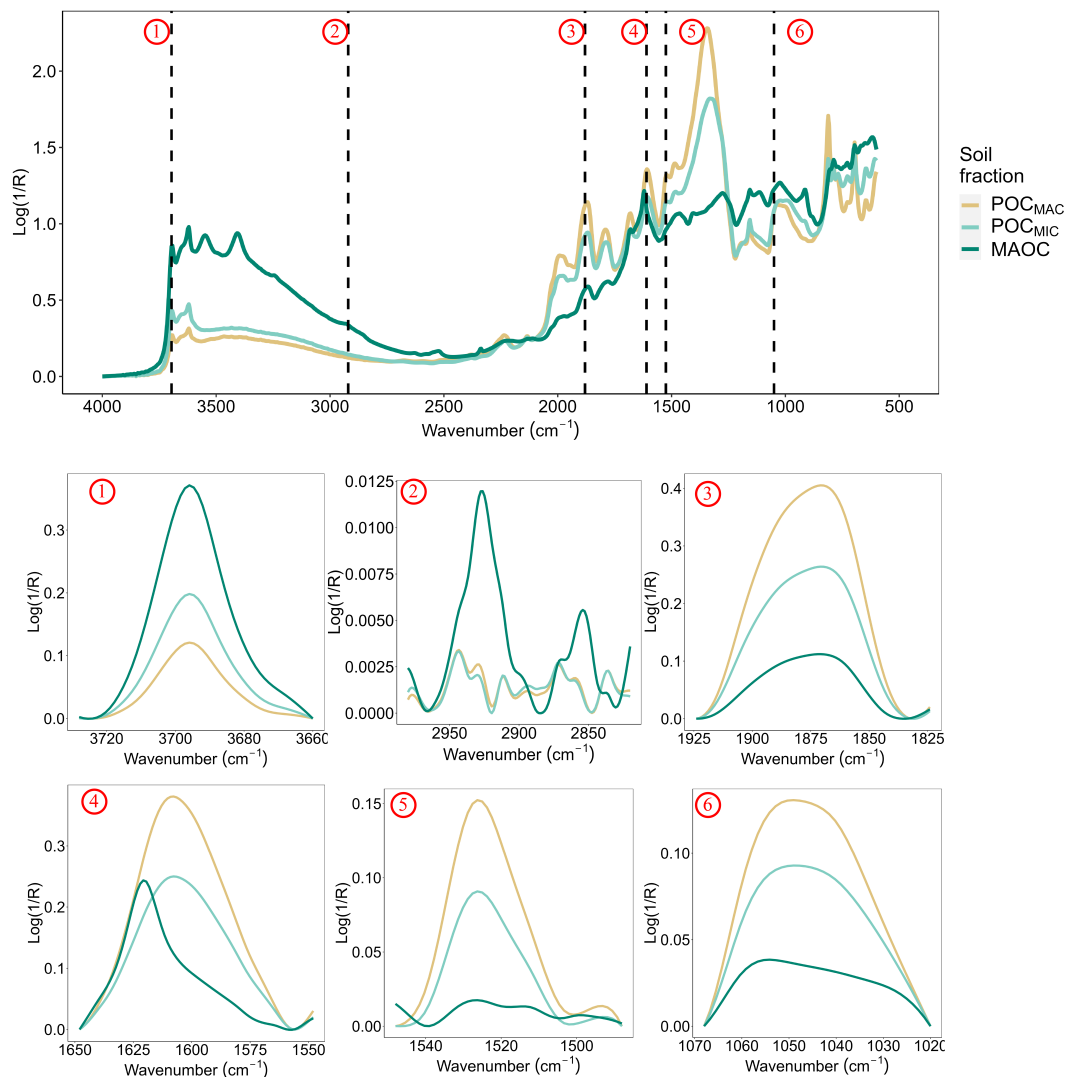
Figure 4. Whole MIR spectra of C fractions (POC$_{mac}$, POC$_{mic}$ and MOAC) for the sandy (S) soil, with key organic and mineralogical
wavenumbers highlighted with numbers 1–6. Representative sections of POC$_{mac}$, POC$_{mic}$ and MOAC spectra are then represented below
with the corresponding numbers. A linear baseline was applied to each section.
