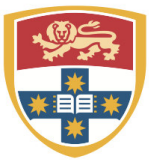
TECHNIQUE INNOVATION IN SOIL CARBON MEASUREMENT

Doctoral Thesis (PhD)
by

Robert Pallasser



THE UNIVERSITY OF SYDNEY

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DECLARATION

I declare that the contents of this study were my own and the ideas were originally conceived. Furthermore, that the work has not been submitted for any degree elsewhere or at any other time.

EXECUTIVE SUMMARY

Increased global industrialisation and deforestation have placed enormous burden on our atmosphere and environment. For no other reason than future proofing soils against major climate variability – a possible side effect of growing atmospheric CO_2, diverting more of this carbon (C) from our air to where it can do considerably more good seems very worthwhile.

This has made soil carbon storage and its measurement such an important and intense area of current research activity. To know and fully understand the impacts of various land management practices on soil carbon building processes requires before anything further is said or done the ability to measure carbon stocks reliably. The enormous challenges are to do this for huge land areas with a sensitivity to see the real changes occurring with an awareness of the spatial, seasonal or other variations that may be as significant.

This research study had set out to advance our understanding of soil carbon and its measurement. It has investigated what has gone before and is currently being done but also considers ideas on the horizon. From this base, several novel approaches have been taken to develop innovative methods of dealing with these immediate questions with an aim to easing the soil carbon data crisis. One of the major problems is the natural variability of carbon in soils over relatively small distances leading to uncertainties in carbon analyses which easily amplify in terms of carbon stocks. To capture this variability using conventional methods available today make on-the-ground measurement prohibitively costly. Specifically to deal with this problem a system named the Soil Carbon Bench (SCB) was developed at the centre of this research to cope with large amounts of soil and in fact to enable carbon analysis of whole cores by trusted combustion. This newly developed apparatus formed the core of the work and in its test-bed form has been tested on carbonaceous calibration materials and was then demonstrated on soil cores recovered from a trial field under lucerne rotation. Its accuracy has been equivalent or better than standard analytical methods and when evaluated in terms of its cost efficiencies and determining carbon stocks on the work to date it has done so with a smaller margin of error and at much lower cost. The relative costs of determining soil C stocks were estimated to be about 1/5 of conventional methods along with improved precision. Soil C data obtained with the SCB had a lower variance and C stocks could be replicated so that total C values per 50cm core were typically within 0.2g or 0.0003 kg/kg of the site mean. The research has succeeded at addressing the benefits of analysing whole cores and paved a way to more efficient carbon surveys that easily respond to any changing protocol requirements as may be recommended by bodies such as the IPCC. There are

numerous other possibilities to test in conjunction with sampling designs and the support of emerging proximal techniques under experimentation.

Another but related area was to elucidate reliable ways to differentiate and determine soil carbon forms which are of great importance when considering carbon pools and storage. Thermal analytical studies were not only an ideal complement to the development of the SCB but provided many insights into the thermal behaviour of soil carbon components relevant to these pools. While it provided useful information related to loss on ignition methods (an important alternative method for large scale soil determination) it has opened up further possibilities for productive investigation that encompass characterising soil components and organic matter (OM) stabilisation. In particular it has shown real potential for the determination of black carbon and bushfire residues not easily detected by other instruments, but important for calibrating rapid soil spectral techniques.

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Presentations and Publications (to date) arising from the study

Peer reviewed publications:

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Robert Pallasser, Budiman Minasny, Alex. B. McBratney 2013. A Carbon Determination System for Whole Soil Cores. Communications in Soil Science and Plant Analysis (in review).

Conference presentations:

Robert Pallasser, Budiman Minasny, Alex McBratney and Hank De Bruyn. Simplified method to assess soil organic matter in landscape and carbon sequestration studies. EGU, Vienna May 2010.

Robert Pallasser, Budiman Minasny and Alex McBratney. Novel use of thermal analyses to meet soil C monitoring in Agriculture. 19th World Soil Congress, Brisbane, August 2010

Pallasser R.J., Keitel C, Minasny B. and McBratney A. Stable Isotopes Monitor Soil C. 11th Australasian Environmental Isotope / 4th Hydrogeology Research Conference, Cairns, July 2011.

Robert Pallasser, Budiman Minasny, Alex. B. McBratney. A novel method for measurement of carbon on whole soil cores. IUSS Global Soil Carbon Conference, Madison WI, June 2013.

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INTRODUCTION

Food and soil security have been perennial topics but the past decade has seen a sharpened focus on soil and its capacity to store more carbon (C) in the form of soil organic matter (SOM). Now the world's soils are coming under ever greater stress to meet the huge demands for food and other land resources which in some cases lead to greater soil degradation. In a downward cycle, wind and water erosion are processes that can further alter the soil C balance. The value of organic matter (largely composed of C) to soil function and its general well-being has long been recognized. Soil and moisture conservation methods as proposed and implemented by visionaries such as H. Bennett and H. Finnell during the 1930s in the drought ravaged states of the US have been classic examples (for further reading see compilation by Helms, 1992). There, much of the poorer crop land had to be returned to native grassland as the only viable way of retaining the fragile top soil. These same concepts have become reinvigorated where many are beginning to realize increased OM as the way to drought proofing their farms (Landline February 2012, Australian Broadcasting Corporation). With the encouragement of worms and other biota, OM promotes stabilization and the development of a soil structure (Angers 1992; Dexter et al., 2008; Six et al., 2000) where the organic-mineral combination has a greater ability to withstand compaction from overgrazing, dispersion and erosion. More recently there has been an added driving force – to reduce atmospheric C and storing it via photosynthesis in agricultural soils. The major incentive has been the concept of C credits operated through various financial instruments for carrying out this function in verifiable ways according to a framework as set out under IPCC guidelines.

Carbon in perspective

Carbon dioxide (CO_2), well known for its capacity to absorb long-wave radiation (so called radiative forcing), has varied within the atmospheric composition in the Earth's past (Berner and Kothavala, 2001; Ehleringer and Cerling 1995). The biosphere has been improving landscapes fit for habitation since the appearance of algae and plants eons ago. The influence of land plants, in particular forests, has had a major role not only in metabolising C (which can be enhanced through CO_2 fertilization) but accelerating the weathering of silicates (organic acids) to release Ca^{++} and Mg^{++} which have been crucial to the removal of mineralized C from the system over time (see also Berner and Kothavala, 2001 and their related references). Global warming and climate change are very complex processes that involve other trace gases, water vapour (very efficient at trapping heat) and albedo-type mechanisms such as stratospheric cloud and ice formation (Andrews et al., 2012). While some amount is essential to life as we know it, the relatively abundant atmospheric CO_2

distributed around the globe has predictable anthropogenic sources lending itself to much better control and management.

Records for atmospheric CO_2 concentrations since the Industrial Revolution are evidence of the accelerating trend which has been from primarily fossil fuel energy usage (coal and petroleum) especially since the 1950s. The fossil fuel reserves can now be considered as having re-entered the C cycle through human exploitation. However the problem is bigger than only the increasing levels of atmospheric C now nudging 400ppmv. Atmospheric CO_2 (figure 1) would be predicted to be more isotopically depleted through the [d]Suess effect but is only nearing –8 ‰ relative to the Vienna PeeDee Belemnite (VPDB) limestone standard (Ghosh and Brand, 2004). Apart from additional primary sources and cycling via the biosphere (expected to be fairly balanced) the isotopic impact from fossil sourced C over this past 150 year period is not fully reflected in the current composition. This signals another process at work – the dissolution and exchange with the ocean's dissolved inorganic C (DIC) store. According to Raven and Falkowski (1999) up to a third of the CO_2 emitted to the atmosphere since the industrial revolution has dissolved in the ocean, which provides some offset mechanism but there are serious limitations. These involve ocean stratification, limits to removal in the form of CO_3 and importantly acidification that may lead to worse consequences. In addition the well established CO_2 (aq) – bicarbonate – carbonate equilibrium is temperature sensitive representing another potential feedback.

The total mass of soil organic C (SOC) in the surface metre of global soils is in the region of 1600 Pg (Pg = 10^{15} g) and nearly ½ as much again in the form of carbonates (Batjes, 1996). The soil organic C pool is a minor part of the global C inventory (figure 1) but in cooperation with the biosphere is a comparatively active reservoir (for non-geological timeframes) and of major importance to the C cycle. Deforestation is an equally serious contributor where forest fires emit 4 Pg annually (Adams and Attiwill, 2011). Some of these emissions are cycled back into the biosphere and hydrosphere but after all these sinks are accounted for, there still remains a net increase to atmospheric stocks of well over 3 Pg per annum. On a comparative basis world's soils (1600 Pg) rate prominently as ideal sinks to offset these growing emissions where the atmosphere contains a relatively modest 750 Pg C. Soils are particularly well suited for some of this redistribution because many have become C depleted (sources of C) after years of exploitative farming (and stand to gain from increased organic matter) but importantly much of the land area is under management with agriculture covering 30% (Janzen et al., 2004).

[d] The Suess effect describes the increasing isotopic dilution of atmospheric CO_2 by ancient, fossil C.

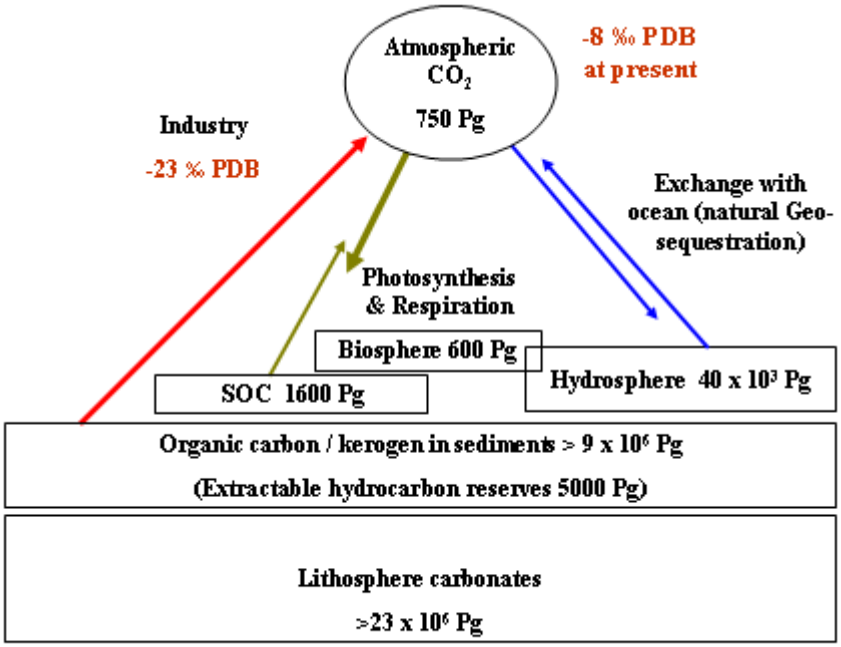

Figure 1. The relative sizes of the global C pools and significant carbon isotopic (^{13}C) compositions. Sources: Mackenzie et al. (2004); Brownlow (1979) and Ghosh and Brand (2003).

[d]Carbon is the vital building block used in plant and animal assimilation and the subsequent forms of diverse organic matter which when broken down to SOM render numerous beneficial properties to soils and its biology.

Increasing organic soil C (through SOM) on an on-going basis can be brought about by encouraging more OM entering the soil or reducing losses of stable C from the soil. However building organic soil C is a slow and difficult process because the majority of organic matter (e.g. crop residue) is fairly promptly (months) returned to the atmosphere from the soil's surface through microbial respiration with only a small fraction preserved. SOM may be considered the biologically degraded residue of OM where nutrients have been balanced and are readily available to plants when required but SOM may be subject to further microbial degradation. This process of humification is widely acknowledged but not particularly well quantified and understood.

Improving the soil's fertility and selecting the crops with favourable root ratios are one way of increasing soil biomass although there are limits to how much this can be raised, rainfall

[d]The element carbon (C) with a crustal abundance only 320ppm readily combines in a diverse range of organic and inorganic compounds and has formed substantial reservoirs around the earth's surface.

being an obvious constraint (Ugalde et al., 2007). While in itself not a nutrient, C has been positively correlated with the macronutrients N, S and P (Kirkby et al., 2011; Kirkby et al., 2013). Net increases in SOM appear to be very dependent upon these nutrient inputs but particularly their proportions (analogous to [d]Redfield ratios). Apart from supplying the balanced nutrients needed for plant growth it appears to be more important in controlling SOM formation by providing the optimal conditions for soil microbes to stabilise SOM (Kirkby et al., 2013). Disequilibrium in these macronutrients is most likely the main driver in positive priming – a serious unwanted consequence of adding high organic C material to soils. Another set of considerations when boosting inputs (e.g. fertilizer) are the C costs of production (Schlesinger, 1993), nitrification (changes to pH) due to top dressing or legume rotation and the final outcome on crop productivity (i.e. foliage vs fruit).

This makes loss reduction an equally important component for C sequestration so that SOM currently held can be maintained and not depleted (through oxidation or priming). Furthermore it is more readily achieved and the associated costs are likely to be lower for example, during minimal tillage where in addition stubble and other residues may be retained near the soil surface. The merits of tillage (primarily weed control) vs direct drilling have long been debated (Faulkner, 1945; Carew, 1949) but current data has shown the practices most likely to boost and maintain higher steady-states of SOM to be conservation tillage methods (Angers et al., 1997; VandenBygaart et al., 2003 and others) and supporting perennial grasslands (Post and Kwon, 2000).

Measurement – one can only manage what can be measured

While SOM assimilation represents some of the difficulties in advancing soil C as a means of sequestration, the major obstacle has been a vacuum in the capacity to accurately measure and monitor soil C over the required scale. In order to make properly informed decisions about various C management with some degree of certainty and within some margin of error the analytical method needs to be able to determine the real changes in soil C stocks that are not the result of natural variation – seasonal or spatial, but due to the C management itself. Such knowledge will allow effective assessment of the various practices applied in all the soil types, climates and land uses.

These are the analytical challenges involved:

- The broad landscapes that have to be covered reliably
- Determining the best sampling schemes to capture the natural C variability

[d] Redfield ratios describe the limiting nutrient balances required by simple marine life forms first recognised by Redfield (1958).

- Having the required sensitivity to quantify the real changes in C stocks (excluding seasonal movements from long term C gains)
- Deliver all these in a cost effective way while maintaining the overall objectives

A number of very promising proximal systems have emerged in the past few years designed to acquire data directly in the field (e.g. Veris on-the-go NIR spectroscopy, hand-held NIR and MIR devices). However most of these systems need to be tied to large calibration sets and have no advantage for the determination of actual C stocks over the traditional analyser. This is because they similarly only sample discrete points of the soil's surface and in many cases can be used more effectively as laboratory benchtop instruments where frequent calibrations can be carried out and where condition are the least variable. However these systems do provide rapid data acquisition allowing high resolution covariate data layers to be mapped e.g., for soil C.

The need to measure broad areas immediately turns attention to remote sensing as the obvious solution. While some good correlations have been obtained by remote sensing such as with Quickbird imagery (2m resolution) where the 'ground truth' soil C was determined for <20cm (Aynekulu et al., 2011) the following must be considered. The more costly hyperspectral methods generally are limited to the near surface, may be obscured by ground cover, have uncertainties related with signal to noise (Gomez et al., 2011) or lack predictive accuracy (Selige et al., 2006). The latter found soil C stocks to be overestimated below levels of 1.5% C and that would include many of our landscapes. It has been appreciated for some time that soil properties (soil C being one) generally cannot be as readily interpolated as continuous geographic quantities, e.g., hydrology (Burrough, 1983) placing the emphasis on direct soil sampling and measurement.

The use of empirical models to determine soil C stocks as a function of inputs (management and conditions) have been promoted as a cost effective means because it avoids some of the big expenditure – that associated with comprehensive sampling and analysis. There are still the measurement requirements to initiate these less direct approaches to simulate the theoretical behaviour of SOM under relevant conditions. However soils and organic matter vary considerably from point to point and there are unrealistic assumptions made such as the homogeneity of degradation in each pool (Poirier et al., 2005). On-the-ground measurement of soil C stocks remains the most direct and reliable way to read real changes occurring at that site as a consequence of management.

All these discussed methods are based however on (are calibrated against) the reliable dry combustion method which extracts all of the C prior to quantification. The problem is that

this technology is only limited to analysing small amounts of material (<0.5 g), being designed as very precise instruments for laboratory determinations. This then means for the measurement of C stocks based on incongruously small aliquots removed from the landscape under some sampling design, the number of required analyses may still become cost prohibitive. Bulking and further sub-sampling of soils combined with powerful statistical tools may ease this but then there are the added time/costs of processing.

This overall lack of an appropriate and reliable soil C determination method that meets the agricultural scale has been an immediate restriction that has prolonged the soil C data crisis and hindered the development of other vital aspects: the importance of dynamic pools and the best management for real and effective soil C increases. As a result, a new look at ways soil C can be better determined have been the priority focus in this study. Two central hypotheses were set:

1. **To demonstrate how large volumes of soil and whole cores can be analysed for C by a trusted method and that these can overcome the problems associated with soil C variability**
2. **How significant are the different soil C fractions when measuring C stocks and what better approaches can be applied during agricultural C sequestration?**

In the following sections, the range of existing methods for determining soil C stocks and pools are reviewed and evaluated and then followed through with the development of apparatus / methods to answer these specific questions.

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CHAPTER 1 – THE ANALYSIS OF TOTAL SOIL CARBON: INDUSTRY STANDARD METHODS

Wet chemical oxidation of organic matter

Although carbon determination by combustion was successfully demonstrated in 1831 by Liebig (Kohn, 1951), the quantification of soil C by wet chemical oxidation has continued to the present day in some laboratories because of low cost and simplicity. This soil C determination method known as Walkley and Black (WB) relies on oxidation of soil OM by a corrosive reagent, potassium dichromate ($K_2Cr_2O_7.2H_2O$) that is acid catalysed (H_2SO_4). It has been credited to these researchers because of the refinements they made (Walkley and Black, 1934; Walkley, 1947) to existing techniques. It still remains a standard method for soils (Rayment and Lyons, 2011) and is used where the more expensive but accurate dry combustion systems have as yet not superseded or in remote locations without access to such equipment (McCarty et al., 2010). In this reaction soil C is lost as CO_2 and the amount of C originally present can be determined gravimetrically or by titration of the remaining solution. From these quantities, SOC can be calculated using a conversion factor (varies depending on the efficiency of the method used). It is generally regarded that WB based methods tend to underestimate SOC although some good agreement with dry combustion has also been reported. Tabatabai and Bremner (1970) obtained a 1:1 correlation (method of Allison, 1960) but care was taken to trap out interfering chloride ions. When CO_3^{2-} and Cl^- are present in soils, these ions can interfere with the process necessitating correction of the results. Wet oxidation has since been further refined (Heanes, 1984) so that the reaction is held at 135 ^oC in a heated block to increase the efficiency of C extraction / oxidation. A compact chronology of the improvements made to wet oxidation and their various merits is presented in a review on methods by Chatterjee et al. (2009). However it has been generally found that the different wet chemical treatments either under- or overestimate OM. Overall, while these wet chemical oxidation methods are cheap and the materials readily procured, they are unsatisfactory in terms of the accuracies obtained (variable results in comparison to automated dry combustion) as well as being messy and time consuming. As an alternative, hydrogen peroxide (acid catalysed) and sodium hypochlorite can also be employed as oxidising agents without the disadvantage of leaving chemical residues for special disposal such as Cr solutions. It must be emphasised that unlike in other methods soil carbonates are not simultaneously decomposed by wet oxidation does not simultaneously decompose soil carbonate present (Zimmermann et al., 2007a; Conyers et al., 2011) while it does occur using other analysis methods.

Dry combustion by elemental analysis

This dry combustion method (also referred to as Dumas for his work in obtaining nitrogen measurements from organic materials) relies on the conversion of carbonaceous material to CO_2 with its subsequent measurement. At the core of the method (including LECO, the commonly associated or quoted manufacturer) is the presence of copper oxide in the combustion train (Fieser and Fieser, 1956; Kohn 1951). As stated earlier, the principles necessary for quantitative dry combustion were established before the 20th C but the development and use of C analysers as we know them now only occurred in laboratories after the 1960s coinciding with the appearance of microprocessors and detectors to measure the product CO_2. While quantitative analysis by combustion was the most precise, prior to this time it required complicated manual systems of glass apparatus for conversion and trapping, often not practical for routine laboratory analysis. Such installations were found in laboratories concerned with specialist research activities such as: determining conversion factors for soils (Read, 1921), plant tissue (Worsley and Nutman, 1931) and in later years for micro C analysis in waters and sediments (Salonen, 1979) or the isolation of C traces from lunar samples (Kaplan et al., 1970). In 1921, Read published an ingenious dry combustion method which relied on a gravimetric technique to determine the amount of C formed from the combustion (with same principles) of OM (diagram of apparatus published in his paper shown on figure 1-1). The resulting data successfully produced OM to C conversion factors to support loss-on-ignition (LOI) analyses of soils. It was somewhat complicated (numerous traps, loading procedure, a separate sweep-out phase after combustion) and analysed only 1g soil but it was very accurate. A number of the design elements are still contemporary and it is surprising that the method did not appear to have been propagated /developed.

