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NATIONAL SOIL CARBON RESEARCH PROGRAMME: FIELD AND LABORATORY METHODOLOGIES

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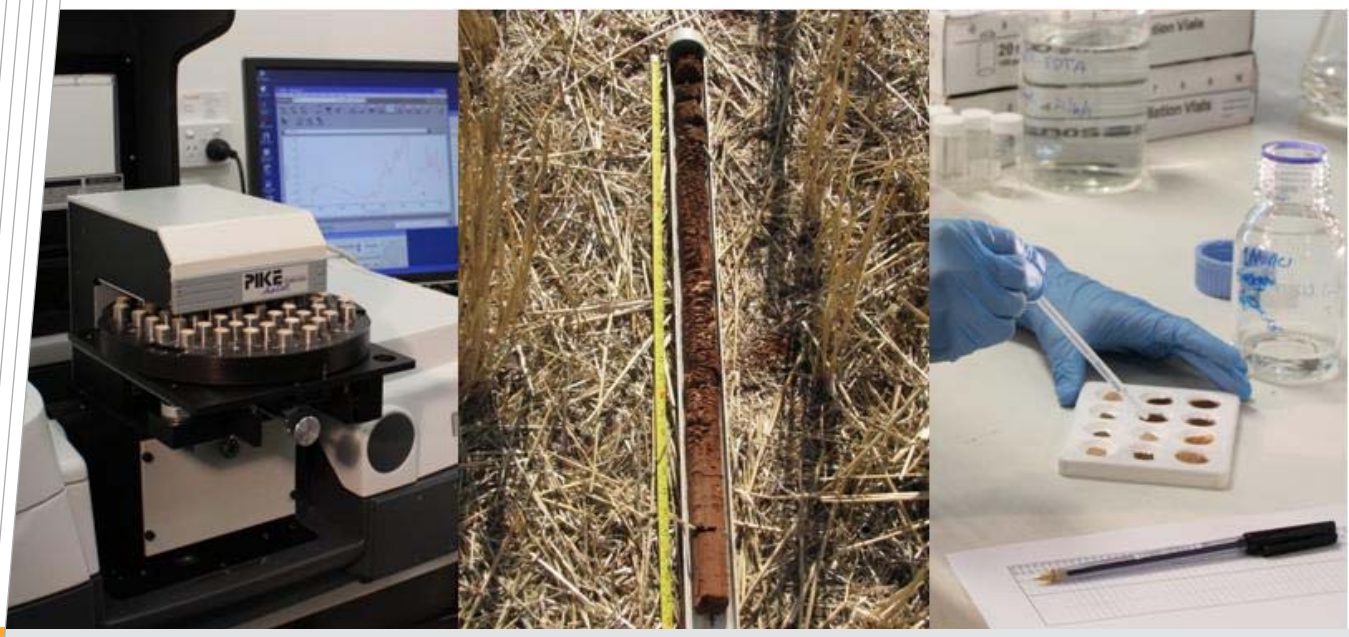

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Cover Photograph

Description: (left) Soil samples ready for MIR spectroscopic analysis (Photographer: R Nicoll © 2011 CSIRO). (middle) Soil core removed from wheat field near Williamstown, South Australia (Photographer: N Watkins © 2009 CSIRO). (right) Soil samples being tested for presence of carbonates (Photographer: R Nicoll © 2011 CSIRO).

Executive Summary

This report documents the methodologies currently used in the National Soil Carbon Research Programme (SCaRP) for sample collection and analysis. The programme has been designed to examine variations in soil organic carbon (SOC) content and composition in the 0 to 30 cm layer of soil due to agricultural management practices in numerous regions around Australia. For all samples, SOC content is measured directly and its distribution amongst three major SOC fractions (e.g. particulate-C, humus-C and char-C) is estimated by a combination of physical size fractionation and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy aided by mid-infrared red (MIR) spectroscopy combined with partial least squares regression analysis. Detailed management histories are collected at all sites along with other potential determinants of SOC content and composition such as climate, soil type and topography.

Contents

1. INTRODUCTION

The national Soil Carbon Research Programme (SCaRP) has been designed to examine variations in soil organic carbon (SOC) content and composition under different agricultural management practices in numerous regions around Australia. Specific research questions varied based upon the major agricultural practices and soil types in each region of the country. As such, the process by which sites were selected varied amongst regional project partners and these processes will be detailed in subsequent publications arising from the project.

In order to facilitate as much consistency as possible across the regional projects, the actual soil sampling and all laboratory analyses have been standardised. Soil sampling protocols and site information data collection are described in section 2. All samples are then analysed for total organic carbon (TOC) with calcareous samples being pre-treated with sulphurous acid first to remove inorganic carbon (section 4). Approximately 5-10% of all samples will be physically fractionated by size separation (section 5) followed by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy (section 6) to determine the amount of char in each of the two size fractions. All samples are also analysed by mid-infrared (MIR) spectroscopy (section 7). The MIR results will be used in conjunction with the analytical data (TOC and its allocation to fractions) and a partial least squares (PLS) statistical approach to assess the ability of MIR spectroscopy to predict the contents of SOC and the organic carbon fractions present in the samples. Where statistically valid predictive capabilities are developed, estimates of the allocation of SOC to its component fractions will be derived from the acquired MIR spectra. Graphical flowcharts of the analytical processes are given in Appendix 1.

2. SOIL SAMPLING

This programme has chosen to sample soils on a 25 × 25 m grid basis as representative sampling sites of any given agricultural management on a particular soil type. By sampling from a small area on each paddock, errors due to encountering a soil type that is not representative of the targeted soil are minimized; however, how representative the sampling site is of the entire paddock will be unknown. As SCaRP was not set up to baseline carbon contents on paddocks or farms, the issue of representativeness of the sampling site to the paddock is not important. It only matters that the sampling site is a random representation of the management × soil type combination under investigation.