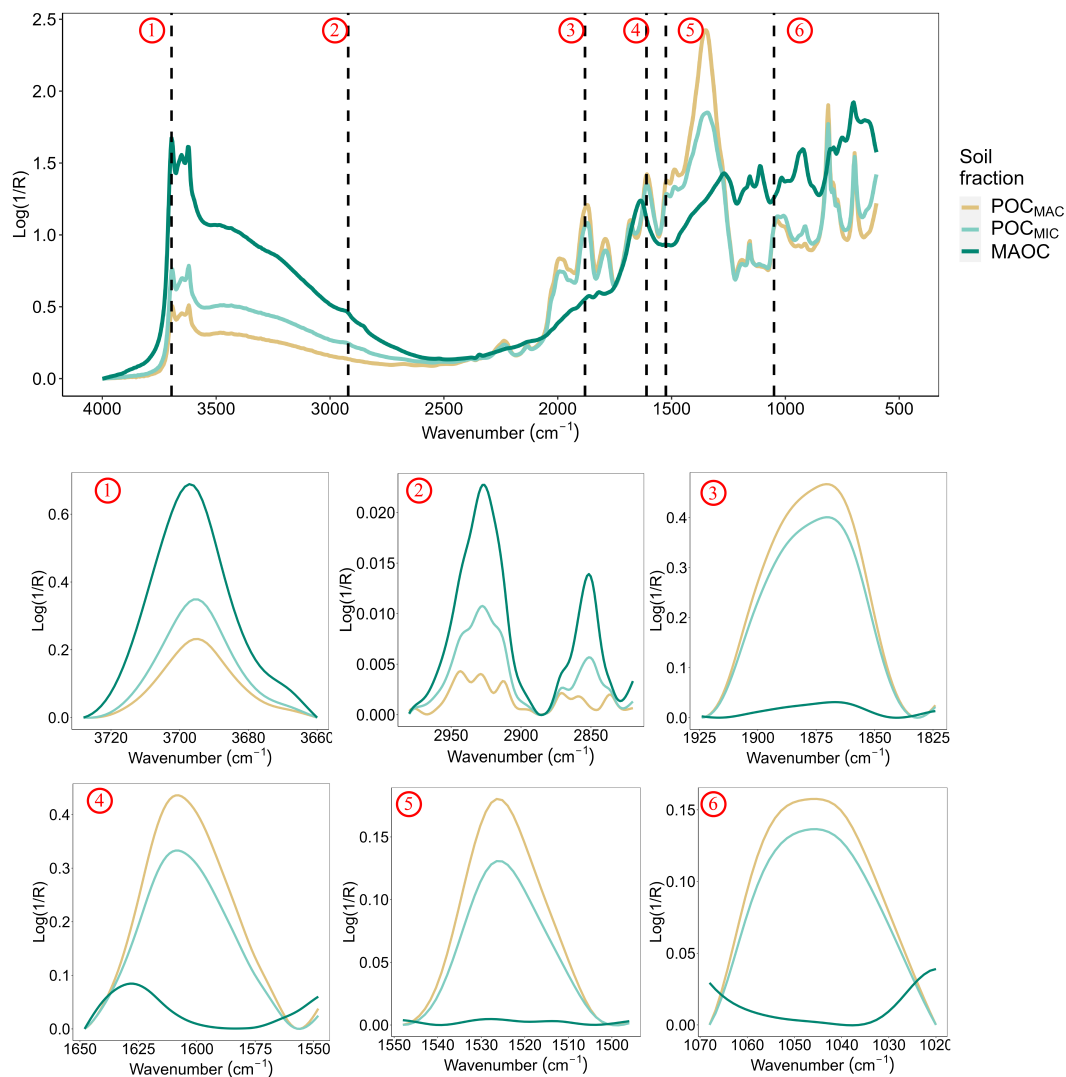
Figure 5. Whole MIR spectra of C fractions (POC_mac, POC_mic and MOAC) for the SCL soil, with key organic and mineralogical wavenumbers highlighted with numbers 1–6. Representative sections of POC_mac, POC_mic and MOAC spectra are then represented below with the corresponding numbers. A linear baseline was applied to each section.