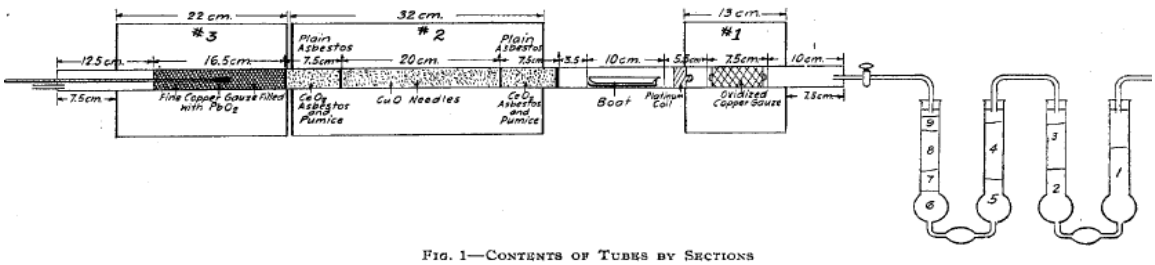

FIG. 1—CONTENTS OF TUBES BY SECTIONS

1—Calcium chloride
2—12-mesh soda lime
3—30-mesh soda lime
4—30-mesh soda lime
5—60-mesh soda lime
6—Calcium chloride
7—Short fibered asbestos
8—Phosphorus pentoxide
9—Asbestos

Figure 1-1 Soil carbon analysis apparatus (taken from Read, 1921).

Most modern automated dry combustion instrumentation analyse very small aliquots, usually less than 1 gram, and trap / release oxidised products for quantitative determination by usually infrared and / or thermal conductivity detectors. The operating principles have much in common with the modules used in gas chromatography. Variable combinations of the

elements C, N, H, O and S can be analysed concurrently but in soil science most interest is in C and N. A number of comparisons between various analysers and between methods have been made in terms of differences in operation and outputs etc. (e.g. Smith and Tabatabai, 2004; Chatterjee et al., 2009). These reviews have taken into account the relative costs and intricacies of the various methods currently available. While some differences in precision may occur (e.g. depending on the combinations of detectors employed) overall, as a soil C determination method, elemental analysis has been indicated as the most optimal by virtue of its greater accuracy and hence reliability in determining C stocks.

The capital costs for such analysers can be in excess of AUD $50,000 and the running costs are also high ($5-10 per sample) due to expensive ultra high purity gases required because of the small amounts of analyte in question. While they are much more efficient (compared to wet chemistry) at extracting 100% of the C in the soil aliquot and its quantification (e.g. %C) there is a strong dependence on instrument maintenance, calibration and how they are operated. That is, the setting up of method files that define combustion periods, temperatures and oxygen flows appropriate to the type of sample material. Results can depend on the sample size i.e. the respective concentration of elements present, for example C and N can be quite different in char rich material.

Inorganic carbon determination

Automated dry combustion methods are usually operated at temperatures in excess of 650 ^{o}C so are unable to discriminate between organic and inorganic C. Furthermore the time and temperatures required for thermal decomposition of carbonates depends on their form (composition and structure), distribution in the matrix and total quantity. Soil carbonates are usually Ca, Mg or $FeCO_3$ while $NaCO_3$ is found associated with salt deposition. Loeppert and Suarez (1996) describe the various occurrences and traditional methods of analysis. The currently used methods available to quantify this primary form of soil C still relies on reacting the anion with an acid to form gaseous CO_2 which can be analysed directly by pressure calcimeter (Sherrod et al., 2002), gas chromatograph, by LECO RC 412 (Girard and Klassen, 2001; Gazulla et al., 2012) or by difference in conjunction with elemental analysis (Rayment and Lyons, 2011). Some related methods have been found quite effective at removing CO_3 such as fumigation (Harris et al., 2001; Amundson et al., 1988).

Loss on ignition (LOI)

LOI is a well established (Rather, 1917; Read 1921) method of dry combustion where the soil is heated (oxidised) in a muffle furnace with a small opening to atmospheric air. Capital outlay is minimal requiring only an oven (muffle furnace) and a balance and as a technique is easy to apply. It is therefore still in widespread use but its reliability has been questioned repeatedly (Mook and Hoskin, 1981; Konen et al 2002; Pribyl, 2010; Sutherland, 1998; Chatterjee et al., 2009; McCarty et al., 2010). First of all the duration of heating has been shown to affect the magnitude of mass loss (Schulte et al., 1991; Matthiessen et al., 2005). Presumably this is related to the movement of oxidising air into and out of the soil matrix where large samples result in lower relative yields. The temperatures applied vary also between laboratories yielding mass changes that can be erroneous due to reactions that are not related to OM (i.e. lattice water, carbonates). At around 450 to 550 ^{o}C the mass changes due to mineral dehydroxylation become a significant contributor in fine grained soils. Typically losses resulting from heating between 105 and 550 ^{o}C (16hrs) are used and then ascribed to OM. Carbon (%) concentrations are then derived by applying a conventional conversion factor (e.g. 0.58 quoted in soil text books). As a result the OH^{-} contribution from such a broad temperature window can lead to over estimation. Therefore depending on the soil type and temperatures (and durations) used, the method can lead to under or over estimation of the C contents. Alternatively, factors can also be empirically determined for a representative cross-section of soils from the locality by processing them with the same LOI protocol and calibrating the results using (more expensive) elemental analyses on a limited number to obtain a 'correction' factor. Konare et al. (2010) reliably determined West African soils by LOI (using the mass losses between 105 and 350 ^{o}C) to serve as a fairly cost effective means of C determination in that region where instrumentation is more difficult to access.

Advanced Methods for In Situ Soil C Measurement

The 'holy grail' in soil C determination is to be able to measure intact soil core or even better in situ soil volumes without disturbance or removal and preferably on a volumetric basis. The benefits that accompany such methods include lower costs associated with sampling / processing of soil, rapid large-area coverage, bulk analyses and very importantly preservation of the sampling site (Gehl and Rice, 2007). Ideally ground should not be removed or destroyed so that the exact same site can be monitored again over time.

In contrast to most spectroscopic techniques where certain wavelengths from an external light source are absorbed by different chemical bonds in the material, emissions type spectroscopy elicit electromagnetic radiation from within the material. These are systems

that use thermal or laser energy to induce responses that can be specifically indicative and quantitative.

Laser Induced Breakdown Spectroscopy (Libs)

LIBS is a spectroscopic technique where laser energy is focused directly onto a sample material (soil) to produce electromagnetic emissions characteristic of the elements present. The method is interesting for this review because it has the potential to measure total in situ carbon and could contribute a major advance.

The breakdown energy is produced by a neodymium doped, yttrium aluminium garnet (YAG) pulsed laser. The energy is delivered to the soil surface using optical fibre and emission signal collected with photo diode array detector. LIBS can be distinguished from NIR / MIR where the quantity of interest is the amount of energy transmitted / absorbed. Instead energy is released at certain wavelengths as a result of the laser ablating (destructive) the sample material. Data are acquired over the LIBS spectral range from a little below 200nm to 800nm. Emission lines specific to elements that include Fe, Si, Mg, Al and C (Cremers et al., 2001) occur more than once, where C has its characteristic lines at 193.03 and 247.88nm.

To date, LIBS development has involved lab based experiments on prepared core faces or pressed buttons containing carefully mixed soil and C powder. In these experiments the moisture and density have been well controlled along with special attention to the distance from the laser focal point. These have been initial laboratory calibration studies that form the basis of any field portable development. In field experiments by Ayyalasomayajula et al. (2012) the specific C lines have been used (in situ) to detect and measure any fugitive CO_2 in surface soil that may be evading geological sequestration. An example of output over the 245 – 250 nm range with the useful C and Fe lines is shown on figure 1-2.

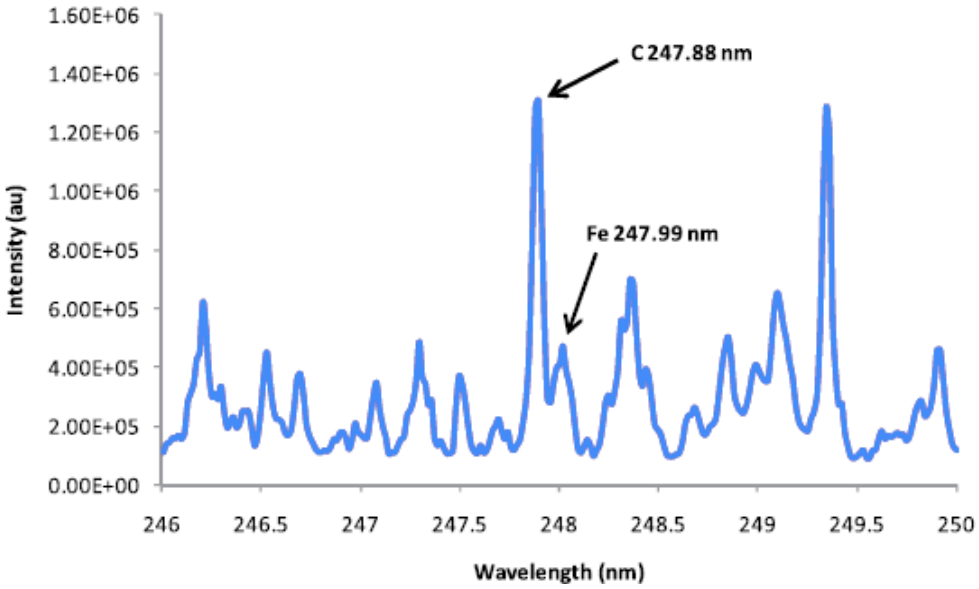

Figure 1-2 LIBS spectrum of whole soil showing the significant emission lines for iron and carbon where the Y-scale are arbitrary units (reproduced from Ayyalasomayajula et al., 2012).

Clearly C can produce a striking emission peak at around 248nm but there is some interference with Fe. The other C line at 193.03 can be out of range for some detectors but according to the literature quantifying proximate spectral lines (i.e. Si or Fe) to obtain ratios with the 248nm C allows the peak areas to be normalised improving the calibration. This more or less univariate approach (corrected using neighbouring elements) has resulted in reasonable correlation with that from dry combustion (Cremers et al., 2001). However due to the multiple and overlapping peaks, a multivariate approach (partial least squares regression or PLSR, described in more detail further on) based on the whole spectra has also been demonstrated by Bricklemyer et al. (2011) who applied it directly to intact cores. Thus this method still relies on calibration using standard method.

Direct application of LIBS can include root material and it may also experience interference due to minerals. It is a point analysis method so for large scale assessment of soil C stocks would again require large numbers of analyses within a framework of sampling design and statistical modelling. However the potential for easy field use and rapid analysis turnover compensates for the numbers required. It is also quite sensitive with a lower limit of detection around 0.03% C and reasonable precision of 4-5% on replicate analyses. In addition there is also the possibility of differentiating lime by the amount of Ca present which can also be determined by LIBS (Bricklemyer et al., 2011).

Inelastic Neutron Scatter (INS)

While INS has been employed previously for the measurement of water (hydrogen) in geological applications the more recent development of this technology has been direct measurement of atomic carbon and oxygen during well logging (summarised in Bond et al., 2010). It has made it possible to determine C/O ratios in proximally located material such as in formations adjacent to wells to assess oil-field fluids.

In the case of C determination the principle of operation relies on the pulsed electromechanical production of fast and thermal neutrons at 14 MeV which then collide with atoms in the material. Between these short pulses, gamma emissions with a distinctive 4.43 MeV (for C) resulting from inelastic thermal neutron capture are subsequently detected. The process has been schematically described on figure 1-3.

Application of the method to the direct measurement of soil C has been very limited probably due to a lack of knowledge in this area. Initial experiments based at medical facilities (Wielopolski et al., 2000) have now developed into a prototype systems that can be used to test soils in situ (Wielopolski et al., 2010; 2011). While still developmental, it stands out from other methods by being non-destructive, as there is no soil removal and no ablation nor combustion. The way signal is generated and acquired and the area that can be covered approaches an on-the-go capability.

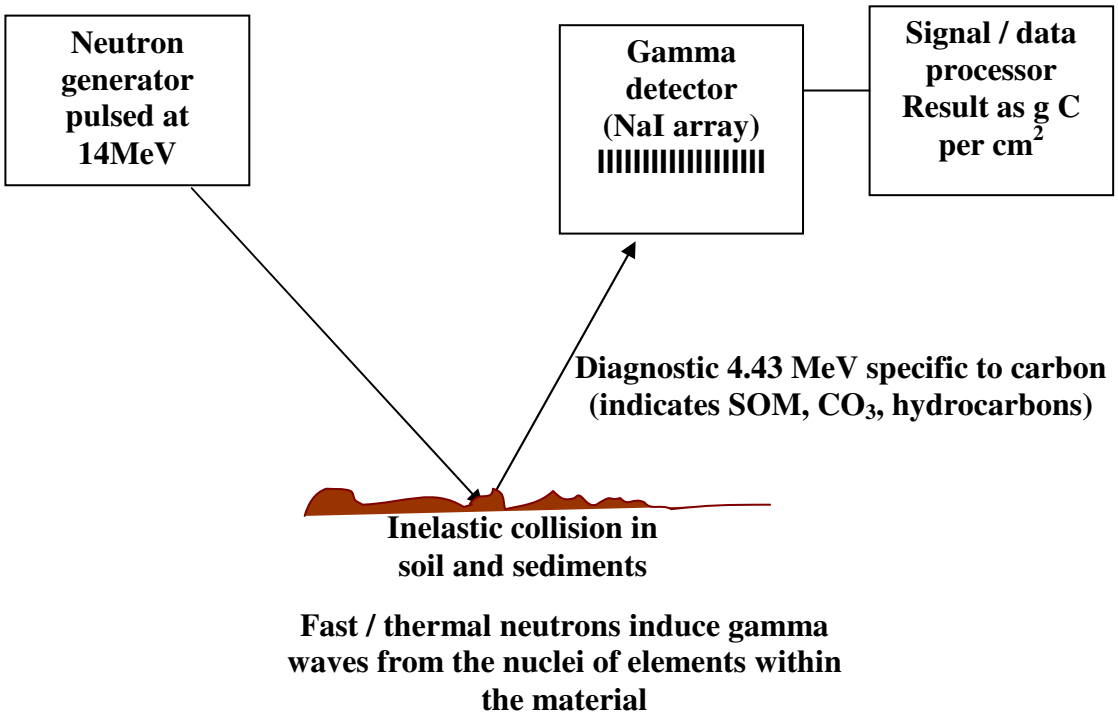

Figure 1-3 Schematic diagram of the prototype system used for soils described by Wielopolski et al., 2010; 2012 .

On the question of the most promising alternatives, according to R. Lal (Ohio State University) the application of the inelastic neutron scatter technique to soils represents the future in soil C determination (pers. comm., July 2012). According to their recent paper Wielopolski et al. (2011) there are still several issues to address:

a) The mass / volume relationship for INS and DC are at odds
b) C varies with depth and laterally so intensive scanning is required to reliably indicate changes over time

The real advantage is that heterogeneities in soil C are easily integrated by the $1m^2$ footprint (controlled by size of detector array where each acquisition period takes 60 min) and a depth penetration currently at 0.3m. Results from this preliminary work (on soil which included >2mm) indicated that soil processing may not be required which is a major advantage although, root C and total C may need to be correlated / calibrated separately. INS offers the potential advantage of C responses that are independent of mass (and bulk density) providing an absolute C value for a given volume per area which makes it a good fit with IPCC guidelines (IPCC, 2006). This is an important consideration because it circumvents issues associated with compaction. INS can also be used for other elements, Ca being one of them, enabling correction for soil carbonate. Although, element-specific responses are reliant on discrete energy inputs which vary according to the element.

Differentiating Soil Carbon Forms

This aspect is important to consider in how it relates to soil sampling and processing procedures employed for soil C surveys. It enables a clearer definition of soil C within the framework of C sequestration, SOM dynamics and global change. It revolves around the issue that the variety of C forms, react and mineralise at different rates (Table 1-1). From a sequestration point of view accumulating a greater proportion of more resistant material is obviously more desirable. These have been extensively discussed in more recent reports examining soil C sequestration and what protocols might be adopted (Walcott et al., 2009; Capon et al., 2010)

Carbon forms – their diversity

Calcium and magnesium carbonates can be significant in soils and according to Batjes (1996) almost half of the soil C stocks can be attributed to this source. These are thought to be stable over millennial timescales but this ignores the possibility of carbonate amendment or subsequent changes to pH (resulting from C building strategies) that may alter the equilibrium of inorganic soil C stocks. Apart from other mineralised forms, such HCO_3^- (aq) and CO_2 in the soil pores, the un-mineralised C is within a continuum of related organic matter of varying stabilities. Under certain Eh (i.e. oxidation potential measured in mV

against a reference hydrogen electrode) conditions other gases can be evolved in trace amounts (Smith and Dodwell, 1973) but this is more of concern to GHG emissions studies for the various land use practices. Conventional soil sampling processes themselves are likely to incur loss and alteration of volatile and less stable organic matter such as gases, short-chain aliphatic acids and some carbohydrates. The major focus must be on the primary quantities that make up soil C stocks and the dynamics of its major pools.

Table 1-1 A simplified representation of soil organic C forms and residence times (after Walcott et al., 2009 and their references).

Active Pool	Recent SOM	Annual
Slow Pool	Humified SOM	Decadal
Passive / inert Pool	Mineral complexed SOM and pyrogenic C	Centennial

Soil organic matter (SOM)

SOM is a collection of heterogeneous organic materials that are related in some way. It comprises varying proportions of carbohydrates, lipids and proteins primarily derived from plants and the soil microbes that utilise them. While simple sugars contain about 42% C and an average of the main plant organs typically closer to 49% C (representing the range for recent OM), this value increases with maturation of SOM in the soil (to over 58%) through the loss of H (but decreases relative to nutrients, discussed later). SOM itself was traditionally seen as strongly aromatic (based on older methods of analysis) until ^{13}C-NMR revealed that carbohydrate was dominant (Barron et al., 1980). Further advances in the technique allowed other chemical structures (proteins, carbohydrates, lignin and aliphatic material) to be assigned (Nelson et al., 1999).

At any one time, SOM is likely to comprise varying proportions of carbohydrates, lipids and proteins primarily derived from plants and the soil microbes that utilise them. Both recent (labile) and mature OM impart important soil functions. Humification of OM is accompanied by increased N and P relative to C and is an important factor contributing to soil fertility. Labile OM high in N directly supports soil biology and nutrient cycling whereas C-rich mulches such as straw lead to competition for limited N as microbes decompose biomass with possible priming effects for the remaining stable OM. While humified OM appears important to long term fertility, a certain proportion of labile OM has been positively correlated with crop yield (Stine and Weil, 2002). This has led to several schemes (e.g. Blair et al., 1995; Hoyle et al., 2006; Weil et al., 2003) that make use of selective oxidation with mild reagents (e.g. 0.025 M $KMnO_4$) to relatively quantify bio-available organic matter.

Important plant components such as lignin can persist in soils for significant periods. Lignin is similar in composition to cellulose and comprises about 25% of woody plant tissue providing strength to cell walls. Carbohydrates decompose to simpler sugars but the relatively strongly bonded units in lignin make it very resistant to decay. This coupled with its relative abundance from plant material means that along with its breakdown products become a significant part of OM in soils and sediments. When lignin eventually breaks down it adds to humic substances (HSs) via the intermediates; unsaturated aromatic alcohols and polyhydroxy carboxylic acids adding to the HS pool derived from various organic sources (Brownlow, 1979). Chemical degradation of lignin by CuO oxidation has been used to study the degree of OM humification (Koegel-Knabner and Ziegler, 1993) but ideas on the precursors for humic substances (HSs) continue to evolve and include plant starches (refer to online article by Susic, 2003. It is still not clear whether the collection of heterogeneous SOM structures fit a polymer model (coiled macromolecules) or supra-molecular model (clusters of smaller units connected by H bonds), as discussed by Sutton and Sposito (2005) who also point to a two compartment system (MacCarthy et al., 2001) made up of more resistant and less resistant humic substances. According to Hanninen (2010), humification may be a de-aromatisation process resulting in predominantly aliphatic compositions. Irrespective of their exact nature, these humic materials are likely to vary in stability (residence times) depending on the environmental conditions and degree of protection (i.e. landscape and soil type). The latter influence has been thoroughly articulated by Schmidt et al. (2011) who review the diverse forms of SOM.

Another prominent soil C group that is not so readily determined is pyrogenic or black carbon (BC) so called because of its often charcoal appearance. These comprise soots, chars and aromatised C remaining after woody material is heated to high temperatures such as through wildfires where conditions become oxygen poor resulting in partial combustion and pyrolysis. BC can also be beneficial having chelating properties due to residual charge acquired after oxidation of phenyl groups producing some ion exchange capacity. Charred C may be of lower significance in some soils but is likely to be widespread in many parts of Australia. Their components tend to be more stable in soils and therefore serve as a (more) effective soil C storage medium and because of this, BC should be measured as a separate type of soil C when considering whole SOM cycling.

Black C reports as organic C in standard C (elemental) analysers. As a necessity there have been a number of methods developed / used to specifically determine black carbon from soils as summarised by Hammes et al. (2007; 2008). These range from laborious microscopy and density separation to selective digestion / oxidation processes (e.g., Kurth et al., 2006)

with varying effectiveness in leaving all the BC as residue. They all operate on the principle that BC which is more resistant, remains after the removal of labile OM and can then be determined by elemental analysis. The CSIRO soils laboratory (Adelaide) previously used a UV-photo oxidation procedure to remove less stable OM (Skjernstad et al., 1996; 2002) followed by C analysis. Hydrogen pyrolysis (HyPy) is a current method that utilises high pressure H_2 to hydrogenate and remove labile OM in addition to *labile* aromatic C (ring sizes <7) for later elemental analysis of the residue (Roberts et al., 1995). These all share in common fairly involved processes coupled with often two or more sets of elemental analyses. The ^{13}C-NMR technique, which has its own complexities, is more direct by providing a ratio of aromatised to aliphatic C and as such is often used to complement / demonstrate the results of the other methods being developed.