At a predetermined location in each chosen paddock, a 25 × 25 m grid is laid out with each 5 m intersection delineated (Figure 1). This 25 × 25 m area constitutes the sample site. From the 36 possible sample collection locations present within the sampling site, 10 are chosen randomly to be sampled. Specific sample collection locations may be rejected upon field inspection as unsuitable (e.g. because compression of the soil). For the case of row crops, the grid should be orientated in such a way that a random distribution of row/inter-row soil is sampled. At each of the random sample collection locations, soil samples are collected from the 0-10, 10-20 and 20-30 cm depth intervals using a corer with a minimum internal diameter of 40 mm. The 0 cm depth is set at the surface of the mineral soil, so any organic layers or surface residues are excluded in this programme. For 95% of the sampling units, the 10 cores from each depth will be composited into one bulk sample for further analysis. However, at 5% of the sample units each of the cores will be kept separate as an assessment of variability within that 25 × 25 m sampling unit.

If, when sampling, large rocks, trees, bedrock outcrops or subsurface bedrock within 30 cm of the surface are encountered at any of the predetermined sample collection locations, then consideration of the areal extent of non-soil area is needed to properly calculate SOC content of the 25 × 25 m sampling unit. Whenever 10 cores could not be recovered for any depth, the proportion of the sampling unit containing rocks and/or trees (P_{rt}) is recorded.

In addition to collection of soil cores for carbon determination at each sampling site, a minimum of 3 bulk density measurements were made for each depth interval. The precise method for measuring bulk density was determined by the individual soil sampling crews based on the nature of the soils being sampled.

2.1 Management history

A requisite for sampling any given site was that the farm owner/manager provides 10 years of detailed management history. The management history data is absolutely critical to the programme. Without this data, there is no way to ascribe differences in SOC content and composition within and between regions to management specific factors. The site information sheet (Appendix 2) covers major items such as crop type, yield, tillage, stubble management, irrigation, fertilisers and other amendments/soil conditioners, fallows, cuts for hay, pasture type, pasture yield or stocking density, and grazing management.

3. SAMPLE PREPARATION

3.1 At partner laboratories

Freshly collected field samples are initially air dried, typically at 40 °C for a minimum of 48 hours, and then the sample mass is recorded. Samples are then passed through a 2 mm sieve and the mass of material retained on the sieve (> 2 mm) recorded. Fine textured soils may need to be crushed prior to sieving to ensure that aggregates are not accidently retained in the > 2 mm fraction. It is critical that the crushing method only disrupts soil aggregates and does not break down large plant debris into material than can then pass through the 2 mm sieve. A device such as a jaw-crusher is ideal for this purpose. For very high clay soils, sieving prior to oven drying may be desirable to facilitate efficient sieving. In this case, both the > and < 2 mm material must be air dried after sieving prior to recording their respective masses. A flowchart of sample processing is provided in Figure A1.1.

Bulk density. Entire volumetric soil samples collected for determination of bulk density are oven dried to 105 °C to eliminate all moisture prior to weighing. Alternatively, the entire sample can be air dried and the oven dry equivalent (see section 3.2) weight is determined on a subsample.

$$Bulk\ density\ \left(\frac{g}{cm^3}\right) = \frac{whole\ dry\ soil\ mass}{core\ volume}$$

3.2 At Adelaide lab

The majority of samples are received at the Adelaide lab as approximately 500 g of air dried < 2 mm soil material. Each sample is quantitatively split down to a 30-40 g subsample using a riffle box (Civilab, 12 × 13mm slotted box) to minimize bias in selecting the subsample for subsequent analyses. About 10 g of the split material is set aside in a glass scintillation vial for fine grinding and subsequent analyses.

Oven dry equivalent (*ODE*) *mass*. The residual moisture content in the soil samples after air drying is quantified by mass loss after oven drying. A 20 g sample of the same split material is weighed out into an aluminium foil weighing tray, placed in an oven set at 105 °C for 16-24 hr, allowed to cool in a desiccator and then re-weighed.

The difference between the initial and final mass is then recorded and used as the *ODE* correction factor (mass residual water/mass dry soil) in the calculation of all carbon concentrations. In sandy soils, the *ODE* correction factor is typically less than 0.01. However, in high clay soils, there may be as much as 0.10 to 0.15 residual gravimetric water in these samples after air drying. This means that C concentration (mg C g $soil^{-1}$) measured on an air dried sample can be underestimated by up to 10-15% because the air dried mass is not an accurate reflection of the true dry mass of the sample.

Fine grinding. The 10 g subsamples are then finely ground using a Retsch MM400 Mixer Mill, with screwable 35 mL zirconia grinding jars and zirconia yttrium-stabilized grinding balls, set at a mixing oscillation frequency of 28 Hz for 3 minutes. The 180 second duration was found by Baldock and Hawke (2010) to be necessary to minimize variability in MIR spectra due to particle size differences between samples across a wide range of soil textures.

Fizz testing. Prior to organic carbon analysis, all samples are tested for the presence of inorganic carbon. Approximately 0.5 g of a finely ground soil sample is placed in a ceramic or plastic well and a few drops of 1M hydrogen chloride (HCl) are placed directly on the sample. Visible effervescence is recorded as: 0 = none, 1 = slight, 2 = moderate, and 3 = vigorous. Any samples that score 1-3 are then treated as containing inorganic carbon and are pre-treated with sulphurous acid (see section 4) prior to determination of carbon content.

4. DETERMINATION OF CARBON CONTENT

4.1 Measurement of carbon concentration

Elemental analysis of total carbon (TC) is determined by high temperature oxidative combustion followed by non-dispersive infrared detection of CO_2. High temperature combustion, when samples are properly treated to remove carbonates if needed, has been demonstrated to be a more accurate and reproducible method for measuring total organic carbon (TOC) as compared to wet chemical methods (Sanderman et al 2010) especially when samples are from numerous different soil types. The Adelaide laboratory runs three elemental analysers: a LECO C-144 (LECO Corporation, MI, USA), a LECO CNS2000 and an Elementar Vario EL III (Elementar Analysensysteme GmbH, Hanau, Germany). Non calcareous samples are typically run on the CNS2000, while the C-144 is primarily used for calcareous samples. The Elementar is only used for carbon analysis of the accumulated fractions after NMR analysis where there is limited sample size (< 500 mg).

A set of five standard soils have been generated as working standards for carbon and nitrogen analysis in the laboratory. All five of these soils have been rigorously tested against certified standards by repeat analysis on both instruments (Table 1). The long-term mean relative standard deviation (%RSD = 100×s.d./mean) of the primary standards for TC is 0.72%.