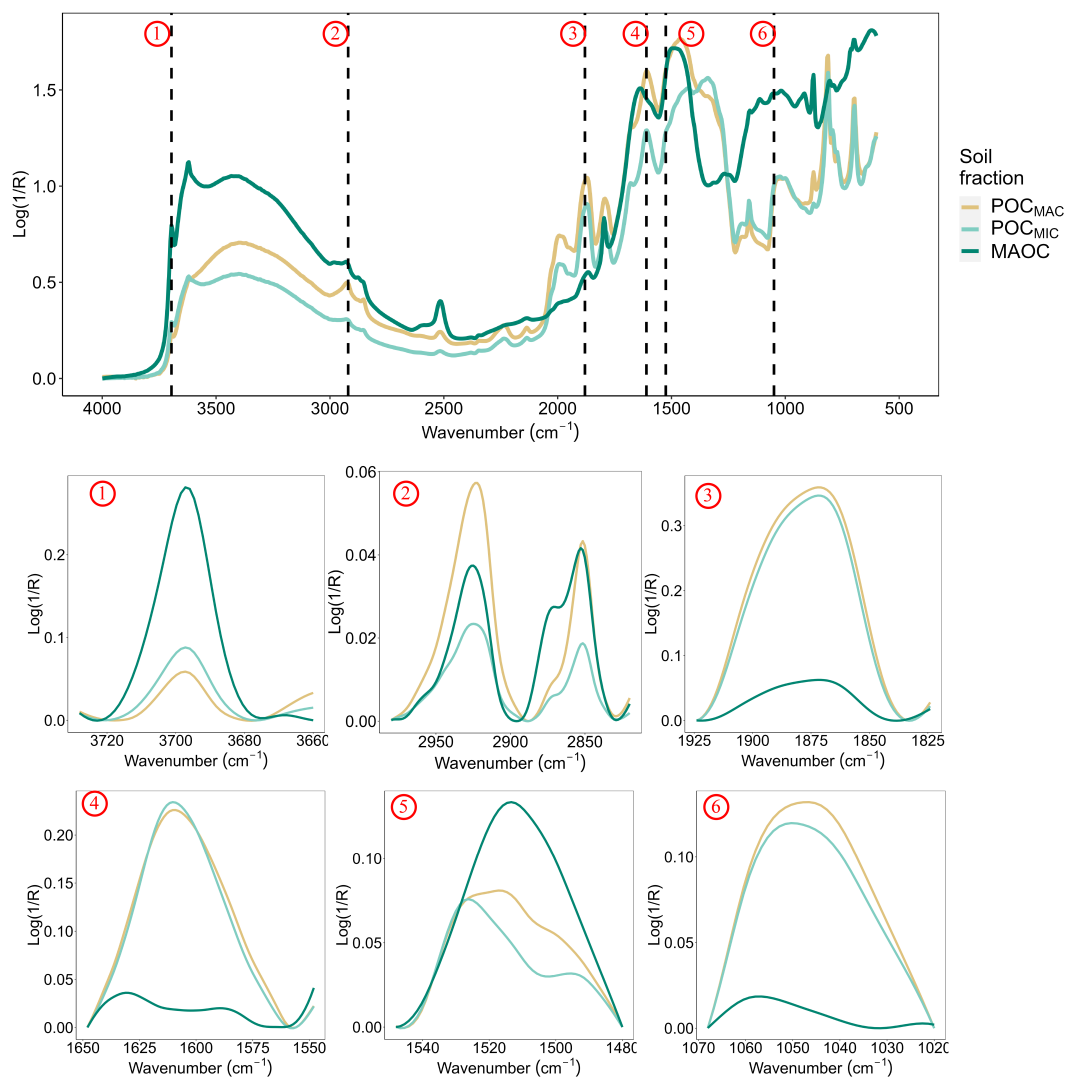
Figure 6. Whole MIR spectra of C fractions (POC$_{mac}$, POC$_{mic}$ and MOAC) for the SL soil, with key organic and mineralogical wavenumbers highlighted with numbers 1–6. Representative sections of POC$_{mac}$, POC$_{mic}$ and MOAC spectra are then represented below with the corresponding numbers.