Wet chemical extraction and separation

In contrast to the wet oxidation procedures used to determine the C content of SOM (discussed in the foregoing sections), extraction methods were long used as a way to remove parts of the SOM. The origin of extracting OM from soils dates back considerably, for example Sprengel, (1826) studied these products and historically humic substances (HSs) were extracted from soils and peats to obtain organic rich liquids. In the laboratory the method involved using alkaline solutions (0.1M NaOH) to extract these organic substances which could then be further separated into humic and fulvic acids by lowering the pH (Leeper and Uren, 1993). Alkaline extractions are still carried out today to isolate certain SOM components for their individual quantification and for further detailed study such as solid state ^{13}C NMR or infrared spectroscopy. The basic principles still apply only that the level of refinement with regard to solutions used (NaOH, NaP_2O and Na_2CO_3) and strengths used has become more sophisticated (Tatzber, 2007) enabling the extraction of selected fractions for detailed study. Similarly, instrument methods to separate and measure HSs directly continue to be developed for ion chromatography under various experimental conditions (Hutta et al., 2011). According to studies by Tatzber et al. (2009) humic acid extracts appear to be a representative pool of SOM. However recent studies have questioned such a view, with humic substances merely a product of the extraction procedure rather than an actual component of SOM (Stockmann et al., 2013). Contemporary works have proposed the combination on both physical and chemical fractionation methods (e.g. Zimmermann et al., 2007a). Physical fractionation is mostly used to isolate soil C which is related to soil structure, or protected inside soil aggregates (Angers and Caron, 1998). This requires arbitrary application of energy to disperse the soil aggregates and separation of fractions by density or size.

Soil C and Grainsize: What is the Case for Soil Pre-Processing?

A great deal of time and effort in soil C analysis goes towards soil preparation and processing, although much of it can be mechanised using for example electric puck mills. There are several explanations used to justify the focus on fine size fractions with respect to soil C analysis that prompts a detailed examination.

Soils submitted for elemental analysis (i.e. dry combustion) are generally ground and sieved to pass 100 or sometimes 50μm particle size. Historically the basis for refining soils to these small particle sizes prior to dry combustion appears to be uniformity (Rayment and Lyons 2011; Smith and Tabatabai, 2004). This has evolved as the standard method for soil processing over the last few decades mainly because the sample capacities of these instruments are so minuscule (more appropriate to the analysis of pure substances) that including coarser material lowers homogeneity and potentially the precision of analyses. Due to such studies as by Tabatabai and Bremner (1970) who originally recommended using <150μm sieve fractions for reasons of precision, such processing methods seem to have become entrenched as a standard. Moreover, their data actually reflected similar reproducibility for the <420μm fraction with C present throughout all size fractions although commonly tends to be more abundant in the fine silt particle sizes (Anderson et al. 1981; Ladd et al., 1985). What does this mean for the quantitative accuracy (% total C) of a given unit of soil using only that particular fraction? Should values be corrected (a dilution) for the mass of coarser material present (so called gravel correction) and how much C is contained in the discarded portion? These are questions seldom dealt with – but obviously it must affect the final outcome and only through consistent methodology can any data be comparable (Hedley et al., 2012).

Clearly, aside from chars, SOM is a diverse set of organic materials with different stabilities as indicated by wet chemical and other analyses. The issue of how OM is humified (degraded and matured) and how it is preserved in various soil types and mineral components is an important aspect because it has a bearing on how to proceed with soil treatment before analysis especially in the context of C sequestration where long lived SOM is more desirable. There is some evidence that occluded C is older and or possibly more stable. Contact with clays, incorporation into microaggregates and mineral defects offers some physical and chemical protection for OM (Balesdent et al., 2000; McCarthy et al., 2008; Skjemstad at al., 1996; Wilson et al., 2009). This chemical protection probably increases with maturation (given a constant soil environment). Intuitively, sandy soils are expected to be poor in C and fine grained soils higher because of greater moisture, nutrients

and retention. This has been supported by various studies where resistant plant remnants were concentrated in the sandy particle sizes and the microbial wastes in the clays (Ladd et al., 1996). Similarly greater amounts of microbial biomass and aliphatic compounds have been noted in the finer particle sizes (Spain et al., 1990; Baldock et al., 1992; Nelson and Baldock, 2005) and these aliphatic compounds appear to be more recalcitrant according to Baldock et al. (1997). Such fine particles have greater surface areas to support microbes, as have mineral hydroxides and oxy-hydroxides (Oades, 1989). Furthermore the tiny soil interstices may be water filled so that diffusion of O_2 is much slower (Baldock et al., 2004) than through open pores - an important consideration in the context of biodegradation of OM. Thus fine and adsorptive particles offer both places to be attached and physical protection from more rapid chemical degradation.

There is a sound basis for the separation of coarse organic material from soils. The removal of material >2mm (McKenzie et al., 2000) excludes recent OM such as roots along with gravel sized material. These are also termed surface plant residues (SPR) and buried plant residues (BPR) according to the separation scheme described by Baldock et al. (2004). If included as part of sequestration inventories, these fast pool (see table 1-1) components can likely complicate any soil C accounting offset scheme. Being present in various states of decomposition they also comprise root material with masses that vary according to land use, i.e. crop type. It is reasonable to expect degrading OM to become more finely divided and therefore a broad fractionation according to grain size by sieving should achieve some separation of recent from *older* C (as with the >2mm and hand picked fragments). Then what of recent microbial wastes and cellular material that pass the finest sieves and as these decompose further, their gradual polymerisation (D. Guest pers. comm. November 2012). A number of SOM fractionation schemes have evolved such as those of Zimmermann et al. (2007a); Baldock et al. (2004) with further development (Janik et al., 2007) to isolate pools with different levels of stability. Attempts have been made to equate these within the framework of the Rothamsted carbon model where each contributes to soils by way of metabolic C cycling, nutrient regulation and C storage. The latter method involves primarily physical techniques comprising sieving and settling (after dispersion and pH adjustment) to yield the following:

Particulate organic carbon (POC)	in size fraction >53µm
Humus organic carbon (HOC)	in size fraction <53µm
Resistant organic carbon (ROC)	by ^{13}C NMR or remaining after UV-photo oxidation of disaggregated soil

where POC is particulate organic C, HOC is more stable humified OM associated with the fine soil particles and ROC is fairly inert C, such as charred material (usually very fine) resulting from wildfires. These subdivisions resemble those in table 1-1.

As SOM develops and ages, C diminishes relative to N, making the C/N value a useful indicator of stabilisation. The trends observed in C/N ratios with decreasing size fractions add support to this (Gerzabek et al., 2006 and references therein) as the example on table 1-2 shows (from Magid et al., 2002). Similarly C/N are expected to decrease down the soil profile reflecting the disappearance of less stable C with advancing humification. The final SOM composition would probably reach C/N values in the order of 5 or 6, a natural floor set by the remaining microbial compositions (Aponte et al., 2010).

Table 1-2 Changes in C/N with soil size fractions reproduced from Magid et al. (2002).

Size fraction (μm)	ρ (g/cm^{-3})	C/N ratio	std error
1000-4000	<1.0	37.30	2.50
	<1.6	21.30	2.80
100-1000	<1.0	26.20	1.40
	<1.6	23.80	1.00
53-100	<1.6	17.20	0.40
	<1.6	13.20	0.30
<53	<1.6	21.00	0.80
	<1.6	7.90	0.30
			(n=4)

Note to put in perspective sand, silt and clay are classified thus:
Fine sand 20 – 200μm
Silt 2 – 20μm
Clay <2μm

SOM Diversity – The Best Technologies

New life into thermal analysis and the LECO models RC 412 and 612

Thermogravimetric analysis (TGA) is an old technique developed in the early part of the 20th century. It was mostly applied to the study of soil mineral decomposition but there were some notable investigations into SOM (Turner and Schnitzer 1962; Schnitzer et al., 1964). TGA is a common piece of instrumentation in chemistry laboratories where it is used to study the thermal stability of polymers and various oxidation (mass gain or loss) reactions of synthetic or natural materials. Along with differential thermal analysis and differential scanning calorimetry which measure heat flow, together these comprise a very useful toolkit in distinguishing the chemistry of substances. With the appearance of more sophisticated software and precise electronics these instruments have attracted renewed interest for application in soil research over the past decade or so with a particular focus towards OM characterisation and measurement. In addition there are more opportunities to couple these with for example, bench top mass spectrometers providing a greater combination of signals

to interpret or to screen out ambiguous information. TGA is such a powerful technique because it allows precise control of reaction conditions (atmosphere and a heating program) while monitoring any slight increase /decrease in mass using a microbalance. It is a simple exercise to estimate for a whole soil with minimal processing the amount of free moisture, inorganic and organic C from a single run (Boyle, 2004; Kasozi et al., 2009). Current research has focused on ways to better analyse TGA results from soils and composts to assist our understanding of SOM dynamics, a major contributor in global change dynamics. There are some inherent difficulties in using TGA for soil analysis related to overlapping events but further investigation and development has been well justified. A key observation from early studies (Schnitzer et al., 1964; Schulten, 1996) was that humic compounds were found to be more thermally stable than their plant derived precursors – and this makes TGA study worthwhile in the context of soil studies. The TGA technique, which has some common principles with LOI, has been taken up experimentally in the current study and is expanded in detail in the following section/s. Because thermal analysis instruments are such fine equipment comprising microbalance sensitive to 0.0001mg and precise heating furnaces they come at a capital cost in the region of AUD$35,000 or more.

The LECO models RC 412 and RC 612 (temperature programmable C analysers) were designed to differentiate C in industrial applications by continuous or sequential oxidation. They use a controlled heating rate to characterise the thermal breakdown of a material (refractory continuum) and can be likened to the TGA method by their programmable heating. Instead of sensitively monitoring mass change it monitors the evolution of C (CO_2) and H (water) using infrared detectors. This enables the interpretation of particularly organic related events where both water and CO_2 are the main products. It appears to be potentially one of most useful approaches for the simultaneous characterisation and quantification of SOM by thermal means. While such instruments may have been designed around industrial applications it has been largely under-utilised (or overlooked) for distinguishing inorganic C from SOM and within SOM with very few exceptions. Schwartz (1994) and then Beyer et al. (1998) demonstrated the automated separation of carbonates and SOM. Only since the work of Kristensen (1990), who devised a TGA based index where relatively refractory-C was expressed against labile material as an indicator of SOM maturity, has an equally simple (and effective) approach been put forward. This separation into thermal regions by Kristensen (i.e. Rp or Exo 1, 2, 3 etc.) for each mass change was never fully rationalised which was one of the main criticisms of the method according to Plante et al., 2011 (and others). This instrument circumvents this issue to a large extent by producing discrete C-only signals which can be varied by the heating program. Beyer et al. (1998) already recognised that the method might potentially detect differences in OM turnover /

humification characteristics between soils (example of a mineral soil on figure 1-4). These are not current generation instruments and may be partly the reason they have not been used to further explore the possibility of differentiating SOM or at least separating components such as BC from SOM. This has only been exceeded by a more recent development that uses multi element scanning thermal analysis (MESTA) and has been tested on coals and chars. This allows diverse C to be separated thermally while concurrently being measured by continuous elemental analysis.

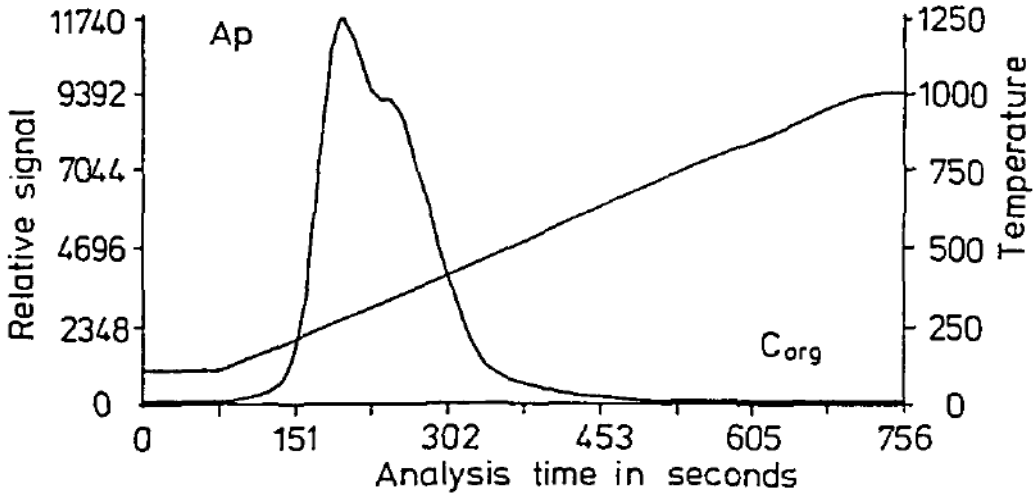

Figure 1-4 SOM analysis by Leco model RC 412 showing C pattern as it is released over a temperature program of 13 ^{o}C per min (reproduced from Bayer et al., 1998).

Infrared Spectroscopy

Historically, the nuances in colour have been an important descriptor for soils providing a means of inferring certain characteristics such as presence of Fe, hydration state or even organic matter providing some basis for classification. The use of the Munsell chart to help describe soil properties is a good example (Minasny and Hartemink, 2011). In the same way that the visible spectrum is used to distinguish and infer a great deal about the nature and composition of a material (soil), the same applies to other parts of the electromagnetic spectrum, only that the human eye cannot detect them. A material absorbs specific frequencies over the infrared spectrum which gives away much about its chemical composition through particular bonds vibrations from within functional groups. There are a number of regions over the visible - near infra red (Vis-NIR) band (400-700-2500 nm) and especially throughout the mid infra red or MIR (2500-25000 nm) wavelength band that relate to the mineral and organic constituents found in soils. This has presented huge opportunities in being able to directly identify and quantify numerous chemical (and physical) properties

from whole soils without complex and lengthy procedures. In fact it has the capacity to be a single analytical tool to measure and predict a number of agricultural soil properties and numerous studies over the last decade or so have indicated its great potential (Janik et al., 1998; Janik et al., 2007 and others).

As a field portable spectrometer, NIR has proved more reliable in comparison to MIR where spectra are much more particle size and moisture dependent (Kusumo et al., 2011; Reeves, 2010). Importantly the NIR spectral range is not influenced by quartz minerals (Merry and Janik, 2001), a significant component of mineral soils and so removes this as a potential source for any overlapping (and ambiguous) absorptions. Furthermore the higher energy of NIR can be an advantage when penetrating in situ soil where the usual preparation to refine soils, as afforded laboratory MIR analysis, is obviously not practical during field application. The 1650–2500 nm range is often cited as the most relevant for measuring soil organic C (Bellon-Maurel and McBratney, 2011).

MIR spectroscopy is a powerful analytical technique having a great number of vibrational absorbance regions (primary and secondary) hence its prominence in chemistry laboratories. So much so that absorbances in the fingerprint region (1430 - 600 cm^{-1}) can be difficult to interpret due to the complex absorptions. For example the C-C bond has absorptions over a wide range of the region and would not be specifically informative when analysing for example charcoal type materials. In contrast, at the higher energy levels of the group frequency region (4000 - 1430 cm^{-1}) there are some important diagnostic positions relevant to organic materials (diatomic units). The exact frequency / wavelength at which these occur vary according to the compound hosting these bond units. In the analysis of SOM some of the most important bonds are the aliphatic and aromatic C-O double and single bonds (ca. 1670 and 1430 cm^{-1}) (Tatzber et al., 2007; Zimmermann et al, 2007b). The aliphatic C-H bond (2980 - 2850 cm^{-1}) may not be as compound specific and therefore regarded as less diagnostically useful but for TOC analysis is an easily 'picked' indicator of OM abundance and has been used to quantify labile SOM (Demyan et al., 2012). In addition the proximate broad O-H band (over 3000 cm^{-1}), while present in organic compounds, becomes overwhelming where mineral matter, especially clays occur, as in the analysis of whole soils and size fractions. This huge absorption band can distort or obscure the C-H bond vibration (in the context of SOM studies). Beyond this region (4000 to about 6000 cm^{-1}) lie the combination tones (interacting vibrations) and overtone region which simply means the next order harmonics related to the fundamental vibrations in the MIR. One way of dealing with interference due to coinciding absorptions from inorganic and organic bonds was to use ash subtraction (in effect removing the mineral imprint). However, the real benefit of this

approach has recently been reassessed (Reeves, 2012) concluding that the variable ashing effects from different clay minerals is not at all helpful by therefore leaving narrower ranges.

These instruments commonly incorporate a method of diffuse reflectance infrared Fourier transform spectroscopy (or DRIFTS). It is optically designed so that scattered reflection from the soil surface layer is collected by parabolic mirror over a wider angle. This has made possible the analysis of soils placed neat or directly into the sample cups whereas previously it was necessary to dilute the soil with a non absorbing media to counteract specular reflection. Halide salts such as NaCl and KBr are transparent to IR light and have been routinely used by mixing or grinding sample material into KBr at a rate of generally 1 to 5% and pressed into discs. While there can be improvements to specular reflection there are various effects (e.g. attenuated absorbances, sloping baselines) that accompany both the methods and these have been reviewed for soils (see Nguyen et al., 1991; Reeves 2003). Overall the amount of pre-treatment a soil should undergo before analysis is comparatively modest making it an attractive analytical technique for large sample numbers. Soils (and other materials) analysed by FTIR need to be of a particle size commensurate with the incident infrared beam which has a wavelength of 0.8 to 25μm. For this reason soils are usually ground to a fine powder using a timed agate mill and this has become a routine industry practice (Janik et al., 2007; Janik et al., 2009; Gerzabek et al., 2006). Both the refining of particle size and the addition of KBr can improve DRIFTS spectra where the latter is an advantage where very limited material is available for analysis. In addition to measuring total OM in soils it can also be used to determine specific forms of C such as carbonates, condensed C or humified OM (Janik et al., 2007) based on calibrations after separating and analysing these C fractions (e.g. Baldock et al., 2004).

Spectral Data Analysis and Chemometric Models

Most methods of chemical analysis such as gas chromatography can adequately use univariate analysis to quantify the analyte in question. In other words it is based on a unique response (peak area) dependent on the concentration present easily described by a linear algebraic relationship. This is usually not the case in spectroscopic analysis where a number of (spectral) regions can be influenced by the concentration of the analyte but according to the Lambert-Beer law a spectral response can be linearly correlated with concentration. Univariate analysis runs into problems with multidimensional data (numerous responses) and when they are not fully resolved (e.g. poorly separated peaks) as seen with FTIR or LIBS which are best served by a multivariate method.

Taking a principal component analytical (PCA) approach allows relationships to be drawn between two matrices: one with x-variables (running into thousands of spectral reflectance), the other with a y-dependant variable comprising analyte concentration/s (usually equal to the sample size). The number of variables (absorbances) usually far exceed the number of soil samples being studied. The classical method is to compress the number of relevant components to a workable set of data (principal components) the number of which should be robust enough to capture all the important spectral events (rank) associated with the full range of analyte in the samples. This means it is not necessary to identify specific peaks (e.g. C-H or C=O bond stretching) but to encapsulate all the relevant regions for these functional groups (eg., using the Bruker Instruments software). This is because continuous frequency bands can be subtly influenced by the concentration of one component. However to go beyond clustering of associated data and build a mathematical relationship between the spectra and known concentrations requires a partial least squares regression (PLSR) analysis. Rather than just simply predicting a class property, PLSR allows for the prediction of such a continuous variable.

Before measuring unknown samples, calibration spectra have to be obtained and analysed and it is generally accepted that a minimum of 20 to 30 are used and that their characteristics cover the expected range (concentrations, soil types) to ensure a robust model. For example the presence of CO_3 can affect linearity (Reeves, 2009). PLSR analysis is a form of 'supervised' training where those soils with known characteristics or concentration comprise the training set. Data can only be properly analysed quantitatively when spectra of differing baselines or amplitudes (due to optical artefacts) unrelated to concentration can be made comparable. Normalising and smoothing the data can achieve this while acquiring the mathematical derivative (for points) helps overcomes scale and noise issues and emphasises significant spectral features (Wehrens, 2011). From there the data is transformed into a set of Eigen vectors that range in significance with respect to the analyte of interest (rank). Building the model on too many components observes more features than may be required or even includes noise or unrelated events and results in overfitting. The reverse leads to under-fitting. According to Wehrens (2011) there is no hard and fast rule for the correct sequences of data processing (smoothing, differentiation etc.) as this may vary for different instruments / techniques and materials but rather to optimise the process to obtain the most reliable predictive model (lowest standard error of prediction). The aim is to produce a 'best fit model' but it is only as good as the calibration that built it and as pointed out in a review by Bellon-Maurel and McBratney (2011) the value of bias in the data is a good indicator. Other statistical approaches that achieve the same outcome can also be used such as so called rule

based algorithms. These generate association rules by extracting co-occurrences that link, in this situation, spectral information with the concentrations of analyte.

Gravimetric and volumetric carbon concentration

Generally, soil C measurements give a gravimetric concentration (kg C per kg of soil, or also usually expressed in percent mass). However the C stock requirement is reported in kg C per m^2. This requires the knowledge of bulk density:

C stock (kg/m^2) = C concentration (kg/kg) x Bulk density (kg/m^3) x depth (thickness) of soil sample.