Each run of approximately 32 unknown samples is accompanied by a 4-point calibration set at the beginning and end of the run with reference samples run after every 10 unknown samples. The standard soil for each run is chosen to best match expected range in C concentrations based

on initial MIR results (see section 7). Calibration and drift correction, if needed, is performed offline in an Excel spreadsheet. Ten percent of unknown samples are run in duplicate. The long-term mean %RSD of these repeat unknown samples for TC is 1.11%.

All reported carbon concentrations are corrected to an oven-dry equivalent mass by dividing the analytical results on the air dried sampled (mg C g air-dried soil^{-1}) by (1 – *ODE* correction), so that all final carbon data are reported as mg C g oven-dried soil^{-1}.

4.2 Calcareous samples

Any soil sample that may contain carbonates based on results of the fizz test gets analysed twice for carbon determination. The first analysis will determine the TC content and is always performed on the LECO C-144 when carbonates are suspected. Then any inorganic carbon is removed by pre-treatment with sulphurous acid (H_2SO_3), as a 5-6 wt% SO_2 solution, and re-analysed for total organic carbon again on the LECO C-144 carbon analyser. Total inorganic carbon (TIC) can then be calculated by subtracting TOC from TC. The detection limit for TIC measurement is approximately 1.0 mg C g soil^{-1} due to the cumulative errors in TIC determination. When the difference between TC and TOC is less than 1.0 mg C g soil^{-1}, we report that TIC is below detection limits.

We have chosen to use sulphurous acid to remove carbonates because upon heating the acid volatilises avoiding the formation of salts (Fernandes and Krull 2008). For pre-treatment, approximately 0.8 g of a suspected calcareous sample is placed in a nickel lined ceramic LECO boat. This weight needs to be recorded as it is the mass that TOC will be calculated against. Next, after placing the nickel-lined boats containing soil samples on a hot plate set to 100 °C, 1 mL of H_2SO_3 is added to each calcareous sample. When the material dries a further 1 mL of acid is added to the sample and then left to dry. Acid additions continue until there is no more effervescence upon application of the additional H_2SO_3. Once all samples in a batch are dry, they are left to cool overnight before carbon analysis on the LECO C-144. When sulphurous acid treated samples are run on the C analyser, it is recommended to add a wad of zinc wool to the top of the water vapour trap to remove any sulphur that may corrode the system.

4.3 Calculation of carbon content

The total carbon content (Mg C ha^{-1}) of each soil layer is calculated as:

$$OC\ content\ \left(\frac{MgC}{ha}\right) = \%OC\ \left(\frac{mg\ C}{g<2mm}\right) \times gravel\ correction\left(\frac{g<2mm}{g\ soil}\right) \times bulk\ density\ \left(\frac{g\ soil}{cm^3}\right) \times layer\ thickness\ (cm) \times correction\ for\ units\left(\frac{10^8\ cm^2}{ha} \times \frac{Mg}{10^9\ mg}\right) \times correction\ for\ \frac{rocks}{trees}(1 - P_{rt})$$

where P_{rt} is the proportion of the land area within the sampling unit allocated to rocks and/or trees (see section 2).

5. FRACTIONATION

As soil organic matter contains a variety of materials with a wide range of susceptibilities to microbial degradation, various fractionation schemes based upon physical and/or chemical properties have been proposed to isolate SOM into more homogeneous fractions. In this programme, we have adopted a modified version of a fractionation scheme proposed by Skjemstad et al (2004) which isolates a particulate and mineral-associated fractions based upon

size fractionation after sample dispersion, and then further isolates a resistant OM fraction dominated by charcoal present in the two physical fractions by use of solid-state ^{13}C NMR spectroscopy (Figure A1.3 & A1.4). This fractionation scheme defines a particulate, humus and char fraction, which Skjemstad et al (2004) successfully substituted into the Roth-C modelling framework as measureable versions of the resistant plant material (RPM), humified OM (HUM) and inert OM (IOM) pools.

Several improvements from the initial fractionation protocol have been made. First, it was recognized that there is often significant variability between operators when physically separating the soil into the > 50 and < 50 μm size fractions. This operator variability has been almost completely eliminated by the use of an automated vibratory sieve shaker system (Massis et al 2010). Importantly, particulate C recoveries were found to be significantly greater using the automated method likely because less truly particulate material was being broken down and forced through the sieve. Second, it has been now recognized that the particulate fraction (> 50 μm) can contain measureable quantities of char, thus charcoal content of both fractions is now determined and the quantities are bulked together to give the total char-C content.

As the fractionation procedure is very labour and time intensive, 5-10% of the 16,000 samples in the SCaRP project will be physically fractionated. These samples have been carefully chosen to span a range in OC content in each region, so that they can be used as an effective calibration set of samples for developing mid-infrared spectroscopy-based calibration algorithms that will allow the content of SOC its allocation to the particulate, humus and charcoal-like fractions to be predicted from the acquired MIR spectra (section 7).

Each sample selected for fractionation goes through the fractionation procedure twice. The first time through, a 10 g sample is size fractionated and the carbon content of each size fraction is determined. This information is then used to determine how much of each of these two fractions need to be accumulated in order to determine the char content via ^{13}C NMR spectroscopy (see section 6).

5.1 Initial determination of mass and carbon distribution

Approximately 10 g of unground < 2 mm soil material is weighed into a 50 mL centrifuge tube. To this soil, 5 g L^{-1} sodium hexametaphosphate (NaHMP) is added as a dispersant to the 45 mL mark and then the sample is vortexed and placed on a shaker table, set to 180 rpm, overnight. After dispersion, the sample is passed through a 50 μm sieve using an automated wet sieving system (FRITSCH Vibratory Sieve Shaker Analysette 3 PRO). Operating parameters are as follows: 20 sec interval, 3 min minimum sieving time (3 min or until drainage water becomes clear), 2.5 mm amplitude, and a water spray at a rate of approximately 150 mL min^{-1}. Once sieving is complete, the particulate sample retained on the 50 μm sieve is visually inspected to ensure all fine material has passed through the sieve. If not, the system is reset and run again.