3.3 Potential association of mineral and organic compounds with MIR

We have shown that the spectra of the three soils and their organic C fractions contain information on key mineral and organic functional groups. Therefore we quantified the interactions between organic and mineral absorptions and found a strong relationship between CH$_2$ and clay mineral absorptions (Fig. 7). In the sandy and SCL soils as the peak height increased for kaolinitic minerals (3696 cm^{-1}) the peak for CH$_2$ absorption also increased (Fig. 7a). Similarly, as peak height for the smectite absorption increased in the SL soil, so did the CH$_2$ peak (Fig 7b). There was a significant linear relationship between the peak

height of the OH stretching vibration related to clay minerals and the asymmetric stretching vibration of CH$_2$ for both the
kaolinitic (F(1,16)=519.4, p<0.001) and the smectitic soils (F(1,10) = 311.5, p<0.001).

a)
b)
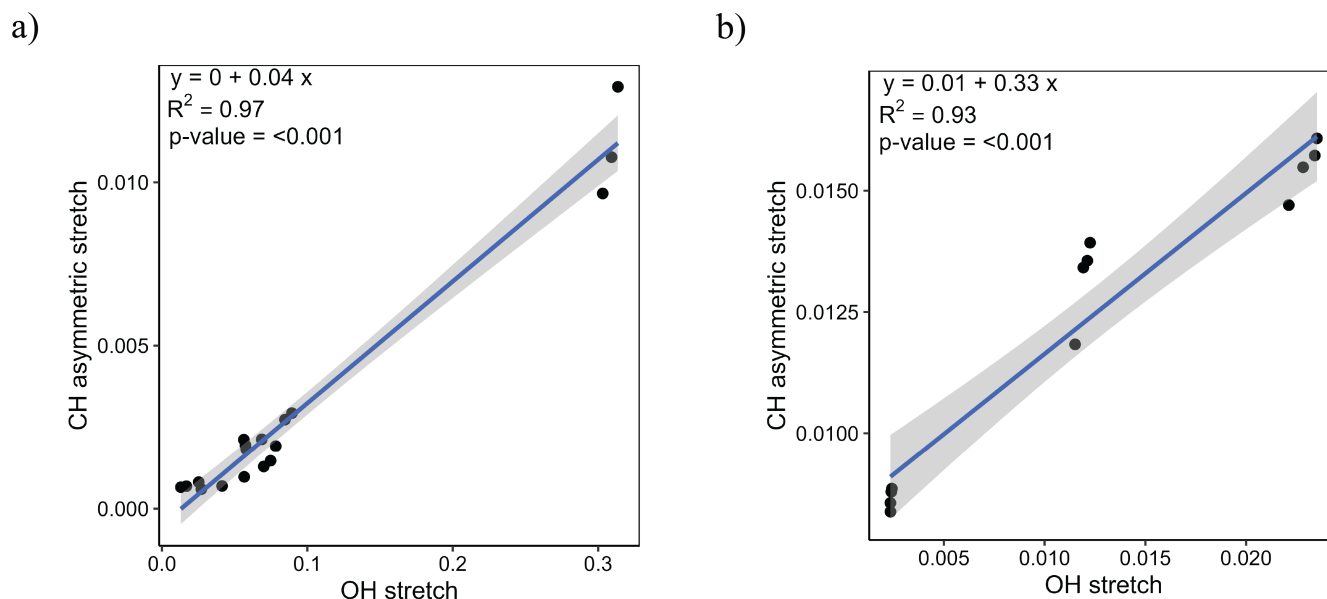
Figure 7. Peak height of mineral and organic absorptions for the C fractions of the three soils tested. a) Mineral peak assigned to Kaolinitic minerals (3696 cm^{-1}) plotted against the peak height for the stretching of alkyl CH$_2$, and b) Peak height of smectite (3622 cm^{-1}) plotted against the peak height for the stretching of alkyl CH$_2$. Grey shaded area indicates the 95% confidence interval of the linear regression model.