The requirement for bulk density measurement is an additional sampling effort and source of uncertainty in conventional analysis. There is also another problem where there could be differences in bulk density from different sampling dates (e.g. due to cultivation practices) which will affect C stock calculation. This issue is discussed in McBratney and Minasny (2010).

NIR spectral calibration is usually performed for prediction of total soil C content (on a gravimetric basis). Several studies also differentiated the prediction into soil organic C, and inorganic C (Bellon-Maurel and McBratney, 2011). In order to reduce the uncertainty in having to estimate bulk density, Bellon Maurel and McBratney (2011) suggested directly predicting the volumetric C concentration ($kg\ m^{-3}$) from spectra. Other researchers have attempted the calibration / prediction of C content (volumetric basis $kg\ m^{-3}$). The group in France (IRSTEA) found that the prediction from NIR spectra on a volumetric basis is slightly lower than gravimetric, however the prediction is still reasonable ($R^2 = 0.66$). Researchers in New Zealand have made a direct prediction of C stocks (in tonne ha^{-1}) from NIR spectra with good result ($R^2 = 0.75$) (Roudier et al., 2013). These efforts have further highlighted the pressing need and potential to develop a method that measures soil C concentration both on the gravimetric and volumetric basis.

Another exciting concept is proximal hyperspectral / multispectral imaging incorporating the advantages of rapid scanning in a way of overcoming the limitations associated with point analyses (Buddenbaum and Steffens, 2012).

It can be concluded that the emergent proximal technologies encompassing in situ nuclear, laser or infra red spectroscopic methods should in due course revolutionise soil C stock determination once fully developed and tested. But in the interim, measurement methods can continue to rely on improved existent techniques that are able to adapt to the varying scales. Similarly, the ability to readily observe patterns of different soil C forms have major

uses in the study of soil C dynamics and in this regard thermal analytical approaches appear to offer a simple and accessible means to do this. The following table summarises the most significant advantages and disadvantages for the methods reviewed.

Table 1-3 Merits of the reviewed systems.

Laboratory methods for soil C analysis	Strengths	Weaknesses
Wet chemical oxidation / extraction	selectively targets OM pools	destructive, inaccurate for TOC, does not react with all the stabilised OM
Standard dry combustion	unequivocal and precise, TOC	unable to distinguish C form, high capital & running costs
Temperature programmable dry combustion	differentiates main C pools	high capital and running costs
Thermal analysis	differentiates mass changes, i.e. possible main C pools	overlapping ambiguous events, opposing endo and exothermic responses
Hyphenated thermal analyses	differentiates main C pools depending on detector	limited by detector used, catalysation issues, high capital costs
In situ / Proximal C analysis		
Proximal inelastic neutron scatter	non destructive, area coverage	still under development, shielding of neutron pulses
Proximal laser induced spectroscopy	rapid and direct	still under development, need to generate laser
Proximal spectroscopy	large area coverage	point analsyes, surface penetrataion, large calibration data sets
Hyperspectral imaging	large area coverage	poor spatial resoltion and limited depth, higher acquisition costs

Note that the group of laboratory based methods generally require soil preparation while proximal methods require large calibration sets.

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CHAPTER 2 –
SOIL CARBON DETERMINATION BY THERMOGRAVIMETRICS

Statement of Contribution of Co-Authors

This chapter has been written as a journal article. The authors listed below have certified that:
1. they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
2. They take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
3. There are no other authors of the publication according to these criteria;
4. potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit, and
5. They agree to the use of the publication in the student's thesis and its publication on the Australasian Research Online database consistent with any limitations set by publisher requirements.

In the case of this chapter 2, the reference for this publication is:

Robert Pallasser, Budiman Minasny and Alex B. McBratney 2013. Soil carbon determination by thermogravimetrics. PeerJ 1:e6; DOI 10.7717/peerj.6. Contributors	Statement of contribution
Robert Pallasser Signature & Date:  29 August 2013	Conceived and designed the experiments, performed the experiments, analysed the data, contributed reagents/materials/analysis tools, wrote the paper.
Budiman Minasny	Conceived and designed the experiments, contributed analysis tools, wrote the paper.
Alex B. McBratney	Experimental design, conducted experiments, data analysis

Introduction

Soils are significant global reservoirs for carbon (C) and are therefore receiving a great deal of attention for their capacity to offset the increasing levels of atmospheric CO_2. In addition there is the immense value of having higher amounts of organic matter (OM) for improved soil function and structure which have a positive outcome in terms of food security through resilience against degradation. To assess the effectiveness of land use practices where temporal changes in soil carbon building can be small, measurement instruments / methods need to be sufficiently sensitive and provide robust values. Added to this, soil carbon content can be highly variable (Goidts, Van Wesemael & Crucifix 2009) from one place to another and also occur in a variety of forms with different stabilities and therefore residence times (McCarthy *et al*. 2008 and references therein), a key issue for soil carbon storage. As a consequence, soil carbon analysis methods have become a lively area of interest, especially considering the need to map large areas and the inherent variability.

Dry combustion by elemental analysis of soils is often used to provide precise total carbon determinations on fairly small amounts sub-sampled. On the other hand the loss-on-ignition (LOI) method, which is widespread due to its low cost simplicity, allows larger amounts to be tested appropriate to the scale of the task at hand. However the technique has attracted a great deal of discussion about its accuracy and equally OM to C conversion factors have been controversial (Pribyl, 2010; Sutherland, 1998 and others). By this technique, previously dried and weighed soils are heated in a muffle furnace to obtain the mass of material lost which can be transformed to % carbon by reported conversion factors. More accurate factors can be derived from a 'calibration' set of soils (via elemental analysis) from the study area which take account of local conditions but there is no set protocol for temperatures (can be 500^{o}C plus) and durations which vary between soil laboratories.

Thermogravimetric analysis (TGA) is a method traditionally used for the mineral (clay and oxides) components of soils, generally after the removal of OM. Although the variety of carbon forms present in soils, which can range in their kinetic behaviour, can also be broadly distinguished (and quantified) using controlled heating rates (see examples in Laird *et al.* 2008). For example $CaCO_3$ which begins to degrade after 600 ^{o}C, is readily separated from organic-C (Kasozi, Nkedi-Kizza & Harris 2009). Thus the thermal distribution of all components in a whole soil, including those that are carbon-bearing, can provide a fingerprint characterisation as well as quantitative information. Applications of this method to tease out differences within the organic fraction in soils have been relatively small but a number of researchers (notable examples Dell'Abate, Benedetti & Sequi 2000; Dell'Abate, Benedetti & Brookes 2003; Siewert 2004; Manning, Lopez-Capel & Barker 2005; references

in the review by Plante, Fernandez & Leifeld 2009) have more recently reported TGA related investigations on either soils or composted materials**.**

In this study TGA and related techniques were applied to a range of whole soils to obtain a better understanding of SOM thermal stability and hence distribution over the heating program and what this means for loss-on-ignition methods. Inorganic reactions were not followed up in detail here but instead were an overall consideration when evaluating thermal methods used to measure organic matter. A database containing comprehensive information on LOI, carbon and texture was also analysed to support the observations from thermal studies and to indicate the influence of clay content when measuring C using LOI methods.

Materials and Methods

Collection and preparation of soil subsamples

A cross-section of soils (36) were obtained from different areas in NSW, Australia (Table 2-1). Soils were extracted from the field using hand or mechanized corer and intervals removed to plastic tubes for transport and storage. Some of the soils included here were collected as part of other studies hence some variation in depths sampled (indicated on Table 2-1). Representative amounts were sub-sampled for replicate analyses (>2 per site) to confirm differences observed for these localities which varied considerably by soil type and the amount of OM present. Samples were air and oven (40 ^{o}C) dried prior to removal of recent organic matter such as visible root material by passing a 2mm sieve (McKenzie 2000). For elemental and thermogravimetric analysis samples were then ground by mortar and pestle and dry sieved (<100 μm) to obtain homogenous material to facilitate the small sample loadings. In addition some soil references (Rayment et al. 2007) acquired through Proficiency Services Ltd. Hamilton, NZ were included in this study. A legacy dataset of soils (CSIRO National Soil Database) containing LOI, dry combustion and textural data (methods according to Rayment & Lyons, 2011) was also used.

Measurement of carbon by dry combustion using elemental analysis (EA)

Carbon concentrations were determined using a Vario-max Elementar CN analyser (Hanau, Germany) with 900 ^{o}C combustion temperatures (for detailed explanation of the dry combustion method, refer to Rayment & Lyons 2011).

Thermogravimetric analysis (TGA)

Pure TGA experiments were conducted on a TA Instruments 2950 thermogravimetric analyser which can heat up to 100 mg of material at variably programmable rates to over 1000 ^{o}C. Typically 60 to 100 mg of soil (equates to several mg OM) were suspended in a platinum pan (tare weight 360 mg) such that comparable amounts of C (ranging from <1% to 15%) were analysed. The incremental mass changes (resolution of 0.0001 mg) recorded over this program were processed using TA Universal Analysis 2000 software.

An oxygen-rich atmosphere was used (60ml/min O2 purging furnace and 40ml/min N2 purging balance) as it provides O2 in excess for SOM conversion to CO_2 which would otherwise degrade in an inert stream and potentially leave a charred residue leading to an incomplete mass loss (possible ghost signals) and underestimation. Evolution of non-combustible components such as interlayer water and OH- units or CO_2 from carbonates are unaffected by the type of atmosphere used during thermal decomposition. Samples were heated to 200 ^{o}C, held for 10 min (surface dehydration) and then ramped at 10 ^{o}C per min to 700 ^{o}C and finally held for another 10 min. Carbonatic soils were heated at the same rate but beyond 700 ^{o}C until completely calcined. Heating rates of 10 ^{o}C per min. from 200 to 600 ^{o}C provided the optimal resolution in soils.

Thermogravimetric analysis / mass spectrometry (TGA/MS)

To look more deeply into the various mass change events, thermal analyses on a small subset of soils were run in combination with mass spectrometry. TGA/MS was carried out with a Setaram setsys 16/18 thermobalance TGA connected via quartz capillary (in 150 ^{o}C jacket) to a Balzers Thermostar quadrupole mass spectrometer set at 70 eV (electron energy). This allowed major ion traces to be obtained (electron impact) along the entire heating program and is presented as mass/charge (m/z). Whole soils (approximately 40 mg) were suspended in a Pt pan and heated at the same rates as above in a stream of aerobic purge gas flowing at 40 ml/min.

Results and Discussion

Typical soil thermal patterns

Plotting the first derivative of mass changes (DTGs) over the heating cycle (time or temperature along x-axis) allows separate events to be more readily distinguished and assists in the measurement of their proportions. Using this technique, soils can exhibit thermal characteristics which may be affected by its type, management or sampling depth as shown in figure 2-1. These relative differences seen in the interval 15 and 70 min. run time (200 to

590 °C) are related primarily to the amounts of carbon (200 to 430 °C) and clay minerals (430 to 590 °C) producing the typical patterns. For example the trends shown here are easily attributed to the regular changes in texture and OM with depth (Conant, Smith & Paustian 2003; McKenzie et al. 2004).

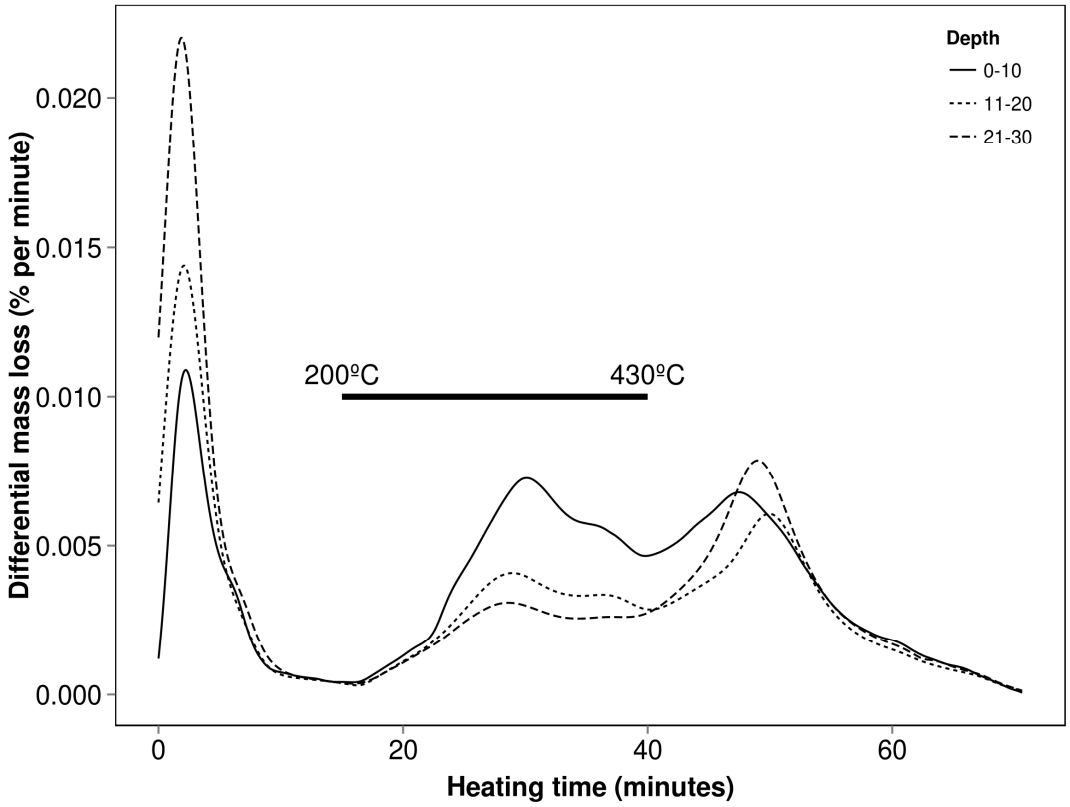

Figure 2-1 Typical soil thermal characteristics at various depths using the differential mass losses (DTG) obtained by TGA (here of a sandy clay loam at one site).

The mineral fractions of soils have been widely studied by thermal methods (Fanning, Keramidas, & El-Desoky 1995; Frost & Vassallo 1996; Hedley,Yuan & Theng 2007) providing a baseline for underlying inorganic reactions from micas and clays when considering organic constituents within whole soils. Mass changes occurring in the 430 to 600 °C interval appear to be dominated by the mineral fraction (reflected in figure 2-1) and furthermore when using an inert gas, mass changes in this interval are not noticeably affected indicating decomposition rather than oxidation. By contrast, mass losses over the thermal region 200 to 430 °C are significantly greater when aerobically purged, consistent with OM. Thermal decomposition of most carbonates is quite separate and obvious from other constituents occurring over 600 °C (Kasozi, Nkedi-Kizza & Harris 2009) with the exception of more soluble types such as siderite which have much lower decomposition temperature at around 500 °C (Alkac & Atalay 2008). During thermal analyses this would contribute to

(and possibly confound) the changes related to clay minerals but generally these carbonates are less common in soils and do not persist below pH of 9.5 (Rayment & Lyons 2011). It should be similarly noted that other minerals such as Goethite or Gibbsite which is more common in tropical soils, decompose in the 210 to 550 ^{o}C region (Kloprogge, Ruan & Frost 2002) along with OM and therefore have the potential to interfere with TGA analyses conducted on whole soils.

Quantitative TGA and dry combustion

A selection of eastern Australian soils were studied for their TGA behaviour and the principal mass losses quantified. Along with relevant soil information these have been assembled on table 1 with respective carbon contents found by dry combustion (elemental analysis). The soils are mostly carbonate free and comprise sandy clay loams (Camden, NSW), Dermosols (Hunter Valley, NSW), Vertisols (Liverpool Plains and Narrabri, NSW) and several local soils ranging in texture. The principal mass loss regions were obtained by the natural separations (condition specific) as described by differential mass loss curves or DTGs corresponding to the two temperature bands that correspond to organic and predominantly inorganic events contained in the 200-430 and 430 to 590 ^{o}C regions respectively (table 2-1). Some soils contained carbonates and this was readily measureable using this technique (indicated by mass changes in the 590 to 750 ^{o}C region).

Table 2-1 List of soils used for these experiments detailing mass losses from thermal analysis.

Soil and origin	**Landuse**	**Depth**	**% C**	**% mass losses**		
		cm		200 - 430^{o}C	430 - 590^{o}C	590 - 750^{o}C
Pilliga Chromosol 2	Crop	surface	0.2	0.7	1.9	
Hunter Valley Dermosol 3	Mixed farming	30-60	0.2	0.9	2.7	
Hunter Valley Dermosol 2	Mixed farming	30-60	0.2	0.9	2.8	0.7
Hunter Valley Dermosol 5	Mixed farming	30-60	0.2	0.7	2.3	
Hunter Valley Dermosol 4b	Mixed farming	60-100	0.2	0.8	1.7	9.8
Lansdowne Kandosol	Mixed farming	0-5	0.2	0.4	0.3	
Hunter Valley Dermosol 4a	Mixed farming	30-60	0.3	1.4	3.0	
Pilliga Chromosol 1	Crop	surface	0.4	1.1	3.1	8.0
Namoi Vertisol 2	Crop	10-30	0.5	1.1	2.5	
Narrabri Vertisol 1b	Crop	16-30	0.6	0.9	1.5	
Camden 2c	Lucerne	21-30	0.8	1.3	3.1	
Hunter Valley Dermosol 8c	Mixed farming	30-60	0.8	0.4	3.4	
Camden 2b	Lucerne	11-20	0.8	1.4	2.9	
Narrabri Vertisol 2b	Crop	16-30	0.8	1.4	2.2	
Narrabri Vertisol 1a	Crop	0-15	0.9	2.1	2.4	
Camden 3c	Lucerne	21-30	0.9	1.4	3.1	
Narrabri Vertisol 2a	Crop	0-15	1.0	1.5	1.5	
Soil standard 1	Reference material	unknown	1.0	1.3	3.8	1.9
Hunter Valley Dermosol 1d	Mixed farming	30-60	1.0	3.7	4.2	2.5
Hunter Valley Dermosol 8b	Mixed farming	15-30	1.1	0.6	3.7	

Namoi Vertisol 1	Crop	0-10	1.4	2.2	2.0	
Hunter Valley Dermosol 7a	Mixed farming	0 to 5	1.5	1.3	4.2	1.7
Hunter Valley Dermosol 1c	Mixed farming	15-30	1.5	2.5	3.7	0.6
Liverpool Plains Vertisol 2	Crop	surface	1.6	3.1	4.1	
Hunter Valley Dermosol 8a	Mixed farming	5-15	1.7	0.9	1.9	
Camden 3b	Lucerne	11-20	1.7	2.1	2.7	
Liverpool Plains Vertisol 1	Pasture	surface	1.8	3.3	4.8	
Hunter Valley Dermosol 7b	Mixed farming	15 to 30	1.8	1.4	3.5	
Camden 2a	Lucerne	0-10	1.8	2.2	2.5	
Camden 1	Lucerne	0-30	2.0	2.0	2.3	
Camden 3a	Lucerne	0-10	2.3	3.1	3.1	
Hunter Valley Dermosol 6	Mixed farming	25	2.4	2.7	2.6	tr
Soil standard 2	Reference material	unknown	2.6	4.5	1.7	
Hunter Valley Dermosol 1b	Mixed farming	5-15	2.6	3.6	5.7	
Hunter Valley Dermosol 1a	Mixed farming	0-5	3.4	6.2	6.6	
Soil standard 3	Reference material	unknown	4.0	7.5	2.0	
Sydney Basin Kandosol	Vegetated	0-5	6.1	9.5	1.5	
Sydney Basin loam	Horticulture	0-5	9.5	13.8	7.1	1.1
Sydney Basin sandy loam	Horticulture	0-5	9.6	15.8	6.3	1.2

The elemental analyses (% C) obtained for these various study soils (n=39) were most closely correlated (figure 2-2) with mass changes occurring over the 200 – 430 ^{o}C indicating this to be the (most) significant thermal region for SOM release. A slope of 0.62 (R2 = 0.95) provides an approximate conversion factor for these different soils that is a little higher than the traditional factor of 0.58 (van Bemmelen 1891) and could be suggesting that not all the C is accounted for by this region alone, in those soils. Nonetheless it highlights the most important part of the thermogram for the bulk of OM and is consistent with what can be observed in thermogravimetric soil characterisations under aerobic conditions. While this region probably encapsulates the most labile OM, any shortfall indicated by this comparison is more thermally recalcitrant C such as black carbon (char) and quite likely, equally resistant organic substances tied up with the expected clay mineral event which follows over the 430 – 590 ^{o}C. Very few of our soils have high C contents but removal of the extreme points on figure 2 lowers the factor to 0.57 (lowers coefficient of determination to 0.84) but it is anticipated a greater number of high C soils would only reinforce that existing trend. A similar approach taken by Gerzabek et al. (2006) on a small set of soils yielded a value close to 0.59 (mass loss maxima 330 and 440 ^{o}C) while Plante et al. (2011) obtained a considerably lower value (0.49) for the slope of regression but the temperature interval included mass losses to 600 ^{o}C (possible contribution from other reactions).