Each size fraction is then washed into pre-weighed 500 mL LDPE bottles. These fractions are frozen and lyophilised until completely dry. The fractions are then weighed and mass recoveries are calculated. Typical mass recoveries are better than 98%. After fine grinding (as per section 3.2), the size fractions from non-calcareous soils can then be analysed for TOC as outlined in section 4.1. If the soil is calcareous, the fractions will have to be pre-treated as described in section 4.2 prior to determination of TOC. Carbon mass recoveries are calculated to assess the success of the fractionation process. Recoveries between 85 and 115% are targeted. When recovery values fall outside this range the TOC analysis and fractionation process are repeated.

5.2 Accumulation for NMR spectroscopy

In order to collect acceptable ^{13}C NMR spectra in a reasonable amount of time, a minimum of about 20 mg C is required in a total sample mass of < 400 mg. Based on the results from the first fractionation step (section 5.1), the mass of soil needed to ensure an accumulation of at least 20 mg C in each size fraction is calculated. These samples are then fractionated as before in 10 g lots and recombined into one accumulated particulate fraction and one accumulated humus fraction.

For the > 50 μm particulate fraction, if possible, OM is concentrated by floating it off from the sand by decanting through a glass fibre filter. Ensure that all OM is transferred even if this means transferring some sand with the OM. Wash accumulated OM off filter into a test tube, freeze, lyophilise and grind to a fine powder. If carbonates are present, then the finely ground particulate sample is treated with sulphurous acid in a ceramic boat as described in section 4.2. After pre-treatment the sample will need to be finely ground again prior to NMR and C analysis.

For the < 50 μm humus fraction, all aliquots of the < 50 μm material are combined and transferred to a large beaker. Saturated aluminium sulphate ($Al_2(SO_4)_3$) is added to flocculate OM and clays. The suspension is left overnight and the supernatant decanted making sure not to disturb the settled sample. The remaining suspension can be further clarified by centrifuging for 10 min at 2000 rpm. The concentrated humus fraction is then frozen and lyophilised. If carbonates are present, the fraction needs to be treated with sulphurous acid prior to further analysis. The pre-treatment procedure is modified slightly for the humus fraction. Here, 3 g of the sample is transferred into a 50 mL centrifuge tube, using as many centrifuge tubes as necessary to pre-treat the entire accumulated humus fraction. H_2SO_3 is then added in 5 mL aliquots with frequent stirring until effervescence is no longer visible. Top up tubes with DI water, vortex mix and centrifuge for 10 min at 2000 rpm. Decant supernatant and wash three times with DI water by vortex mixing, centrifuging and decanting. These samples are now ready for HF pre-treatment (section 6.1) prior to the NMR analysis.

6. NMR ANALYSIS

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy is used as the tool to determine the relative abundance of condensed char-like carbon in both of the physical fractions (> 50 and < 50 μm).

6.1 HF pre-treatment

The < 50 μm humus fractions need to be de-mineralized prior to NMR analysis to both remove potential paramagnetic interferences, primarily from iron containing minerals, and to concentrate the organic carbon. A sequential extraction procedure using 2% hydrofluoric acid (HF) modified slightly from that proposed by Skjemstad et al (1994) has been used for preparation of samples in the SCaRP project.

Briefly, 3 g of soil material is extracted with 45 mL of 2% HF at total of 9 times over a week long period. The first 5 treatments are for 1 hr, the next 3 will be performed overnight and the final treatment over a 48 hour period. For each treatment, 45 mL of 2% HF is added to the sample, vortex mixed and placed on a rotary mixing wheel for the specified period of time. At

the end of that time, samples are centrifuged for 15 min at 2000 rpm. Any organic material floating on the surface is carefully decanted onto a filter and saved to be recombined with the final sample. The remainder of the supernatant is decanted off and the procedure is repeated as needed. After the final treatment, the sample is washed with deionised water 3 times. Aluminium sulphate may be needed to flocculate the sample after the final DI rinse. After rinsing, the sample is recombined with any organic matter that floated off during treatment and then freeze dried prior to further analysis.

Extra care needs to be taken when working with HF due to its highly corrosive and toxic nature. Any laboratory personnel working with HF even at 2% strength needs to be fully trained in safe operating procedures including emergency medical response in case of accidental contact.

6.2 NMR operating conditions

The NMR spectrometer used for all analyses within the SCaRP was a Bruker 200MHz Avance system. Other systems could be used in a similar way to that outlined below but care will need to be taken to ensure the NMR conditions used to acquire spectra are acceptable. Acquisition of NMR spectra is the same for both the > 50 µm particulate organic C and the < 50 µm HF treated humus C.

For the procedure given below it is assumed that the signal acquisition parameters for the spectrometer have been optimised. This optimisation should be completed by an NMR technician or someone with a good understanding of the instrumental requirements (e.g. setting the magic angle, setting the chemical shift values, defining the 90° pulses for both C and H, setting the C and H powers to ensure adequate cross polarisation, etc.). Once these parameters have been set and the acquisition of signal intensity optimised the subsequent procedure can be used.

Samples (100-500 mg) are packed into 7 mm diameter rotors. If the sample size is too small to fill a rotor, spacers are used to fill the remaining rotor volume. Spectra are first obtained for an empty rotor and used as a background signal that is subtracted from the spectra collected for all samples. A sample of glycine is then analysed as an external standard to establish the relationship between signal intensity and the amount of carbon present in a sample. To run a sample the following set of analyses are performed:

An inversion recovery pulse sequence is run and used to calculate the T_1H value of the sample. This value is then used to set the duration of the delay between pulses (d_1) to ensure that signals are detected quantitatively (d_1 should be more than 5 times the value of T_1H).

A variable spin lock NMR analysis is performed to define the value of $T_1\rho H$ for the sample.

A typical cross polarisation analysis is then performed using a contact time of 1 ms and combined with the $T_1\rho H$ analysis and the glycine analysis to calculate the observability of the organic carbon in the sample under the cross polarisation (CP) analysis (Smernik and Oades 2000a; 2000b).