4 Discussion

We used granulometric fractionation with MIR spectroscopy to investigate the organic and mineral compositions of the POC$_{mac}$, POC$_{mic}$ and MAOC fractions in three different soil types. The organic C content in the three soils varied but was small. The analytical measurements showed that most of the organic C in the soils was present as MAOC. There was less organic C in the fractions of POC$_{mic}$ and POC$_{mac}$, respectively.

The MIR spectra could discriminate the different organic C fractions, mainly due to differences in the soil particle size, mineralogy and organic C compounds, which show strong responses in the MIR region. Due to the granulometric fractionation, the key discriminatory wavenumbers related to mineralogy. Absorptions of organic C functional groups played their part in the discrimination, particularly in the MAOC fraction; however, given the small C content of the soils, some of the frequencies assigned to organic C compounds in the POC$_{mac}$ and POC$_{mic}$ fractions showed overlapping mineralogical absorptions.

Absorptions relating to silicates (e.g. quartz) were more pronounced in the POC$_{mac}$ and POC$_{mic}$ fractions than in the MAOC spectra of the sandy soil. The quartz present in the MAOC fraction of the sandy soil might be due to the breakdown of the sand into finer particles during weathering and cultivation. As expected, the absorptions of clay minerals were stronger in the

MAOC fraction than in the POC$_{mic}$ and POC$_{mac}$ fractions, respectively. The smaller absorptions of clay minerals in the POC

210   fractions may suggest the presence of aggregates that did not break during dispersion (Poeplau et al., 2018).

The stretching vibrations of the bonds in the alkyl CH$_2$ (2930, 2860 cm^{-1}) molecule was the most prominent organic C absorption in our soils. CH$_2$ groups originate from different organic molecules, including lipids (fats and oils), cellulose, lignin, and other organic polymers present in plant tissues (Kögel-Knabner, 2002; Kögel-Knabner et al., 1992). As microorganisms break down organic matter, they release simpler molecules into the soil, including alkyl CH$_2$-containing fragments, which may

215   undergo further decomposition (Grandy and Neff, 2008). The findings of Calderón et al. (2011), who characterised the organic C fractions of agricultural soil of Minnesota, accord with our results as they, too, found prominent alkyl CH$_2$ and clay mineral absorptions in the MAOC fraction and stronger absorptions due to quartz in the POC fraction. However, others have attributed the presence of alkyl CH$_2$ primarily to the more labile POC fraction (Demyan et al., 2012; Ramírez et al., 2020) because these molecules are present in compounds that soil microbes can metabolise more readily.

220   The spectra of the MAOC fraction also had absorptions due to C=O (1630, 1610 cm^{-1}) stretching vibration, indicating the presence of amides from proteins in plant and microbial tissues (Kögel-Knabner, 2002). The presence of the amide molecules in the finer fraction suggests that they are mineral-associated, not readily available for decomposition (Demyan et al., 2012).