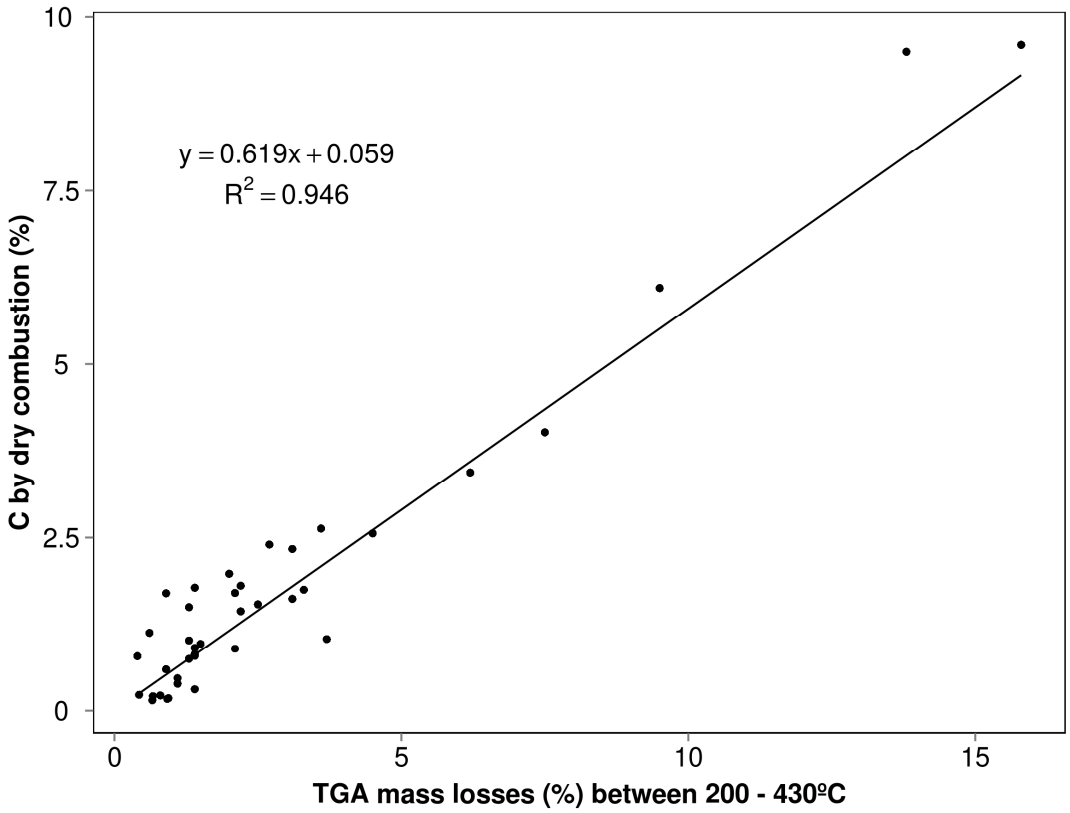

Figure 2-2 Relationship between TGA mass losses between 200 and 430 °C interval and the total carbon content determined by dry combustion for the soils on table 1 (relative to van Bemmelen line obtained by multiplying these mass losses by 0.58).

There is some deviation or scatter of the data points relative to the calculated (TGA 200-430 multiplied by 0.58) van Bemmelen line. Any under-bias (under van Bemmelen line) could be attributed to minor structural water from the 320 °C region although according to Ball (1964) this should be minimal where most of the water loss from clay minerals pre-dried (105 °C) should appear between 450 to 600 °C. Conversely any over-bias (above van Bemmelen line) as most points appear to, is indicative of unaccounted for, more thermally stable C-matter which has not reacted until higher temperatures are reached. Analyses relying on this temperature interval would be relatively diminished by some amount (in comparison to the true value found by dry combustion >600 °C) which could be minor but is an uncertain quantity from soil to soil.

Investigating the thermal distribution of soil carbon - evolved gas analysis (EGA) by TGA/MS

To ascertain what reactions / compositions prevail over the TGA heating cycle, combined thermal experiments were conducted on a small number of whole soils. This could uncover decomposition / oxidation and the significance of C-release beyond 430 ^{o}C which may be quite variable possibly reflected on figure 2-2. To be able to use m/z 44 (CO_2) as a proxy for C release from OM assumes that its relatively low concentrations in whole soils, an excess of oxygen in the purge gas and sufficient active sites ensures quantitative conversion to CO_2 and water (without charring or recondensing). Major ions were recorded as they evolved into the MS from the programmed heating as mineral material and OM degraded / combusted. The kinetic distribution of carbon (m/z 44) could now be observed (unlike the previously combined signals from DTGs) which interestingly continues into the temperatures where clays (m/z 17, 18) degrade as in the example of a Vertisol under crop shown in figure 2-3. The mass changes in the 200 to 430 ^{o}C and then the 430 to 590 ^{o}C region correspond to the destruction of OM and a combination of predominantly inorganic and lesser organic substances respectively. Water and hydroxyl units indicated in the EGAs (m/z 18, 17) peaked around 500 ^{o}C from a falling baseline which follows initial surface dehydration (to 200 ^{o}C). Carbonaceous material indicated by m/z 44 reached a maximum around 350 ^{o}C, which was in keeping with the correlation of mass changes over 200 to 430 ^{o}C with dry combustion on figure 2-2. Other soils have shown similar ion patterns where most of the organic material is destroyed (CO_2 released) over the first thermal region (200-430 ^{o}C) with lesser and variable amounts in the second (430 ^{o}C+). The m/z 17 or 18 ion traces did not indicate the presence / breakdown of hydroxide or oxy-hydroxide minerals that would add to mass losses in the thermal region near 300 ^{o}C as noted in some soils (Boyle 2004; McCarty et al. 2010).

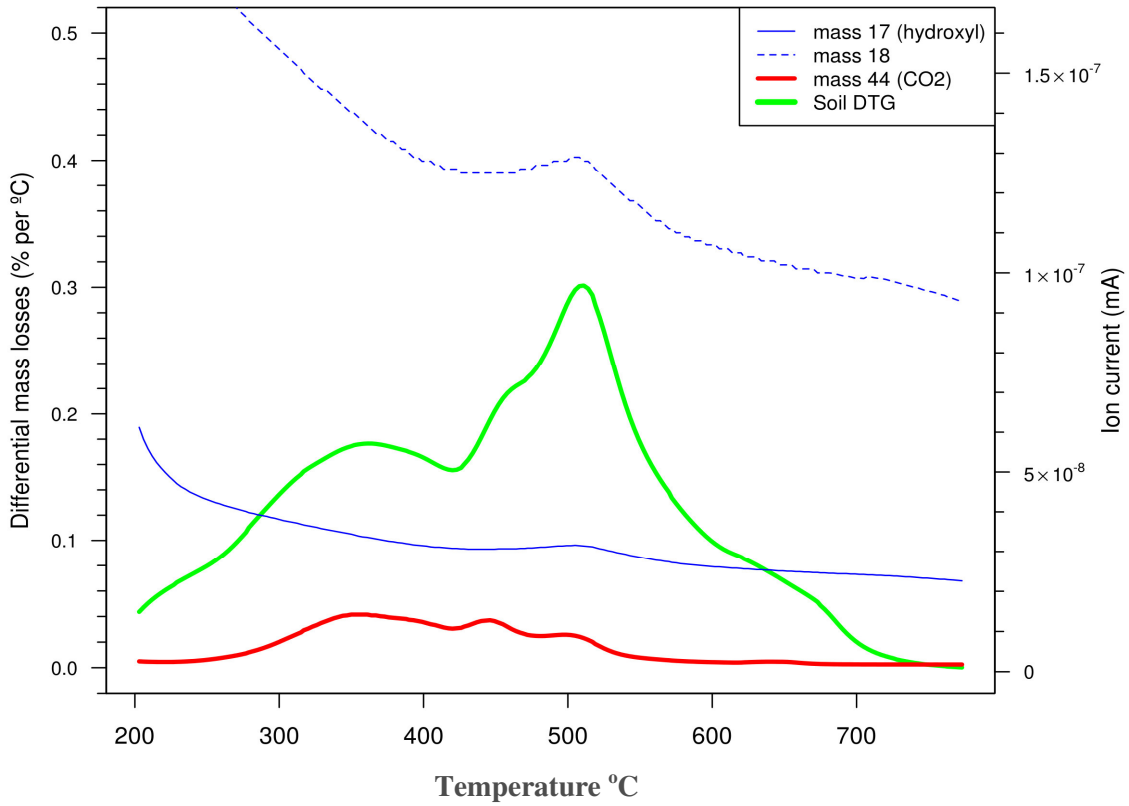

Figure 2-3 Monitoring ion currents (mass 17, 18 and 44) for oxidised products evolving from a cropped Vertisol aerobically heated by TGA program (10 °C/min between 200 and 800 °C) along with the differential mass changes (DTG). Note the continuation of C release from OM (m/z 44) beyond 430 °C where inorganic reactions dominate.

These experiments have further demonstrated that OM persists into the temperature regions where clays lose most of their mass, raising this accompaniment between OM and clay or other minerals such as an organo-mineral complex (Oades 1995; Kleber et al. 2011). Other approaches need to be employed to provide reasonable evidence of the physiochemical linkages between clay and C, often described as a shielding / encapsulation mechanism (Bachmann et al. 2008; Brunn et al. 2008; Flessa et al. 2008; McCarthy et al. 2008) and more lately organic micelles in mineral defects as possible sites (Wilson et al. 2008; Chenu & Plante 1996). Irrespective of this, the OM is probably more recalcitrant (Plante, Pernes & Chenu 2005; Plante et al, 2011), may be thermally similar to char particles but degrades at the same temperatures that the mineral components lose mass (i.e. tightly held water / hydroxyl units) making it difficult to quantify by simple LOI methods.

The relevance of grainsize and implications for loss-on-ignition (LOI) methods

What does this merging of residual SOM and mineral matter mean for LOI determinations? To explore and demonstrate any possible pattern of C-overestimation with grain size, we referred to a legacy database where there was sufficient corresponding dry combustion, LOI and textural data (n=208) (acquired with methods described by Rayment & Lyons, 2011). The % carbon values derived from LOI (using factor 0.58) were plotted against dry combustion (elemental analysis) but separated into <25, 25-50 and >50% clay to highlight these differences (figure 2-4). To obtain greater statistical detail about how the calculated values deviate from the true value (expressed here as % C LOI / % C dry combustion) for the respective textural classes, these have been presented as a box plot on figure 2-5. Clearly the coarser textured soils tend towards parity while the more clay-rich (especially >30%) soils show a greater variability in this relationship indicating quite large overestimation in some cases.

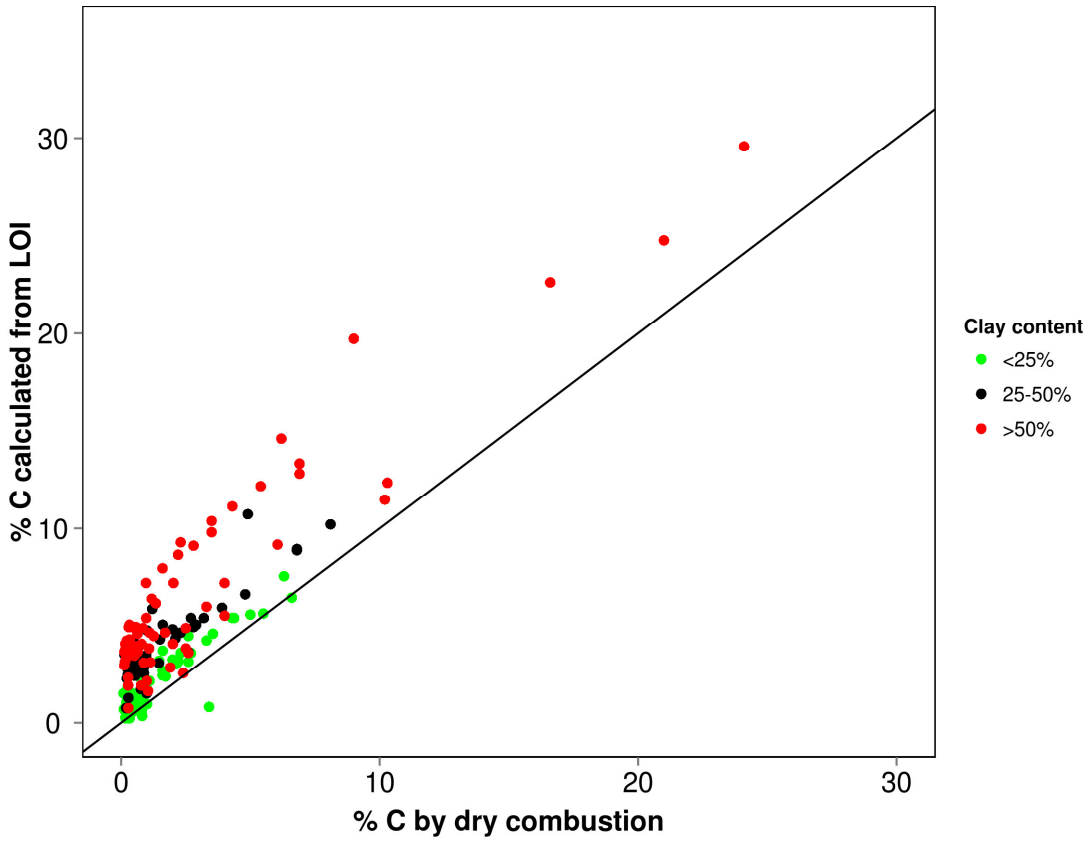

Figure 2-4 Correlation of LOI derived %C (using 0.58) with their values determined by dry combustion relative to 1:1 line.

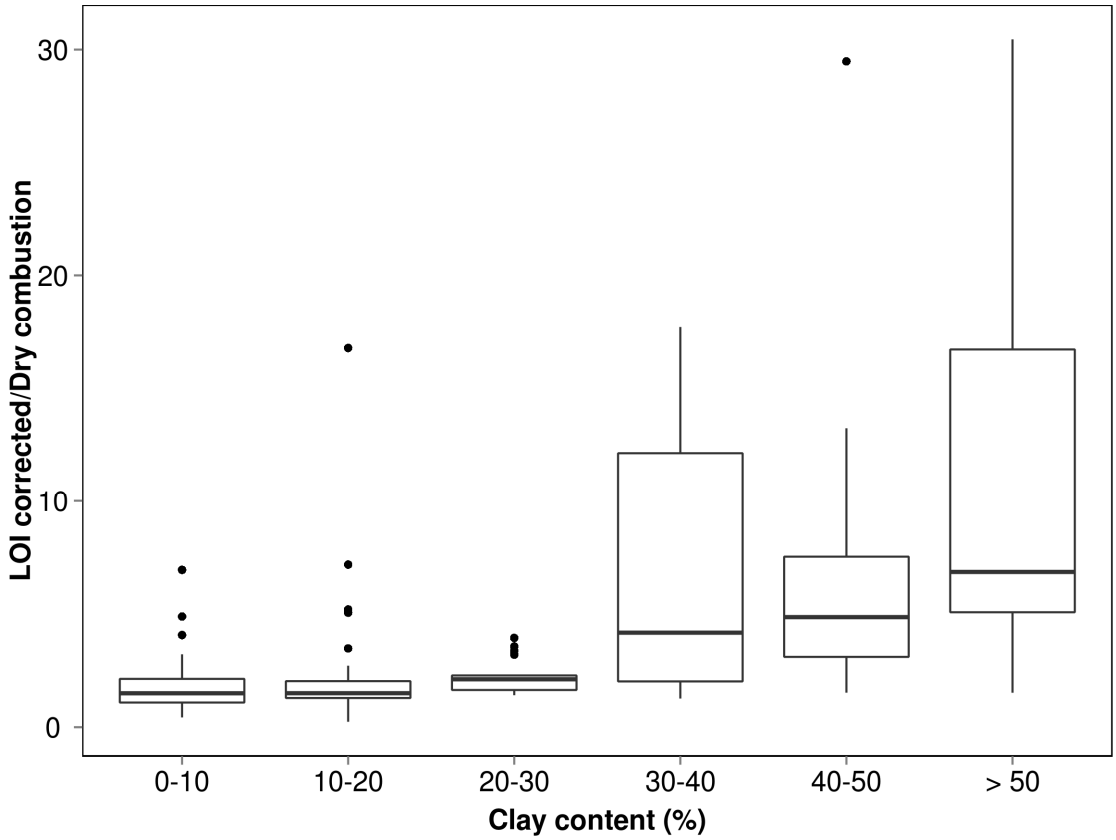

Figure 2-5 Box plot measuring relationship between soil C by LOI and soil C by elemental analysis for each clay class where ends of the boxes are 25th and 75th quantiles and mid-line the median value.

To get some real perspective of how much C is unaccounted for when applying more conservative LOI temperatures or conversely how much erroneous mass is included at higher temperatures, these relative amounts have been assessed using the coupled TGA/MS approach. In the example on figure 2-6 of a Vertisol (under pasture) corresponding ion currents were plotted along with mass changes represented by the DTG. These initially track the differential mass loss curve but deviate beyond about 430 ^{o}C enabling a relative quantification of how much C remains using the m/z 44 ion as all OM is released through combustion. The additional mass losses beyond this temperature which derive primarily from increasing inorganic reactions provides a guide of how much unrelated mass loss needs to be incurred in order to obtain those diminishing increments of OM (i.e. % C). The data on table 2-2 (determined using LoggerPro, Vernier Software and Technology) shows a benefit – sacrifice regimen to assess how worthwhile each subsequent temperature interval is in terms of returning these extra few % C (over and above the 3.46 % mass due to only OM). Obviously this varies considerably depending on the soil texture and OM complexity / type. Interestingly, Siewert (2004) correlated principal soil components with mass changes up to

1000 °C in 10 °C steps. Very significantly C and N were strongest in the range 200 to 500 °C and clay % increased to 550 °C producing a composite plot (correlation coefficients against temperature) showing some resemblance to the ion plots in this study.

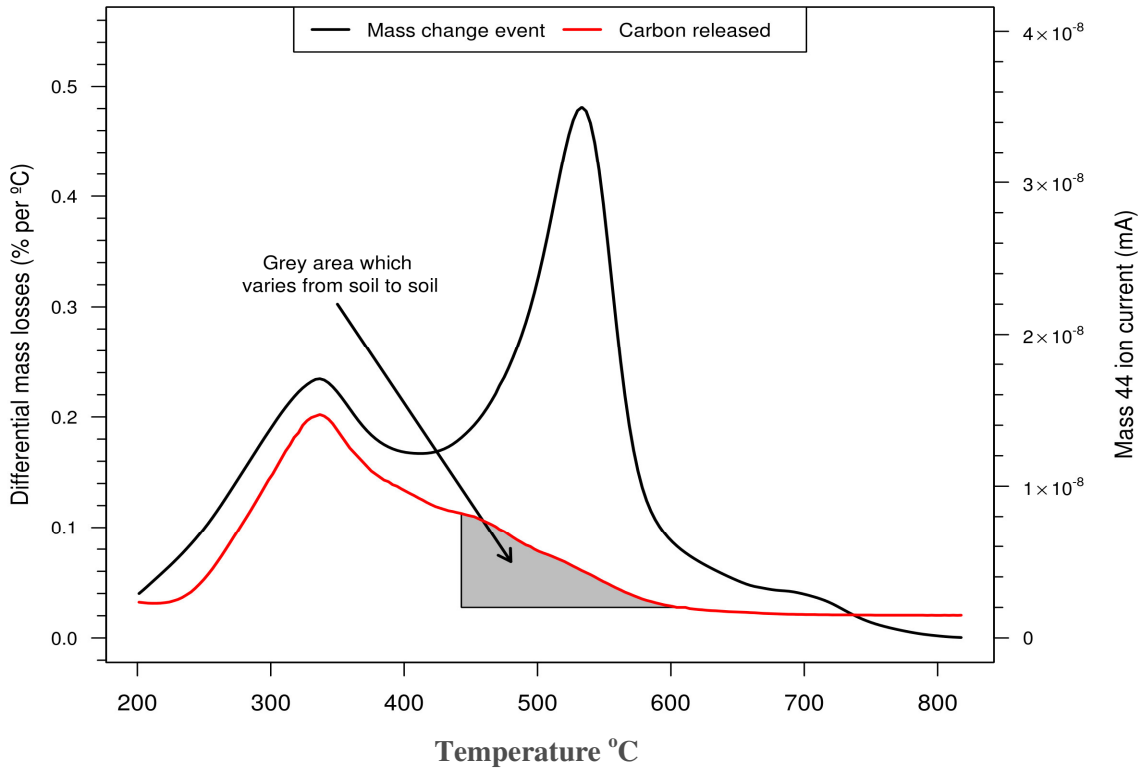

Figure 2-6 Differential mass loss (DTG) and C-trace (m/z 44) for Vertisol under pasture to assess benefit of LOI temperatures over 430 °C which are detailed on table 2.

Table 2-2 Increments of % C (OM) and % mass loss (mostly from mineral) for intervals beyond 430 °C.

Temperature °C	% of total C	Additional % mass loss	Cumulative % mass loss
440	76.5	0.20	3.66
460	81.0	0.39	4.05
480	87.0	0.48	4.53
500	91.3	0.61	5.14
520	95.0	0.84	5.95
540	96.9	0.94	6.89
560	98.9	0.52	7.41

LOI temperatures vary between laboratories (Rayment & Lyons 2011 and their sources; Sutherland 1998) and in many cases are likely to overestimate OM values where temperatures are excessive. It is expected that LOI would yield reliable C determinations on

sandy or peaty soils and not so much for clay-rich soils which may explain previous concerns over the method (Leeper & Uren 1993; Sutherland 1998; Heiri, Lotter & Lemcke 2001). However it is suggested that the method should be fairly reliable provided they adhere to careful heating regimes and observe the mass changes between 200 and 430 ^{o}C which should exclude changes from all inorganic reactions (dehydration and clay collapse i.e. dehydroxylation). Generally the conversion factor of 0.58 has been regarded by many as not universally applicable due to heterogeneities between soils and should be refined for each area or soil type (Sutherland 1998; Kasozi et al. 2009; Pribyl 2010). In their summary on carbon determination methods, Chatterjee et al. (2009) indicate 430 ^{o}C (similarly conservative temperatures suggested by Nelson & Sommers 1996) as a recommended LOI temperature after drying the soil at 105 ^{o}C which should, based on our work, yield a reasonably good estimate of OM and hence TOC. However it can be noted that it excludes any variable amounts of more resistant OM oxidisable at higher temperatures such as charred particles or what might possibly be tied up with the mineral component. TGA combined with other detection techniques clearly reveal the overlap of CO_2 due to organic matter and OH- from inter-crystalline losses which could give rise to overestimation when mass-only changes are considered (as in LOI beyond temperatures of 430 ^{o}C). Conversely, the proportion of residual OM (overlapping with inorganic mass changes) that would be excluded would result in small underestimations but should provide greater accuracy than yields from higher temperature as is often the practice.