If the observability from the cross polarisation experiment is low (< 85%), then a direct polarisation (DP) analysis is performed in an attempt to define the nature of the carbon that is not observed by cross polarisation. Typically, carbon not observed in the cross polarisation experiment is observed in a direct polarisation experiment and includes carbon devoid of protons like charcoal C and some alkyl C capable of exhibiting significant molecular motion.

To determine the amount of charcoal-like C (poly-unsaturated C) present the signal intensity of the CP or DP spectrum (as appropriate depending on observability) is integrated across the chemical shift regions given in Table 2 and a Molecular Mixing Model (Baldock et al 2004) is used to estimate the proportion of the carbon present in the sample that can be allocated to charcoal-like C.

As the analysis of additional samples with the SCaRP continues, the correction factor required to be applied to the Molecular Mixing Model results acquired from the CP NMR analysis to provide values equivalent those obtained from the DP NMR analysis will be quantified across fractions and soils. If possible, a generic correction factor or soil specific factors will be defined so that only the CP analysis is required in an effort to reduce the NMR time required to obtain estimates of the proportion of charcoal-like C present in a sample. Details of the final method used to determine charcoal-like C content will be published at the conclusion of the project.

7. MID-INFRARED SPECTROSCOPY

Mid-Infrared (MIR) spectroscopy in combination with chemometric statistical methods, such as partial least squares (PLS) regression, is increasingly being recognized as a quick and effective tool for measuring numerous soil attributes. In soil carbon research there are a number of examples in which MIR-PLS has been used to successfully predict soil organic carbon content and carbon pool data (Janik et al 2007; Bornemann et al 2010; Zimmerman 2007). For the SCaRP project, where approximately 16,000 samples are being analysed, MIR-PLS is being assessed for its ability to provide rapid and cost-effective predictions of total organic carbon content and its allocation to the carbon fractions on all samples.

7.1 Spectra acquisition

MIR scans are made using approximately 100 mg of finely ground air-dried soils which are placed into 0.9 cm diameter stainless steel auto sampler cups and the surface is levelled. MIR spectra are acquired using a Thermo Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., MA, USA) equipped with a Pike AutoDiff automated diffuse reflectance accessory (Pike Technologies, WI, USA). Each sample is scanned for 60 scans with a KBr beam-splitter and a DTGS detector, with a spectral range of 7800-400 cm^{-1} at 8 cm^{-1} resolution. Spectra are expressed in absorbance (A) units where A = Log $Reflectance^{-1}$. An initial background reference scan is made prior to each sample run using a silicon carbide disc assumed to have a reflectivity of 1 (100%).

7.2 Universal predictions

All spectra are initially converted to GRAMS (*.SPC) format and imported into GRAMS/32 AI^{TM}/6.00 software. An Array Basic program, "Predict" Ver-6.0 (Janik 2006), is used to produce initial predictions from individual soil spectra based upon calibration to a set soil samples spanning a range of Australian soils (Janik et al 2007). Full details of installation and use of this software are available on application. Initial predicted values are obtained for TOC, POM, Char, $CaCO_3$, Total N, pH, Clay, XRF-Si, XRF-Al and XRF-Fe. Associated with each predicted value is an F-ratio value similar to a Mahalanobis Distance (Mahalanobis 1936) which allows an assessment of the reliability of the predicted value to be made.

7.3 Regional calibration

Given that the SCaRP project is sampling many more soil types than included in the calibration set used by Janik (2006), there will be large uncertainties in these initial predictions. Predictions of OC content and pool sizes are particularly error prone when carbonates are present. As such, in the SCaRP project we are attempting to derive regionally specific calibrations wherever possible by physically fractionating (sections 5-6) a calibration set of samples that span a range of OC contents from each region.

The determination of the range and size of calibration sets is an empirical process. All spectra are first imported into The Unscrambler X Ver 10.1 software as default OMNIC (*.spa) files. Spectra are then plotted as line plots to visually inspect the scans. Pre-processing of spectra and carbon fraction reference data, and truncation of the spectral wavelength span used is empirical and is done as required. Principal Component Analysis (PCA) is used to identify any trends in similarity of spectra as well as providing a span of sample variability. The PLS regression procedure is used to produce calibration sets using the test set validation option. Calibration and validation test sets can be produced using Principal Component 1 data and selecting samples on a 2:1 basis. The final form of these calibration sets and prediction equations will be determined when all of the fractionation data is complete. These calibrations and predictions will be documented in a forthcoming publication at the end of the project.

8. REFERENCES

Baldock J, Hawke B (2010) Defining soil sample preparation requirements for MIR spectroscopic analysis using principal components. *19th World Congress of Soil Science. Soil Solutions for a Changing World*. 1-6 August 2010, Brisbane, Australia.

Baldock JA, Masiello CA, Gélinas Y, Hedges JI (2004) Cycling and composition of organic matter in terrestrial and marine ecosystems. *Marine Chemistry* **92**, 39-64.

Bornemann L, Welp G, Amelung W (2010) Particulate Organic Matter at the Field Scale: Rapid Acquisition Using Mid-Infrared Spectroscopy. *Soil Science Society of America Journal* **74**, 1147-56.

Fernandes M, Krull E (2008) How does acid treatment to remove carbonates affect the isotopic and elemental composition of soils and sediments? *Environmental Chemistry* **5**, 33-39.

Janik LJ (2006) Predict Ver 6.0 MIR Soil Analysis Software. CSIRO Land and Water.

Janik LJ, Skjemstad JO, Shepherd KD, Spouncer LR (2007) The prediction of soil carbon fractions using mid infra-red-partial least square analysis. *Australian Journal of Soil Research* **45**, 73-81.

Mahalanobis, P C (1936). On the generalised distance in statistics. *Proceedings of the National Institute of Sciences of India* **2** (1): 49–55.

Massis A, Szarvas S, Hawke B, Baldock J (2010) Assessment of an automated method for determining particulate organic carbon in soil. *19th World Congress of Soil Science. Soil Solutions for a Changing World*. 1-6 August 2010, Brisbane, Australia.

Sanderman J, Farquharson R, Baldock JA (2010) Soil carbon sequestration potential: A review for Australian agriculture. A report to the Australian Department of Climate Change. 82 pp. Available online at: http://www.csiro.au/resources/Soil-Carbon-Sequestration-Potential-Report.html

Skjemstad JO, Clarke P, Taylor JA, Oades JM, Newman RH (1994) The removal of magnetic materials from surface soils—a solid-state C-13 CP/MAS NMR study. *Australian Journal of Soil Research* **32**, 1215–1229.