The spectra of the POC$_{mic}$ and POC$_{mac}$ fraction showed large absorptions for C=O (1630, 1610 cm^{-1}) stretching and C–O (1045 cm^{-1}) bending vibrations, which can indicate the presence of amides and carbohydrates (Verchot et al., 2011; Pietikäinen

225   et al., 2000). However, there are overlaps in these regions with absorptions of Si–O (Bock and Su, 1970; Spitzer and Kleinman, 1961), and Al–O vibrations (Nguyen et al., 1991). Given the very low C content of the POC$_{mic}$ and POC$_{mac}$ fractions, and the absence or small absorptions for the same assignments in the MAOC spectra, we suggest that the absorptions in these regions are largely due to the quartz and kaolinite present in C fraction. Although MIR is a useful tool to identify mineral and organic vibrations in soils, absorbances of organic molecules in the double bond and fingerprint regions can overlap with

230   mineral absorptions making functional assignments of organics molecules difficult, particularly for mineral soils.

The frequencies associated with the absorption of the alkyl CH$_2$ (2930, 2860 cm^{-1}) vibrations were positively correlated with the absorptions of kaolinite and smectite minerals. This may suggest possible associations and stabilisation of these molecules through adsorption onto clay mineral surfaces (Torn et al., 1997). The more clay and active sites for C to adsorb, the greater the potential to store and stabilise C (Six et al., 2002; Churchman et al., 2020; Schweizer et al., 2021; Vogel et al.,

235   2015). For example, Six et al. (2002) found that soils with 1:1 clay minerals (e.g., kaolinite) showed lower C sequestration than 2:1 clay minerals (e.g., smectite). It follows that we could used the spectra to infer soil C stability. For example, the spectra of the sandy soil sample with only small amounts of kaolinitic clay (<5%) have absorptions mostly due to quartz and small or no visible absorptions due to organic compounds. This soil, therefore, has low C stabilisation potential. The SCL soil sample is also kaolinitic, but it contains more clay. Its spectra show stronger CH$_2$ and kaolinitic absorptions; therefore, it will have

240   a greater potential to stabilise C than the sandy soil. The SL soil sample is smectitic, has 18% clay content and has the most prominent alkyl CH$_2$ absorptions compared to the other soil samples and therefore the greatest C stabilisation capacity.

5 Conclusions

In mineral soils with low C, MIR spectroscopy can discriminate between the C fractions and be used to characterise the mineral and organic composition of each C fraction. Differences between the soil C fractions was largely due to Si−O of quartz O−H bonds of kaolinite and smectite. The most obvious and prevalent organic absorption throughout the soils was the stretching of C−H bonds in the alkyl CH_2 molecule, which was most prevalent in the MAOC fraction. There were other organic absorptions present in the MAOC including amine, alkenes, however these were much less pronounced. The POC_{mic} and POC_{mac} showed absorptions for amide I and amide II, as well as carbohydrates, however, given very low C content of these fractions we suggest these are Si−O related absorptions. Determining the functionality of organic molecules in the double bond and fingerprint region of C fractions with <0.1% C is difficult as there can be overlap with mineral characteristics present in the soil. The MIR spectra were able to quantify the interactions between organic and mineral absorptions of the C fractions, which are key to the stabilisation of C via the adsorption of organic C molecules to clay minerals. We propose that MIR spectroscopy could help to infer C stabilisation in mineral soils. However, further research is needed to quantitatively assess the adsorption of C to minerals, its stability, and changes to the MIR spectra.

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Code availability. The code used for the analyses presented in this work is available from the corresponding author on reasonable request.

Data availability. he dataset used to replicate the methods and findings from this paper will be available from Zenodo on acceptance

Author contributions. RAVR conceived the research and with LW planned the experiments. LW performed the experiments with FS and with RVR the data analysis and interpretation. LW and RAVR wrote and edited the manuscript with input from FS.

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