It is herein proposed that the C distributed in these two main thermal bands could be determined separately by elemental analysis on duplicate soils by oxidising at 430 and then again at 550 ^{o}C, the difference in C content between the two, potentially representing the more stable portion. Further studies to map the thermal distribution of C in diverse soils, depths and land-uses by either TGA/MS or TGA/FTIR is encouraged to uncover any relationships with OM maturity or the presence of other C forms such as biochars or those that may be mineral bound. Such combined analyses assist in the interpretation of mass change events where whole soils can contain numerous constituents such as iron or aluminium oxides and clays minerals as well as OM.

Conclusions

Thermal analytical techniques have been a useful tool in elucidating the distribution of carbonaceous materials in soils. They have exposed some of the weaknesses of LOI methods but on the other hand serve an excellent guide to allow its more reliable application. It has demonstrated that care needs to be exercised when applying LOI with an awareness of

the relevant thermal regions and reactions of the various soil components as seen using TGA and adjunct techniques. The soil data from this study has indicated that traditional conversion factors may provide a reasonable carbon estimate for finer textured soils using LOI methods as long as temperatures are constrained between 200 and 430 ^{o}C. Data acquired using higher temperature methods (e.g. 550 ^{o}C) would require more intensive site-specific calibration which should automatically factor in textural variations.

Thermal methods readily divide organic and inorganic C, as is well known, but could potentially be used to distinguish other kinetic pools such as biochars which make it well suited to the assessment of management practices for C sequestration. Further work into the carbonaceous material associated with different particle size fractions and mineral matrices could be very valuable also.

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CHAPTER 3 - A CARBON DETERMINATION SYSTEM FOR WHOLE SOIL CORES

Key aims of the method

Dry combustion of soils has been the benchmark method for soil C and N determination. Whilst destructive, it is most direct because it extracts this element by converting it to a single and pure form, CO_2. This method has generally been regarded as difficult to carry out on samples much larger than a few grams due to incomplete conversion and in particular the huge [d]volumes of gas generated. However the issue surrounding such small amounts is that it necessitates greater sample pre-processing e.g., selection, grinding, bulking and sub-sampling (added cost and labour) which all can lead to deviations from the true composition and a loss of 'representativeness'.

The hypotheses central to this study that need to be addressed in this and the next section are:

1. That large aliquots namely the contents of whole cores can be reliably determined
2. Use them to overcome or improve the uncertainties caused by soil C variability
3. Incorporate cost efficiencies vital for any large scale measurement of soil C stocks

Large aliquots in the following studies (including next chapter) refer to amounts in the region of 300 or 400 gram which is hundreds of times the soil that is currently analysed for the determination of soil C stocks (see review). The method should also be able to measure directly both soil C concentration (mass basis, kg/kg) and C stock (volume basis, kg/m^3 or kg/m^2).

A successful method should deliver:

Reliable C data with requisite levels of precision

Ease of application

Cost effectiveness

Readily duplicated or verified soil C data

Once demonstrated in field trials, this technique can also provide support for the reliable development of other methods such as on–the–go Vis – NIR and MIR, the emerging inelastic neutron scatter method as well as providing a ground-truth to spectral GIS approaches that incorporate spatial statistics.

[d] Rule of thumb: 1 mole of gas occupies 24.4L at STP

Methodology and Design Concept

Classically, C has been determined in a range of materials by combustion at temperatures usually over 800 ^{o}C (Bisutti et al., 2004) and then determining the product by weight (gravimetric), titration or some other means. Combustion is carried out in O_2 rich atmospheres to achieve complete oxidation and any minor CO formed can be further converted catalytically. Anaerobic atmospheres produce pyrolysis products and are inappropriate for analysis of C.

Alternative methods of oxidizing C to CO_2 and quantitatively capturing all the product gas can be achieved by either:

a) oxidizing C in a constant volume reactor and measuring its partial pressure,
b) purging and trapping CO_2 as combustion proceeds and measuring pressure in a constant volume or,
c) analysing or quantifying the resultant CO_2 during the conversion period

These were all considered as part of the proposal but for this study a fixed volume vessel / furnace (a), was regarded unsatisfactory for the samples sizes intended, safety and procurement. In addition, analysis turnover will be restricted by cooling periods between samples which can also affect quantification (taking of pressure readings) as temperature varies. Similarly a purge and trap system (b) requires messy cold traps and high transient pressures. The last option offered numerous advantages as well as simplicity.

Description of test bench - individual components

The soil carbon bench (SCB) was designed around these four main modules:

1. Enclosed and refractory flow-through reactor tube with gas supply,
2. Furnace mounted on rolling stock (mobile heat source),
3. Split flow arrangement leading to measurement systems (e.g. gas chromatograph) and
4. Data acquisition system and computer (signal collection and processing)

These have been set out schematically on figure 3-1 and shown photographically (plates 3-1). Each module is discussed in some detail below.

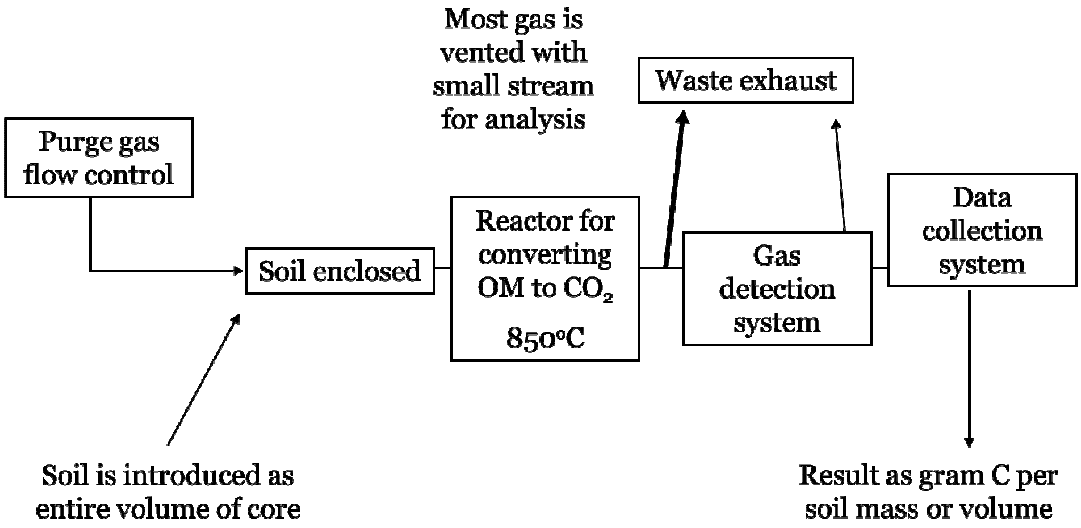

Figure 3-1 Schematic diagram of the important modules that make up the soil carbon bench.

Reactor tube, furnace and catalyst

A purpose built furnace was fabricated with the assistance of Prior Industries Australia (Silverwater, NSW). The heating element consisted of a Kanthal resistance wire wound around a 120mm (od) Mullite tube (Ceramic Industries, Croydon, NSW) over ca. 500mm length (tube length 600mm). This was enclosed in a 380mm diameter mild steel casing and packed out with low density (Kao wool) insulation. This type of low density material facilitates fast heat-up (800 ^{o}C from ambient in ca.45 min.) with lower heat losses (G. Golder and R. Macquart pers. Comm.). This 2 kW furnace was designed so that it could be connected and supplied to any domestic single phase outlet (10 amp).

The power was set with an energy regulator (Prior industries) where temperatures were monitored by K-type thermocouple (positioned midpoint of unit) connected to a multimeter which measures the induced mV signal that is proportional to temperature. After commencement of analyses adjustments to the power regulator were required to maintain appropriate and constant heat levels during analyses as heat was dissipated (into soil core and also lost). Usually thermocouple or thermostatic controllers are used for precise heat control in laboratories but in this case would have been of limited value where heat losses are high due to 'sinking' into soil masses and reciprocation of the furnace. While the oxidation of OM occurs under 500 ^{o}C and $CaCO_3$ decomposition ca. 650 ^{o}C (see section on TGA, Chapter 2), a higher external temperature provided an effective gradient to enable heat to penetrate the matrix of the typically 300 gram soil core masses held within the reactor. The amount of available heat energy (measured as temperature) enclosing the soil also affected the time period required to process / analyse soil C on this scale. The operational

temperature (850 ^{o}C) was accompanied by the distinctive bright orange appearance of its ceramic core making it also possible to visually estimate when the furnace was approaching a good operating temperature. The method of control could ultimately be retrofitted with an embedded thermocouple feedback control device for greater precision and safety but given the lag accompanying heat transfer has made this a lesser priority.

The furnace was mounted on a rolling stock arrangement to allow the heat to be brought to the soil rather than the reverse as in the usual (small-scale) systems. This facilitated soil loading without uncontrolled loss of C but crucially enabled the centralizing of the reactor tube within the mobile heat zone. The parallel track also provided a steady movement around the centralised reactor tube. One disadvantage of the reciprocation method was that some heat dissipated onto the relatively cool parts of the reactor containing the soil and on its return again.

Alternative methods of heating were considered before proceeding with an electric resistance type which was regarded appropriate for test bench purposes. The ratings given in table 3-1 are relative and were based on information gathered during the initial discussion, design and planning processes. The mobile design of the current system overcame what would be considered the main drawbacks of a resistance element furnace which are its slow heating and cooling cycle. By maintaining temperature and moving the heat source to the soil core sample allowed instantaneous combustion (flashing) of volatiles or incipient combustion (in heavy matrices) with some lag as heat penetrated. The soil sample chamber also cooled more quickly after the furnace was returned to its standby position. While there may have been advantages with other forms of heating, predictability (uniform heat) and cost were important considerations.

Table 3-1 Relative merits of different heating apparatus considered for this purpose.

	Heat-up rate	Cool-down rate	Simplicity	Power consumption	Initial cost	Special requirements
Resistance element	mod	slow	high	high	low	N/A
Induction heating	v. fast	fast	low	mod	mod	ferrous material
Radio frequency	v. fast	v. fast	low	high	high	safety shielding
Halogen lamps	v. fast	v. fast	mod	low	mod	N/A
Gas fired	fast	slow	mod	low	low	supply, exhaust

Note that in industry, rotary or tumbler type kilns are used to spread and mix the heat and reactants however, where gas flow and seals are required (as here) these could be more difficult to implement.

The reactor tube constructed of quartz was especially 'blown' to specifications (D. Stathers, ANSTO/CSIRO Lucas Heights). Two main sections, one 85mm (id) x 600mm (to contain soil during combustion) were fused to another 40mm (od) x 600mm interval to contain catalytic material. These were terminated with a 6mm gas outlet and Pyrex flange to accommodate a sealable cap where soil is introduced. Quartz was an ideal reactor material during developmental work because it allowed observation of combustion events (transparent) and withstood innumerable heating and cooling cycles owing to the material's low coefficient of expansion. Transfer of radiant heat and lower losses through conduction (unlike in steel or copper) were also advantageous. Catalytic material (Cu turnings converted to CuO) was incorporated for conversion of volatile C to CO_2 but more importantly completing the oxidation of CO formation. Packing of the catalyst held the key to reliable performance by preventing excess back pressure and maintaining a steady sampling stream which is paramount for quantitative analysis (although higher O_2 partial pressures do assist combustion). Inclusion of catalyst served both OH&S and the analytical objectives.

An examination of the Gibbs free energy values for the reaction:

$$Cu + ½O_2 \leftrightarrow CuO$$

over the temperature range the Cu is exposed to (in the SCB) assists in predicting that at operating temperature oxygen is more readily given up to any passing CO than when cooling (in oxygenated stream) which favours re-oxidation (i.e. ΔG^o is more negative). Values calculated from enthalpies and entropies at different temperatures have been added to an Ellingham-type diagram drawn up for N and C (see figure 3-7 toward end of this section).

The gas supply

There were two primary requirements for gas flow with the SCB. The first is to oxygenate reactive sites in the solid support (catalyst) in situ and provide an excess of O_2 for the decomposition and complete combustion of OM. The second important role of gas pressure was to move combustion products out of the system where they can be analysed and safely disposed of. In commercial analysers where several 100 mg material are burnt, O_2 (ultra high grade) is introduced and locked in for the period of conversion. This is followed by expulsion of reaction products by an inert gas usually helium which is an advantage for the detection system but then becomes a much more complicated process. Ideally if these functions could be performed by one gas it simplifies many aspects of the procedure. Air is the obvious candidate (21% O_2 and 78% N_2 inert carrier) because it can oxidise, is readily available and is safe. Industrial grade air was trialled initially at flows from 0.2L to 2L min^{-1} but there were disadvantages that accompany the use of this gas (see also under subsections:

combustion efficiency and *nitrogen*). Poor conversion was experienced with air-only purging (see plates of soil and char below) unless higher pressures were used (increased pO_2) which caused intermittent seal problems (remembering it was designed as a low pressure flow through apparatus with inbuilt overpressure release). Any bursting hazard was managed by having a continuous open vent for the bulk of the gas stream and a spring tethered flange at the inlet.

Subsequently 100% O_2 in the form of industrial grade oxygen was supplied at similar flow rates to the system to overcome these oxidation problems. This almost instantly solved any difficulties relating to the kinetics and completeness of conversion and could be done at one atmosphere pressure. Using industrial or welding grade oxygen has other advantages. It is cost effective ($20 per cylinder refill) and readily available also in rural areas. [d] Pure oxygen became both carrier and oxidant, making it very convenient for operation but safety considerations needed to be observed. Experimental methods in the past have generally been concerned with analysing low amounts of C in samples and then the background levels in the gas supply may be significant. To remove any uncertainty in these situations researchers 'cleaned it up' by passing it through traps (e.g. molecular sieve) or preheating the oxygen supply (Salonen, 1979). In the current situation, C bearing contaminants may have been present in this lower grade gas. Although any contamination is a constant, the oxygen used to convert and purge OM from the soil cores was also tested to assess if it contained impurities that might contribute C to the overall signal which arises from either CO_2 or hydrocarbons if present at low levels. Impurities were not evident in any blank runs where only gas was passed through furnace and theoretically for the purge volumes used per soil core (1L per min. for 5 min.) even 100 ppm impurity would only add 1/2 ml to the total – an insignificant amount even in low C soils.

Monitoring and measuring gases – the alternative analysis devices

The range of gas sensing devices was reviewed and evaluated in terms of suitability, longevity and cost. A number of alternative detection methods could have been used to carry out a proof of concept for the SCB up to and including soil C field trials. The . (3-2) compares the various merits of traditional and emerging detectors.

[d] Oxygen is an accelerant that can be hazardous in some circumstances such as where it is allowed to accumulate in combustible materials.

Table 3-2 Detector attributes for types commonly used in gas determination

Type	Range	Specificity	Robustness	Current suitability
IR (NDIR)	trace to med	CO_2	high	high
Chemical gas sensor	trace	CO, CO_2,	low	high
TCD	low to high	all gases	mod	high
Cold wire	low to high	all gases	v high	high
Discharge ionisation	trace	HCs, He	mod	low
Flame ionisation	trace	HCs, CO, N_2O	v high	low
Electron capture	trace	N_2O, CO_2, ClHCs	mod	low

Infra Red

For C measurement via the CO_2 form, infrared absorption is the obvious method of detection. This is because asymmetric, diatomic gases absorb energy in the infrared region making it very sensitive to trace amounts. Water vapour, CH_4, N_2O and CO_2 are strong absorbers, hence their reputation as GHGs. These devices typically operate at ppm levels or low %. In order to incorporate this detection system into a combustion system where huge volumes of CO_2 are produced would require dilution of the sample stream and introduces a potential source for error. Dispersive infrared is where the IR beam is broken up (optically) into a continuum of wave lengths for a given range (as determined by the source and beamsplitter), and enables the entire spectrum to be scanned and collected. By contrast non-dispersive infrared gas detectors (NDIR) are so 'tuned' that the broad region around the major absorption band for CO_2 (4.24μm) are detected and quantified. In addition, to perform optimally the gas should be as dry as possible (Smith and Tabatabai , 2004).

Chemical Gas Sensors

These sensors operate on the basis of gas diffusion through a membrane and a chemical reaction resulting in either an amperometric or potentiometric change (Dubbe et al., 1995). In the latter the electrical potential is proportional to the gas partial pressure where the relationship can be described by the Nernst function (Marr, 2004). Being fairly gas specific, these detectors allow acquisition of data in real time because there is no intermediate step such as GC separation. This developing technology was attractive due to the gas specificity but suffers from being restricted to trace levels and saturation limiting service life (poisoning). During SBC development an EL–USB –CO data logger (Lascar Electronics Inc.) was used to monitor CO for the purpose of gauging the combustion efficiency of the system as well as health and safety. Amperometric type devices can be gas selective by optimising the electrode potential but some cross selectivity can still occur (LaConti et al., 1971). This phenomenon was evident during SCB operation where hydrogen used as a carrier gas for the GC (flow rate 25ml / min) was also concurrently registered by the device.

Care was required not to mistake these two sources, however more seriously, greater exposure to such other reactive gases further limits the life of the electrodes.

Thermal conductivity detector (TCD)

Like all materials, gases have a particular heat transfer capacity or thermal conductivity (TC). These can be measured in absolute terms (for example CO_2 dissipates 0.0154 watts per metre Kelvin compared to 0.0240 for air). However when discussing gas chromatography it is more convenient to express TC values relative to air and these have been set out for common gases on table 3-3.. Generally the lighter (smaller) a molecule is, the greater its ability to remove heat. This property makes it possible to observe differences in gas composition by measuring changes to electrical resistance resulting from heat dissipation. Thus the relative thermal conductivity of a gas can be measured by the resistance of a fine filament (examples on plate 3-1) within a stationary or mobile gas stream and this principle has been successfully employed in gas flow measurement devices. Usual TC filaments are composed of rhenium and sometimes tungsten or nickel but these are all susceptible to oxidation. Normally TC sensors are incorporated in such a way that one or a set of filaments is held in a reference stream of pure, non-oxidative gas, commonly He, while the other set is positioned in the sample stream which is primarily the same gas with variable concentration of impurity gas which periodically alters the electrical resistance properties of the filament wire (through heat conduction) as these gases come and go. Electronically these sets of filament wires (matched sets at close to 110 ohm for TCDs) are arranged to form a Wheatstone bridge circuit. An impurity gas having a different TC to the reference gas results in an imbalance across the bridge measured as a small difference in potential (mV). Understandably where the sample gas has an equivalent or similar TC to the reference it becomes in effect invisible to the circuit. Oxidation is the biggest operational concern with this type of device, (Gow Mac Instrument Co and personal experience).

Plate 3-1 Example of a TCD filament (left) and a thermistor bead on the right. (Source: AGC Instruments)

Thermistor filaments (have higher resistances values) are also thermally sensitive elements but have a negative electrical characteristic (i.e. more conductive when warmer) and operate well at ambient temperatures. These are manufactured from a mixture of metal oxides (Mn, Ni, Co), sintered in an oxidising atmosphere and glass coated to provide resistance to further oxidation although, these should not be used around H_2 atmospheres (advice from Gow Mac Instrument Co., New Jersey). Variations of this type of device can be found in low temperature (Cold Wire) applications as well as gas flow monitoring e.g. some digital flow meters and spirometers.

Table 3-3 Thermal conductivities for common gases expressed relative to air (source: Gow Mac Instrument Co.).

Gas	27°C	93°C
Air	1.00	1.00
Ar	0.68	0.68
CO_2	0.64	0.71
CO	0.96	0.97
CH_4	1.31	1.42
N_2O	0.66	0.76
NO	0.99	1.00
NO_2	-	2.68
O_2	1.023	1.031
N_2	0.99	0.99
He	5.73	5.50
H_2	6.94	6.78

The thermistor filaments used in this experimental work had resistance values of 2200 ohms. These elements were similarly fed into a Wheatstone bridge arrangement to provide a stable baseline signal and a response to slight changes in resistivity due to altered gas composition. The cold wire sensor was powered by 3 V (DC) which supplied a low mA current where signals usually under 10 mV were produced by CO_2 depending on the organic material, carrier gas type and volume passing through the cell (figure 3-2).

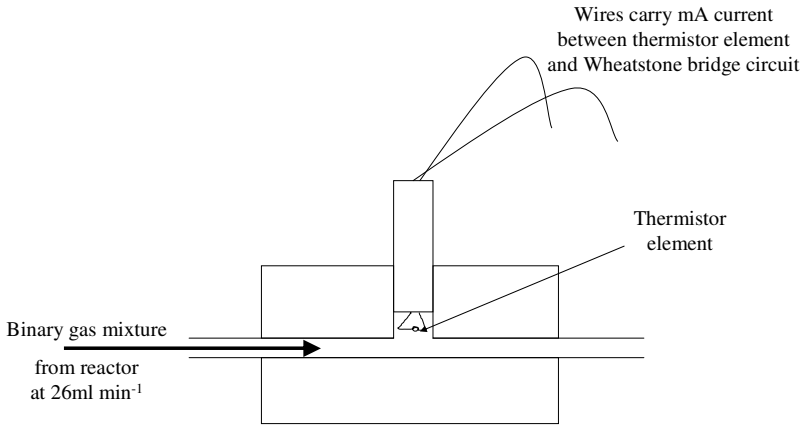

Figure 3-2 The Cold Wire (thermistor) cell used in process monitoring and C determination.