Skjemstad JO, Spouncer LR, Cowie B, Swift RS (2004) Calibration of the Rothamsted organic carbon turnover model (RothC ver 26.3), using measurable soil organic carbon pools. *Australian Journal of Soil Research* **42**, 79-88.

Smernik RJ, Oades JM (2000a) The use of spin counting for determining quantitation in solid state 13C NMR spectra of natural organic matter. 1. Model systems and the effects of paramagnetic impurities. *Geoderma* **96**, 101-129.

Smernik RJ, Oades JM (2000b) The use of spin counting for determining quantitation in solid state 13C NMR spectra of natural organic matter. 2. HF-treated soil fractions. *Geoderma* **96**, 159-171.

Zimmermann M, Leifeld J, Fuhrer J (2007) Quantifying soil organic carbon fractions by infrared-spectroscopy. *Soil Biology and Biochemistry* **39**, 224-231.

9. TABLES

Table 1. Accepted values of working standards for total C and N analysis (mean ± 1 s.d. of 25 repeat analyses at 5 different masses reported).

Soil name	TC (mg g^{-1})	TN (mg g^{-1})
Hamilton[a]	56.30 ± 0.42	4.47 ± 0.04
Waite[a]	25.39 ± 0.48	2.01 ± 0.04
Ottobourne[a]	24.46 ± 0.31	1.90 ± 0.03
Waikerie[a]	07.66 ± 0.22	0.66 ± 0.06
Hart[b]	30.78 ± 0.39	1.73 ± 0.03
$CaCO_3$ std[c]	23.98 ± 0.64	n.a.

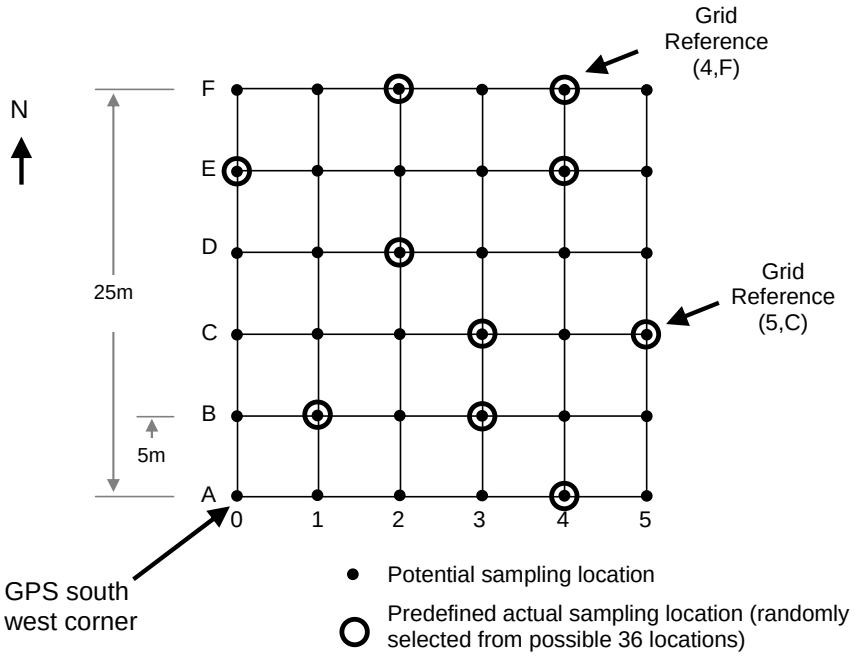
[a]non-calcareous soil, so TC = TOC
[b]Hart soil contains approximately 30% inorganic carbon
[c]25% mixture of pure $CaCO_3$ and acid washed sand

Table 2. Integrated NMR spectral regions, the assignment of each region to a general carbon functional group, and the calculation used to derive the final values after allocating spinning side bands (SSB) back to their parent resonance.

Region (ppm)	Assignment	Calculation
-100 - -55	Alkyl SBB	
-55 - -45	N-Alkyl SBB	
-10 - -40	O-Alkyl SBB	
0 - 45	Alkyl	(0 - 45) + 2 x (-100 - -55) - (215 - 245)
45 - 60	N-Alkyl/Methoxyl	(45 - 60) + 2 x (-55 - -45) - (245 - 265)
60 - 95	O-Alkyl	(60 - 95) + 2 x (-10 - -40) - (265 - 290)
95 - 110	Di-O-Alkyl	(95 - 110)
110 - 145	Aryl	(110 - 145) + 2 x (215 - 245) - (-100 - -55)
145 - 165	O-Aryl	(145 - 165) + 2 x (245 - 265) - (-55 - -45)
165 - 190	Amide/Carboxyl	(165 - 190) + 2 x (265 - 290) - (-10 - -40)
190 - 215	Ketone	(190 - 215)
215 - 245	Aryl SSB	
245 - 265	O-Aryl SSB	
265 - 290	Amide/Carboxyl SSB	

10. FIGURES

Figure 1. Soil sampling grid design.