To sum up, chemical gas sensors can cost less that $150 and are highly sensitive (ppm) but absorb gases being measured leading to saturation, shortened service life and also possibly changed sensitivity with time. This makes such devices not sufficiently robust for this work where gas volumes can be enormous. Cheap and portable NDIR cells can also be purchased for a few hundred dollars but these are constructed to monitor work-spaces for levels of CO_2 under 10%.

For the purpose of demonstrating the concepts and answering the questions posed in this study, thermal conductivity devices (constructed and arranged into a bridge circuit) offer the most effective, versatile and low cost alternative to implement. However it remains perfectly feasible to incorporate other types of detector systems provided they have a satisfactory sensitivity range and are resilient against degradation. The disadvantage of fixing a FTIR cell to the SCB (approx. cost AUD$15,000 and a little less for NDIR) would be a requirement to dilute the sample gas flow and if not carefully controlled can be a source of error during quantification.

Soil carbon test bench operating procedure

The process involved transferring the (>800 °C) heat by means of the rolling stock system directly to the (previously weighed) soil material held within the 85mm diameter quartz reactor tube as the oxygen flows at slightly over 1 atmosphere pressure. The furnace was manually reciprocated along the rail bed between sample (soil / calibration materials) position A (Plate 3-2) and catalytic copper turnings at the other end (position B). In standby the catalyst heated up again in readiness for oxidation. Conversion of carbonaceous material was carried out using between 0.5 and 1.5 L min^{-1} O_2 but 1 L min^{-1} was found to be optimal so became the standard rate. This provided adequate oxidation throughout the hot bed of soil inside the quartz reactor without too much overheating caused by gas exiting at too high a rate. This also served as the principal carrier gas transferring product to the detection cells and ultimately the vent.

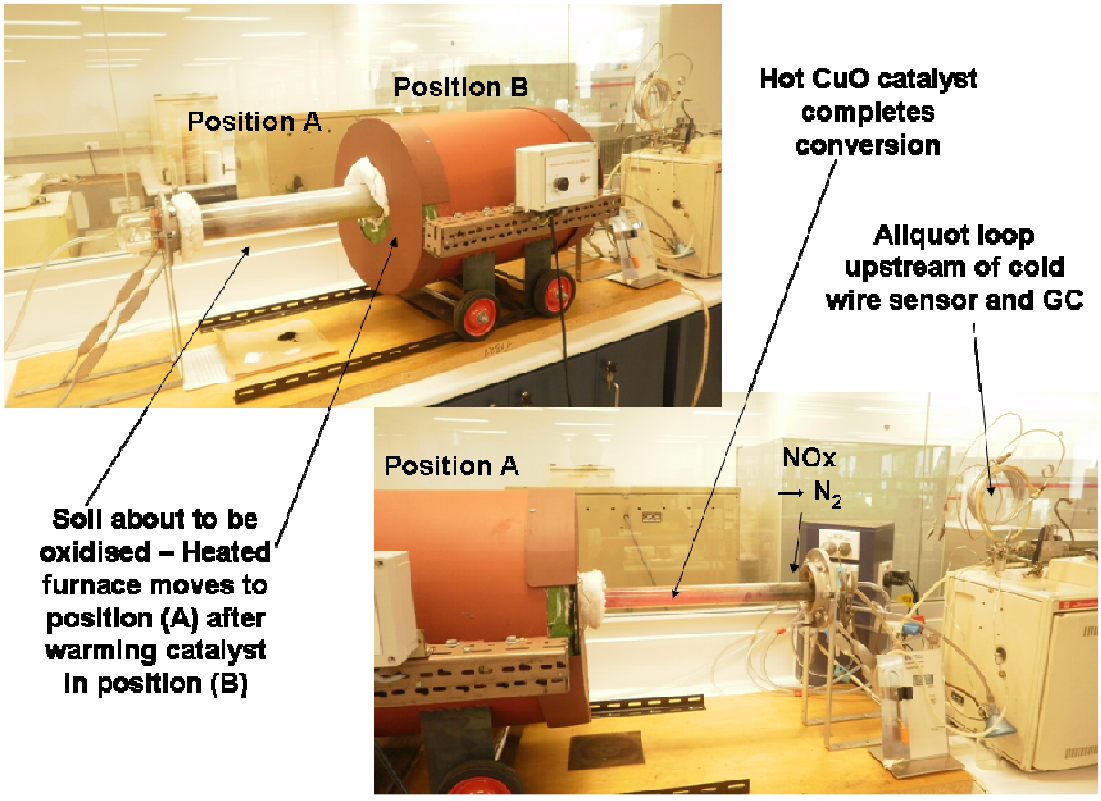

Plate 3-2 Photographs of the SCB in standby and combustion positions.

Clean copper turnings (approx. 2mm average thickness) were lightly packed into the catalytic part of the reactor (see plate 3-2) to oxidise volatile OM and CO (after preconditioning it in situ) and reduce NO_2 (at 'cool' end). It also acted as a filter to arrest any particulates.

Oxidising of OM

Ignition temperatures for OM are often discussed in the literature as low as 200 ^{o}C or so. The TGA work (Chapter 2) has provided a reliable guide to the various reactions (including self ignition) occurring during the combustion of soils. Combustion of OM is an exothermic reaction requiring a minimum temperature (in the absence of a naked flame) to exceed the required activation energy. Typically in a stream of oxygenated atmosphere this self ignition for OM begins around 230 to 250 ^{o}C (figure 3-3) indicated by the differential (rate of mass loss) curve. Onset times (i.e. start of mass change event) depend on heating rates, amount of insulating matrix and adequate O_2. Thermally resistant (refractory) OM can also be present such as charred C, and these require a higher degree of heat energy for ignition to occur. Carbonates if present begin to decompose to gaseous CO_2 above about 650 ^{o}C and need to be considered / tested during dry combustion methods for soils.

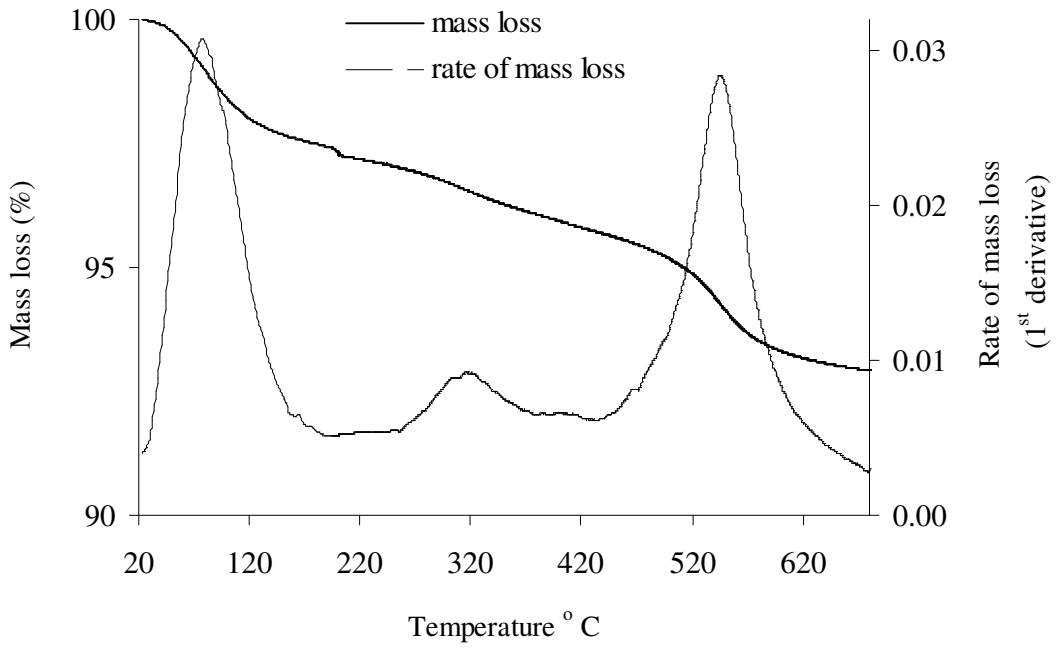

Figure 3-3 Thermogram of soil heated at 10 ^{o}C min^{-1} to show major mass loss events.

The discrete mass loss events above relate to the following predictable and simple set of products (gases) released during the heating of soils:

<200 ^{o}C	water vapour
230 – 430 ^{o}C	CO_2, some water, CO, NOx
430 – 600 ^{o}C and over	water, CO_2

Combustion efficiency

The appearance of the residue can tell a great deal about the heating conditions. For example, the photographs below are of a soil (plate 3-3a) and foils on which pure charcoals (plate 3-3b) had been oxidised. The gradation in the level of oxidation to the right is fairly obvious in the soil and reflected insufficient heat and partial pressure of oxygen. Similarly the foils on the right are clean because these were combusted with pure O_2 while those with grey residue have not completely converted because air flow was used. There were several other measures that could be used to gauge the completeness of conversion and include:

- Gas composition,
- Elemental analysis of residue,
- Re-combustion and signal
- Co-linearity of CO2 vs mass loaded

Plate 3-3 Combustion of charcoal on foil (a) and soil (b) under variable O_2 conditions.

Spot checks on processed soil residue (ashed) by elemental analysis yielded only background signal as are obtained for blank analyses and re-combustion did not indicate any residual C either.

Carbon Monoxide

While all the C may be extracted from the soil (as indicated above) it does not automatically follow that it has been quantitatively converted to oxidised product. A good diagnostic indicator of combustion (quality) and oxygen sufficiency is the level of carbon monoxide (CO) produced. Monitoring CO concentrations by GC was difficult (see next section) with respect to gas separation and TCD sensitivity which is limited to % and over. This may have been possible under pyrolysis conditions where much higher levels can be reached. Instead, a chemical gas sensor (USB pen type) which can detect CO down to a few ppm was

positioned in proximity to the gas outlet which recorded every 10 seconds. The levels during SCB operation depended on various factors (precise location of sensor, the amount of C burnt when air was still used, even other gases used in the vicinity). Overall the combustions were reasonably well managed, where CO levels were typically under 500 ppm as shown on the example of output (figure 3-4).

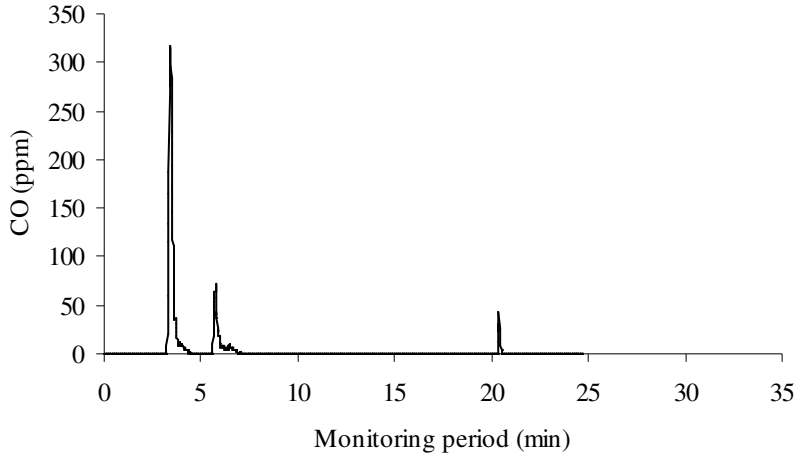

Figure 3-4 Levels of CO over typical experimental session as measured by CGS sensor.

Developing the Methodology

To assist in monitoring combustions and then devising the most effective methodology for determining C from soil cores, the SCB system was connected to a gas chromatograph (GC) fitted with a thermal conductivity detector. A second hand Shimadzu model 8A GC was acquired for this purpose. Being older and of basic design it lent itself very readily to modifications and adaptation with the experimental SCB rig (see plate 3-2). The instrument was fitted with dual injectors / column capability and a thermal conductivity detector. Connecting a GC had a two fold purpose:

i) monitoring various processes during experimental development i.e., completeness of combustion, excess O_2, other gases and
ii) determination of the amount of C being produced by the system either from calibration material (sucrose, charcoal, standards) or soil C.

It was not possible to simply pipe gas to the detector and there were several reasons for this. First gas mixtures need to be separated prior to detection for proper analysis, second, low pressure flow (from the SCB) must enter the high pressure GC systems and thirdly the amount of oxidising gas to the TCD detector must be kept to a minimum (the stream is predominantly excess O_2).

Therefore certain modifications to the instrument were required to be able to use it to monitor the combustion of soils and involved:
-fitting an 8-port Valco valve to the carrier gas supply to obtain 'snapshot' analyses during soil combustions
-fitting of Cold Wire (thermistor) sensor upstream to monitor the combustion event
-connecting to the valve various volume sizes which are constructed of stainless steel or aluminium tubing which (referred to in GC parlance as sampling loops)

The concept was that the loops capture fixed volumes of combustion product for transfer to the GC column where gases are resolved (separated) prior to detection.
The GC separation column was made from a 2m length of ¼" (6mm) tubing, moderately packed with Porapak® type Q polymer (80/100 mesh). Its primary purpose was to isolate the CO_2 from all other gases in the mixture of products from soil (and C standards) combustion for accurate determination. To achieve this, an operating temperature of 40 ^oC isothermal was satisfactory but separation efficiency depended chiefly on the size of gas aliquot sub-sampled from the exit stream. Overloading is a familiar problem in chromatography resulting in a lack of resolution and the inability to analyse individual components by univariate means. Loop volumes below about 40 ml could be resolved (60m was oversaturated) into their gas constituents (CO_2 and residual O_2) from the SCB system. Before operating, the column was baked at 140 ^oC with carrier flow to remove contaminants, mainly water. Monitoring the gases by chromatograph was done to gauge how oxidation was proceeding. It was readily observable that excess oxygen signals were inversely proportional to the amount of C loaded but peaks were 'clipped' because this gas was overloaded on the separation column. The TCD is 'broad spectrum' detector capable of responding to any type of gas (or even mixture) provided it has a thermal conductivity sufficiently different to the reference gas and it is present above the minimum level of sensitivity. In these experiments, H_2 was used as reference gas (rates of 25ml min^{-1}), being low cost and having a thermal conductivity that contrasts well against the analyte gases (see table 3-3). In the case of TCD this lies around 0.01% (vol / vol) so that trace quantities <100 ppmv are less likely to be recorded. The possible gases resulting from combustion usually include CO_2, residual O_2, N_2 and traces of CO (and much less likely hydrocarbons such as CH_4 within an oxidising atmosphere). These gases can be detected by TCD at % concentrations however gases other than CO_2 and excess oxygen were not noted.

These combusted quantities of soil were relatively large (and soils are good heat insulators) which meant the quantity of heat had to be sufficiently high to penetrate the soil mass so that the temperature at the soil centre could extract and convert all C present. The cold wire

(connected up stream of the chromatograph) proved to be an interesting and invaluable means of observing the full duration of the combustion event. It provided (in near real time) an indication of commencement to completion as the C was being evolved from the SCB after which time the signal returned to baseline. The GC and cold wire outputs were recorded simultaneously (Logger Pro 3.8.4) as shown on figure 3-5. Note, as the temperature rose within the soil, any incipient oxidation due to refractory C continued to report at the detector until fully converted. This safeguarded against selective conversion where recalcitrant forms are not fully combusted because of insufficient exposure time. This addresses concerns about some of the more recalcitrant OM remaining at even 700^{o}C as noted by Wang and Anderson (1998) and that complete conversion is time dependent (Chichester and Chaison, 1992).
More thermally resistant C produced attenuated peak shapes where for example carbonate was not distinguishable from SOM but was very elongated due to the slow decomposition of CO_3. Therefore the completeness of combustion of the soils (synthetic soils used charcoal as the C source) could also be directly observed by the cold wire trace (i.e., return to baseline) but was also reflected in the oxidised appearance of spent soil and coherent area data. It demonstrated that heat was transferred effectively when the furnace temperature (as measured by K-type thermocouple on the internal annulus of the heater core) was operated at 800 - 850 ^{o}C.

A secondary aims using the SCB proposed at the outset was to analyse by GC the oxidation products at several successive heat levels (300, 400, 500 ^{o}C etc.) as one might with thermogravimetric methods. However the coarser temperature control along with the sheer mass of soil (leading to variable and uncertain heat transfer) meant such experiments were best assigned to TGA studies (chapter 2) which can carry out slow continuous heating at variable rates. In other words the SCB was best suited to instantaneous combustion of contained carbonaceous matter (determination of the total) rather than thermal differentiation although recalcitrance in combustion was reflected in signal delays.

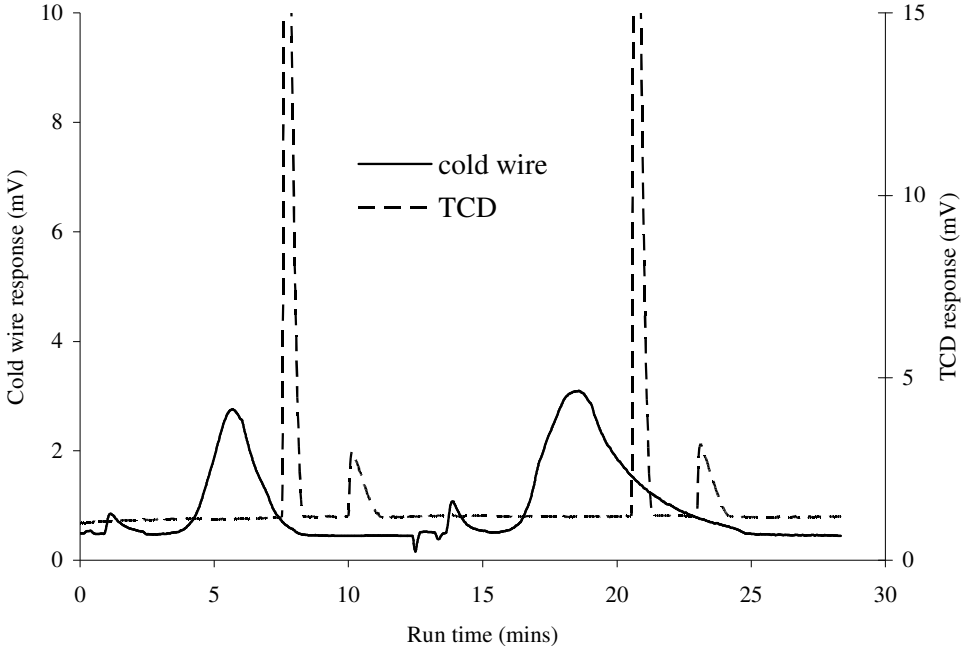

Figure 3-5 Composite signal outputs: cold wire sensor monitors the bulk gas composition and the TCD detector via the 6.5ml sampling loop and GC column.

The response areas obtained from the cold wire sensor when correlated with the mass of C loaded into the SCB system produced a linear relationship (discussed further under calibration section 3.3) indicative of quantitative combustion. However, the response areas recorded from the GC detector (shown on figure 3-6) were linear up to about one gram total C but levelled thereafter. This pointed to an oversaturation effect within the sampling loops even though the GC base line separations were easily achieved and the peaks within range as exemplified by the TCD output on figure 3-5. The extent of linearity (GC area data) may have been a little influenced by sampling loop volume but appeared to be mainly controlled by the volume of gases generated which are then not fully captured. The sampling loop / GC method could be used for soil C determination where the amount of C is under a gram or if sub-sampled from a static, fixed volume reactor vessel.

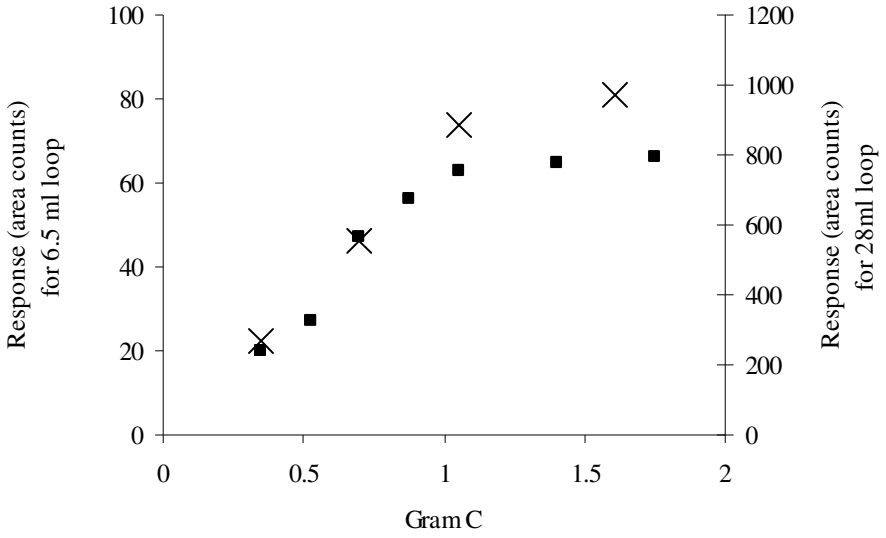

Figure 3-6 Comparing area responses for the 6.5 and 28ml sampling loop volumes.