APPENDIX 1. SAMPLE PROCESSING FLOWCHARTS

Figure A1.1 Initial sample processing

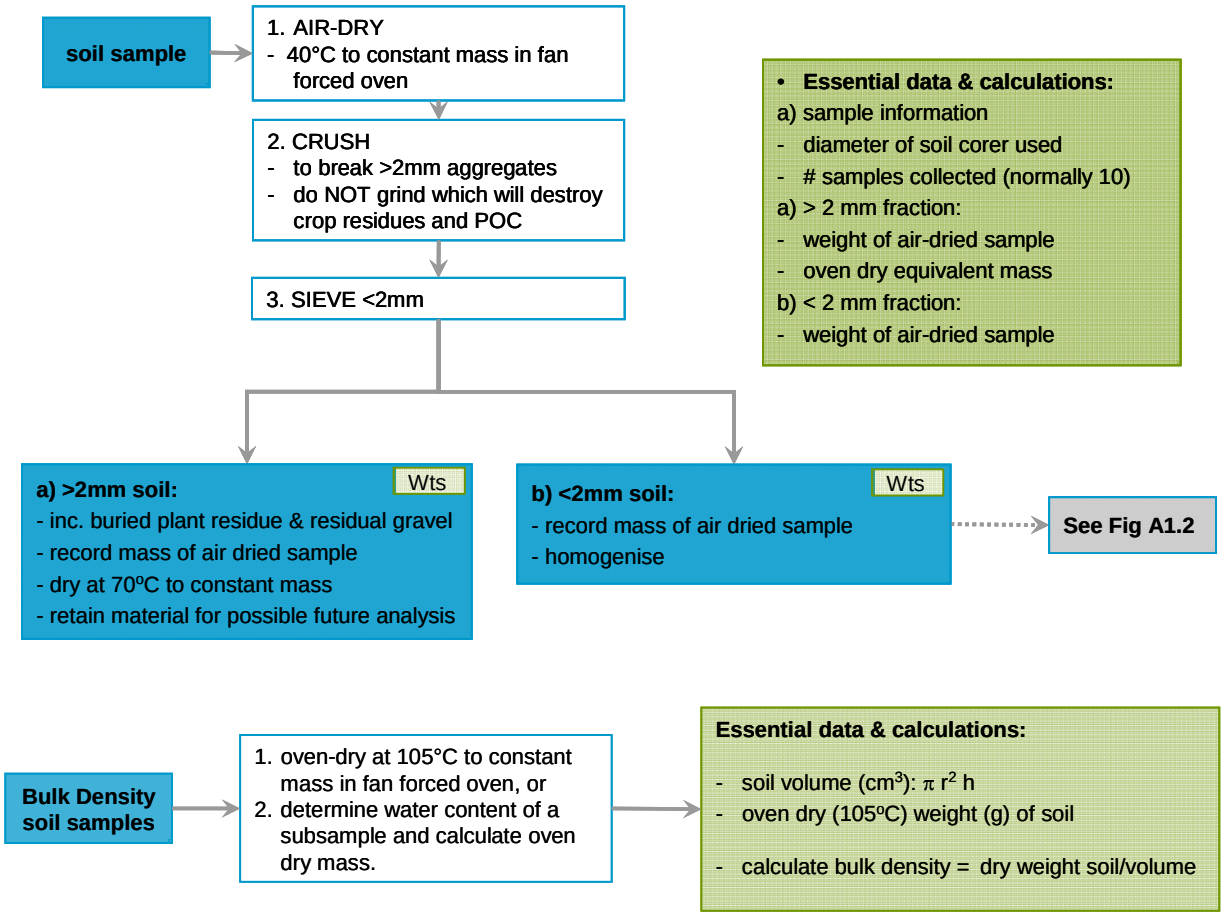


Figure A1.2 Analysis pipeline

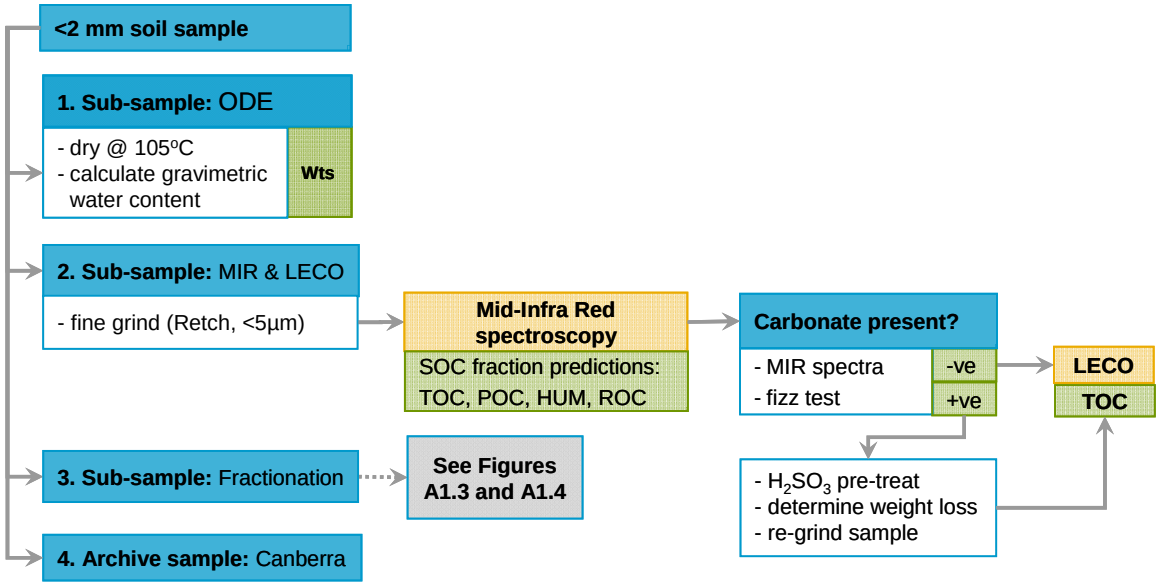


Figure A1.3 Fractionation Part 1: Mass and carbon content

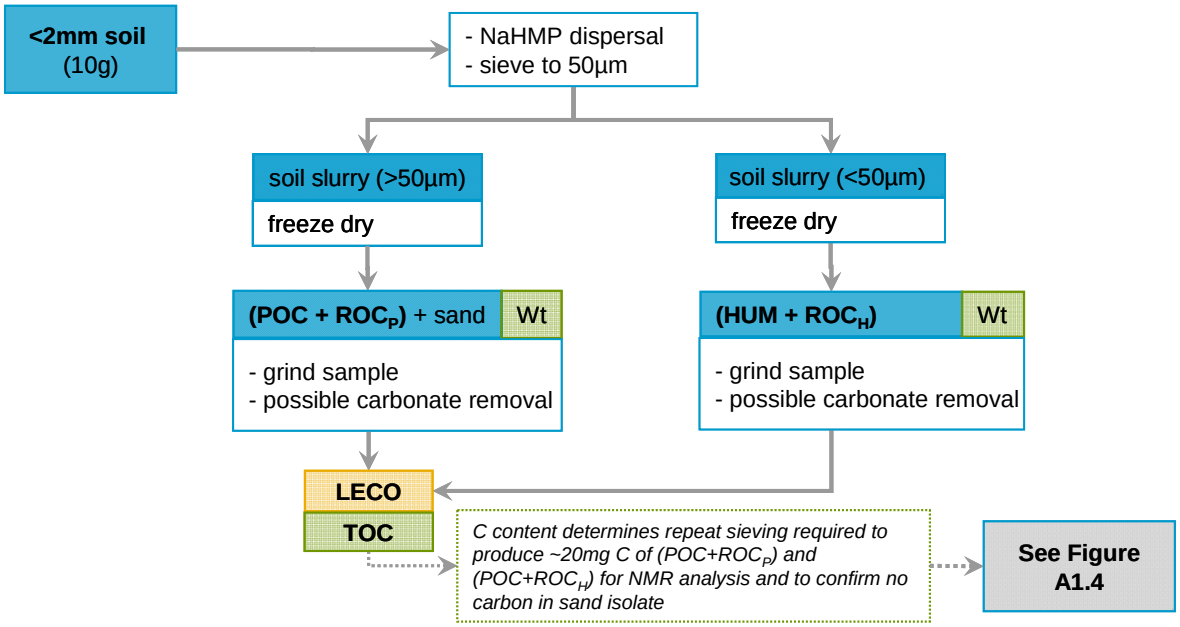
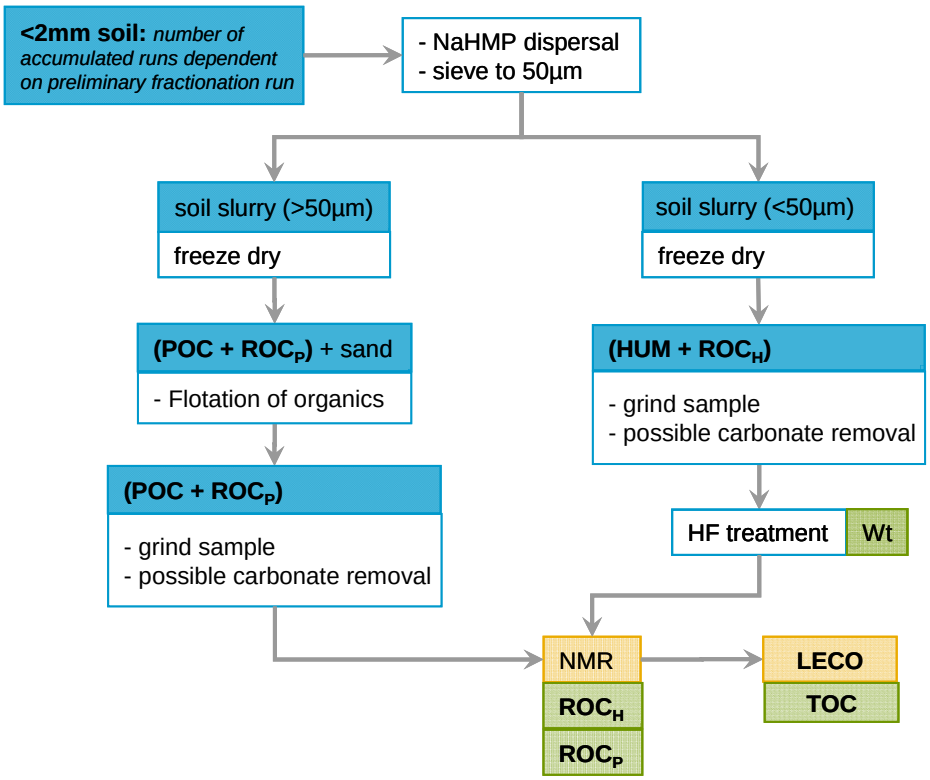


Figure A1.4 Fractionation Part 2: Determination of Resistant OC



APPENDIX 2. SCARP SITE INFORMATION SHEET

Farmer's name & contact details:		Location: Road, paddock name
Date:		GPS location (Easting/Northing):
Paddock size(ha):	Year of land clearance:	Sample collected by:

Land use history and management

Year since present		1	2	3	4	5	6	7	8	9	10
Cropping Enter code											
0 = no crop, 1=cereal, 2=oilseed, 3=grain legume, 4=sugarcane, 5=cotton, 6=non-root vegetables, 7=root vegetables, 8=maize, 9=sorghum, 10=poppies, 11=cereal hay											
Crop Yield Tons/ha											
Tillage Enter code											
0 = Zero till (no workings other than sowing), 1 = minimum till (1 working in addition to sowing), 2 = conventional (2 or more workings)											
Stubble management Enter code											
0 = residue retained on surface, 1 = residue retained by worked in, 2 = residue grazed, 3 = residue baled and removed, 4 = residue burnt											
Pasture Enter code											
0 = no pasture, 1 = annual pasture - grass dominant (>75%), 2 = annual pasture - legumes dominant (>75%), 3 = annual pasture - mixed grass/legume, 4 = perennial pasture - grass dominant (>75%), 5 = perennial pasture - legume dominant (>75%), 6 = perennial pasture - mixed grass/legume, 7 = mixed annual/perennial – grass dominant (>75%), 8 = mixed annual/perennial – legume dominant (>75%), 9 = mixed annual/perennial – missed grass/legume											
Pasture Yield Tons dry matter/ha											
Grazing management Enter code											
0 = no grazing, 1 = set stocking, 2 = rotational grazing											
Cut for Hay (crop or pasture) No=0, Yes=1											
Long Fallow (>8 months) No=0, Yes=1											
Irrigation No=0, Yes=1											
Fertiliser (enter rates in kg/ha)	N										
	P										
	K										
Soil conditioners Enter code											
0 = no soil conditioners added, 1 = agricultural lime, 2 = gypsum											

Soil information

Soil type	Australian Soil Classification – Order:		
Internal diameter of soil sampling tube (mm)			
Number cores / sample from each depth	0-10 cm:	10-20cm:	20-30cm:
Number depths (usually 3 but may be 6 where deep samples are retained)			
Total no. samples (3 composite or 30 individuals			
Sample numbers /codes	Start:	Finish:	

NOTES

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Web: www.csiro.au/flagships

CSIRO and the Flagships program

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