Flow rates and the analytical stream

The key aspect of this system would have to have been that most of the gas was vented and a small but representative stream was split at a T-junction, diverted and used for analysis. This needed to be a constant ratio to be quantitative but the benefits were an interpretable signal (to 'see' the combustion) due to C within a dynamic operating range and minimisation of noise producing inputs (moisture, pressure and temperature changes) over the entire process. The fractional stream also means that some delay and attenuation of the event as indicated at the sensor was experienced because the linear velocity of this flow is reduced relative to the bulk stream.

The use of a single gas to oxidise the OM in the soils and move products through the system had simplified the process with fewer cylinders, valves and lines required (apart from the H_2 used for the GC) and facilitated the setting up of the sampling stream. The gross flows of 1L min^{-1} oxygen through the SCB were found to provide a good balance between heat transfer away (where the gas exits the rig) and an acceptable turnaround in analyses. In addition the majority of gases were vented from the work area to waste (vacuum system vented to roof of building). The possible gases generated from this chemical system that combines OM and excess O_2 can include:

CO	highly poisonous (accompanies incomplete combustion)
CO_2	asphyxiant
OCNs	unlikely or very low
NOx	toxic and forms nitric acid
CH_4	asphyxiant, not important unless pyrolysis conditions

The gas flow pressure was set by adjusting the regulator (at the cylinder) to 20psig however there was some flexibility provided it was sufficiently positive to produce a flow. The rate of flow was more critical and set at 1L min^{-1} by means of a gas metering valve while the value was indicated by (Platon) rotameter, both devices located (in this order) upstream of the reactor's gas inlet flange. Early experiments tested variations to this configuration but resulted in poorly controlled flow, excessive pressures and difficulties in setting and monitoring the flows generally. The reactor vessel was designed to 'rupture' and release pressure in the events of too much gas, explosive force due to high amounts of pure organic material or high back pressure.

The sampling stream was controlled by the sum of restrictions acting on the gas coursing past the T-branch, through the cold wire sensor block, sampling valve and loop arrangement to the exit where the rate of flow was measured by HP digital bubble meter. This could also have been done by a secondary valve but the totality of these devices produced an optimal restriction to achieve the efficient 26 ml per min. This flow was directed to the various analytical components for measurement of contained C (in form of CO_2) and proved to be an ideal fraction in terms of reducing the huge total volumes generated from calibration materials and soil cores in addition to the problematic water released from mineral lattices and oxidation of H from OM. The bulk stream of approx. 975 ml min^{-1} compensates when any changes to sampling stream occurs (e.g. blockage due to condensation in extreme cases) and in theory the consistency of flow was regarded as a crucial factor in calibration and measurement. However, in practical terms the SCB system appeared robust enough to cope with small flow fluctuations (e.g. 24 ml instead of the 26 ml) indicated by comparable output.

Nitrogen during analysis

There are two sources of N_2 (inorganic and organic) that need to be considered in combustion analyses. A large volume of inorganic (atmospheric) N_2 passes through the SCB between each sample as a consequence of soil sample loading (current manual operation) and clears along with any traces of oxidised species formed (initial peak cold wire, figure 3-5). As molecular N_2 moves through the heated zone, some 'prompt' NOx can be formed via the Fenimore mechanism (Dean and Bozzelli, 2000) but this is more relevant in the presence of fuel such as OM. Thermodynamically N_2 should be favoured at these furnace temperatures (850 ^oC) used for ignition and most of the concerns associated with prompt NOx formation have been eliminated here by the use of O_2 rather than air as dual carrier / oxidant. There was the added precaution where Cu/CuO extending to the 'cool' zone at the

end of the reactor facilitates reduction of any NOx through re-oxidation of Cu (to form Tenorite).

The organically derived N (so called fuel N) on the other hand (usually equivalent to about 10% of the C present) could potentially interfere with the C signal where these are measured concomitantly. In particular, NO_2 has a high thermal conductivity (table 3-3) and any significant amount would be problematic for C quantification using the cold wire sensor. For example if an equivalent volume of NO_2 was co-eluting with any CO_2 this would diminish its total response by a factor of about 2. NO and NO_2 are reactive gases (Chickos et al., 1973) formed during oxidation of N ions released from OM. There are a number of complex pathways (Dean and Bozzelli, 2000) by which N oxides to NO_2 with NO as an important intermediate stage so that:

$$NO + ½O_2 \leftrightarrow NO_2$$

Calculating the Gibbs free energy (ΔG^o) of reaction allows some prediction to be made on the direction of an equilibrium reaction and therefore whether either NO or NO_2 predominates at a particular temperature. The data (plotted below on figure 3-7) shows that at the temperatures used in the SCB, ΔG^o have positive values meaning that the equilibrium shifts towards the reactant NO rather than NO_2 (which would be minor if present). The reduced Cu at the reactor exit also acts to 'snatch' any stray NOx promoting the molecular form (N_2) beyond that point. Similarly calculated ΔG^o values for NO_2 formation from N_2 are positive and therefore not spontaneous at these temperatures. The thermal conductivities for species NO, N_2O and N_2 are very similar to the reference stream (O_2) as indicated on table 3-3, meaning these gases are effectively 'invisible' relative to the carrier. Comparisons of area counts[☝] acquired for atmospheric N_2 have been made here with the areas for similar volumes of CO_2. On this basis a 300 gram soil core at 2% C would record about 2000 area counts for C and a negligible 5 for most forms of N making it difficult to distinguish from background carrier using the cold wire sensor. Chromatographic retention times for any gases depend on carrier flow, temperature and the type of packing media. On Porapak type Q, as used here, the order of elution expected is according to their boiling points ($N_2 < NO < N_2O < NO_2$) but to be detected by the TCD would need to be present above trace levels. However in these chromatograms such gases could not be distinguished from the overwhelming presence of excess O_2 and CO_2. Ultimately it would be desirable to accurately measure the amount of N (as N_2) contained in soil cores, it being an important macronutrient.

[☝] Absolute area counts are specific to the particular data (A/D) collection system, sampling rates, sensor and amplifier used.

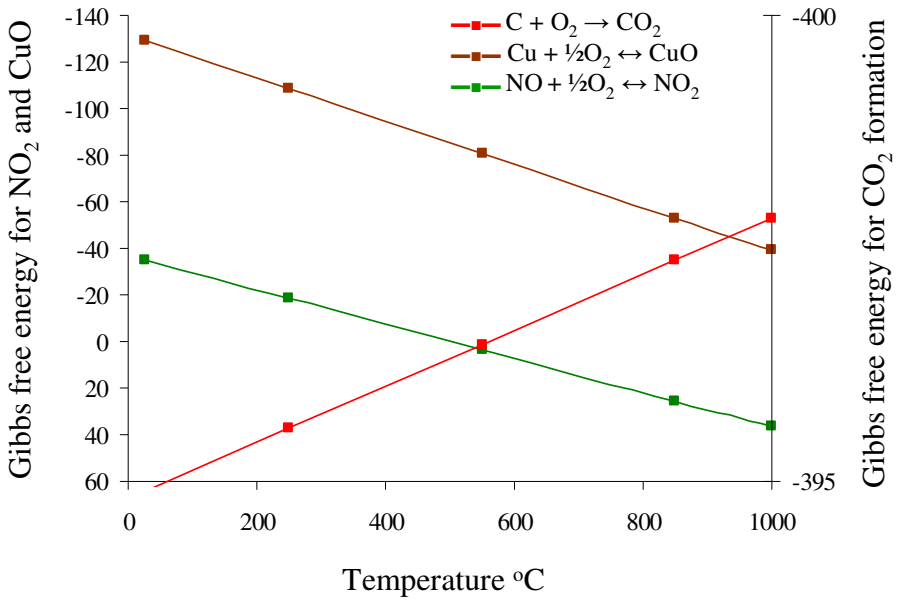

Figure 3-7 Ellingham-type diagram to indicate spontaneity for the reactions and gas formation occurring in the SCB.

Water during analysis

On heating, soils release additional water (several %) beyond the normal drying temperature of 105 °C (i.e. oven dry moisture). Free water, which can be more considerable when sampled after damp periods, is easily removed by air drying and low temperature oven without degrading the OM (although volatile C-containing compounds are almost certainly lost). However there is structural water that is incorporated in the lattice of clay minerals (in addition to OH^- units between phyllosilicate sheets). Greacen (1981) found that the lattice water ranged from 0.02 m^3/ m^3 in sands to 0.1 m^3/m^3 in clays. It is released only after much higher temperatures are attained (430 °C), where OM still continues to decompose / oxidise thus presenting a potential interference for C analysis in any thermal method (see chapter 2) including the SCB. Even NDIR detectors which are tuned to the major CO_2 absorption lines require a dry gas for analysis (Smith and Tabatabai, 2004). Thermal analysis studies (chapter 2) have been a useful way of describing the relative distribution of water-related events over a heating cycle.

The SCB is a bulk C system that operates at over 800 °C and transfers heat into the 300g plus soil mass to degrade and extract carbonaceous material including BC. Elemental analysers

run at equally high temperatures but operate in a somewhat different way, transferring product gases in stages and removing water with hydrophilic traps. This is a cumbersome step and would have meant operating at increased system pressure or otherwise introducing a condenser coil which needs to be dried frequently. While the bulk gas stream was found to be 'dripping' during the analyses of finer grained soils, to date, any precaution in the methodology to trap out water from the analytical stream flow has been avoided owing to the SCBs general configuration. That is, the concept of a fractional stream (40:1) vastly reduces this problem which can cause flow blockage as well as interference at the detector. The experimental soil C data presented here was based on a fractional flow of 26 ml min^{-1}. This appeared to be an optimal flow rate while it probably entailed some condensation on metal surfaces en route to the detector block (followed by cyclical drying as gas purges through) but appeared manageable. The architecture of the cell (refer to figure 3-2) also makes it less susceptible to contamination from a 'dirty' gas stream where the filament wire is raised away a little from its direct path.

However, analyses conducted on such amounts of soils were expected to yield water quantities resulting from the mineral matter typically around 6g depending on the percentages of clay (refer to clay related TGA mass loss on figure 3-3). Because both CO_2 and water can appear at the detector concurrently tests had to be carried out to gauge any interfering effects due to water vapour from the gas stream on the overall C analyses and the detector baseline. To do this, comparable amounts of water were heated in preconditioned soil matrices (C removed). The resulting traces (example on figure 3-8) did not indicate adverse effects and as discussed would be due mainly to the reduced analytical flow to the detection system substantially dropping the apparent level to more like 0.2 g per soil analysis.

There is also the additional source from the combustion of OM according to one of the basic chemical reactions: carbohydrate + $O_2 \rightarrow CO_2 + H_2O$

Simple carbohydrates from recent OM resemble sugars and starches with the common formula $C_nH_{2n}O_n$ and are a rich source of oxidisable H. The amounts of H (i.e. potential water) tend to decrease with maturation / preservation of OM (Schnitzer et al., 1964) and are much lower from charred materials but these differences are likely to be irrelevant in comparison to the amounts of clay minerals present.

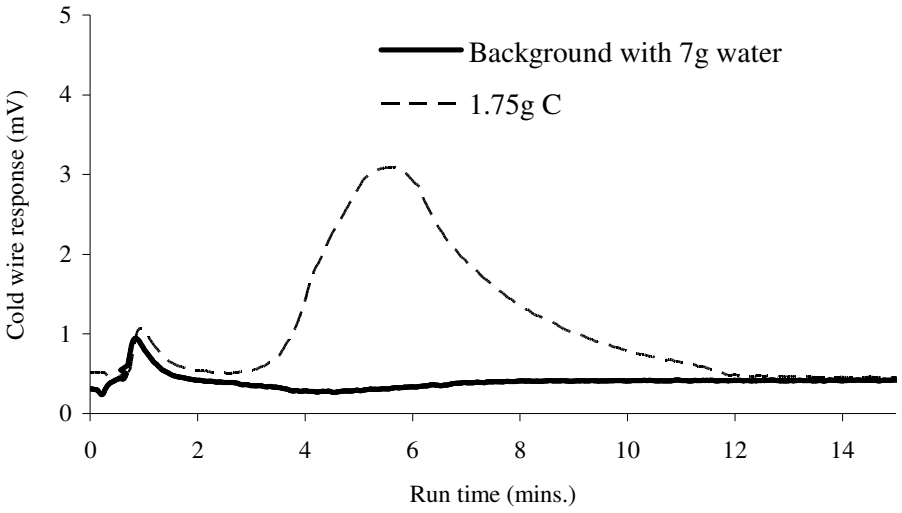

Figure 3-8 Cold wire trace to test effect of usual water contents (background burnt soil and water).

Calibration and Performance evaluation of the SCB

Calibrating the SCB

The calibration work was based on the combustion of known amounts of pure carbonaceous materials. These became the internal standards and were primarily sucrose (42% C) and charcoal (70% C) which were readily available. Combustion initially of neat material was replaced by 'synthetically' prepared (i.e. organic bearing) soils where these pure substances were mixed into previously 'cleaned' sandy clay loam (from Lansdowne). There were two main reasons for this:

a) pure materials can result in explosive combustion events and while the detected peak shapes are favourable gas can be lost and
b) experiments needed to simulate the extraction of C from soil matrices as much as possible because the calibration had to be applicable to (unknown) soils.

The calibrations summarised by the charts on figure 3-9a and 3-9b span 0.1 to 3.5 gram C to cover the typical soil contents expected in subsequent field trials. While most attention was given to lower end calibration, an upper limit for the system has not been reached. Instrument responses were acquired with a sampling rate of 1Hz and the data system (Logger Pro3.8.4, Vernier Software & Technology, Oregon) set to 20mV range. Most importantly the preset analytical stream was measured at 26 ml min^{-1} and while not used numerically it must remain constant.

On the second chart a narrower region has been expanded (range where most activity has been carried out) showing C values re-calculated using the derived calibration function and respective areas along with error bars representing the standard error of the mean (SEM). This area response data was obtained by the cold wire sensor where linearity extends between the origin and the maximum amount of C tested.

The C compositions of calibration materials were also determined by elemental analysis (Vario Max) as part of the standardisation process. While carbonate yielded the expected 12 % C there were serious variations in charcoal results that could not be assigned to moisture (due to hygroscopic properties). It was concluded that the commercial analyser was easily oversaturated where C occurred in high concentration and care was required when analysing black C material.

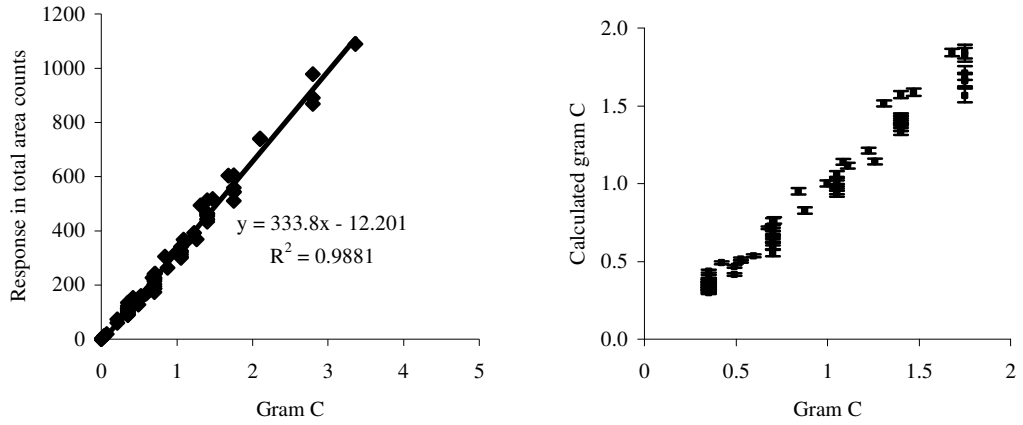

Figure 3-9 Calibration chart for the SCB correlating areas with amount of C (a) and error bars (b).

Performance parameters

Standard methods were used to characterise the performance of the SCB analytical system. The Standard error of prediction (SEP) was calculated so that:

$SEP = [1/n\ (measured\text{-}standard)^2]^{0.5}$

The calculated standard error of prediction (SEP) based on the calibration data was determined at 0.7 g C /kg which provides a measure of the analytical uncertainty when applying the slope function to 'unknown' soils. Sources of error that may contribute to analytical results might be:

SEM = Std deviation / √n where n is number of calibrations at given mass

-unsteady balance when weighing out small amounts of soil with high C content,
-leakage of gas or blockage of flow which changes the relative magnitude of the analytical stream and / or
-incomplete oxidation of C in the soil.

In practical terms the lowest concentration that can be reliably analysed can be determined by running standards at successively lower concentration and plotting the results (and standard deviations). It can also be useful to compare the signals obtained for known amounts against baseline noise from blanks. However it is not necessary to successively reduce the amount of analyte (gram C) to determine the lower limit of detection (LLD). The limit of detection is derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure (IUPAC, 2006). There are a number of different "detection limits" that are commonly used, one of them being the method detection limit (MDL). The method detection limit (MDL) is a statistical approach based on replicate analyses (table 3-4) at one particular concentration (U.S. Environmental Protection Agency, 1997; Oblinger Childress et al., 1999). This parameter in effect quantifies the amount of signal unrelated to the analyte which is then used to determine LLD.

MDL = Std deviation (replicate analyses) x student t coefficient,

where the replicate analyses are carried out on the same (low) concentration over several days to capture all possible variables and the student's coefficient (t-distribution, $\alpha = 0.01$) given by the number of replicates. Accordingly, the MDL and LLD were determined for the SCB based on repeated aliquots of soil containing 0.35 gram C (representing low end of scale) analysed over different days. The standard deviation for the test values in table 3-4 (0.03) with the T-test value for n=9 at 99% confidence level, yielded the MDL of 0.085g C. MDL provides some measure of the variance due to extraneous signal and changes to the MDL over time can alert to problems with the analytical system (it may also be useful to carry this out for different levels of concentration).

More useful information on this subject can be found on the following report by the US Geological Survey (Childress et al., 1999):
http://water.usgs.gov/owq/OFR_99-193/detection.html

Table 3-4 Detector response data (recorded as area counts) based on replicate (0.35 g C) analyses to yield final C determinations (univariate analysis) then used to obtain MDL value.

Analysed	Response	Yield
Gram C	Peak area	Gram C
0.35	101	0.30
0.35	115	0.34
0.35	108	0.32
0.35	112	0.34
0.35	123	0.37
0.35	134	0.40
0.35	107	0.32
0.35	114	0.34
0.35	113	0.34

Commonly a concentration 2.5 times the MDL value is regarded as the LLD and below this the analytical results are deemed unreliable and could report a false positive. In this case the LLD was 0.17g C and if we take a look at the instrument output (figure 3-10) for soils containing C at similarly low levels (MDL 0.085) it is clear that system perturbations and noise become significant factors.

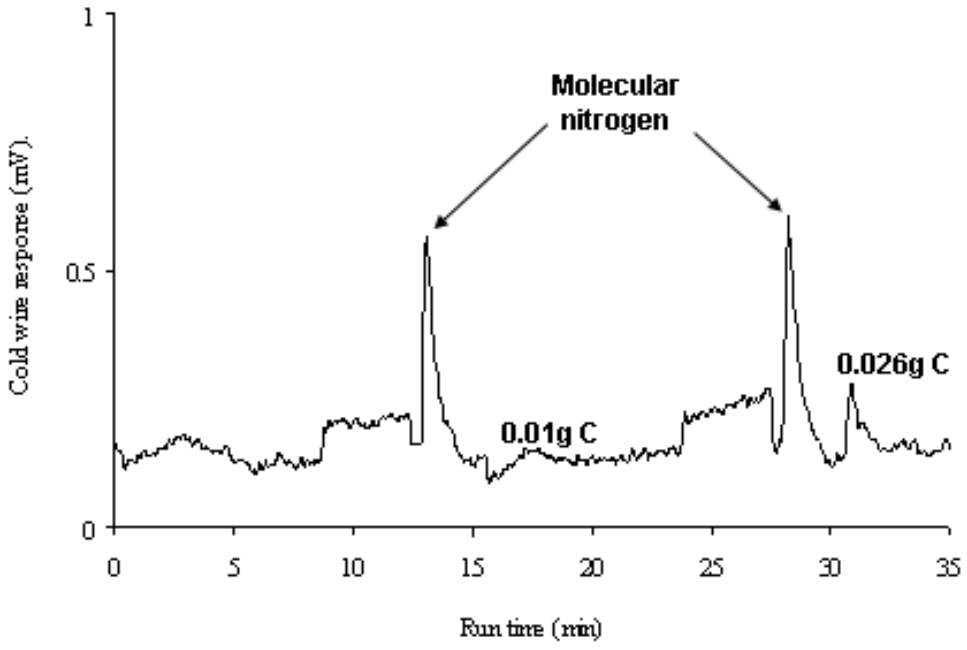

Figure 3-10 Instrument response for low total C masses (one gram of 1.02 % and 2.6% soil C standards). N_2 peaks derive from atmosphere during sample introduction.

SUMMARISING THE SCB

GC was a useful companion in developing the SCB – although it was limited in sensitivity for CO and currently unable to measure N_2.

Soil C can be determined with the GC on calibrated sampling loop volumes below a critical amount of total C in the sample which is about one gram = 1% in 100g.

The thermistor sensor proved very responsive to the amount of C in the bulk gas resulting from the combustion of simulated and natural soils over a 0.1 to 3.5 % range.

The SCB is a test bench that forms a template for commercialisation and / or the possibility to up-scale further if required.

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CHAPTER 4 - FIELD TRIALS: SOIL CARBON BENCH FOR WHOLE CORE ANALYSIS

One of the major problems confronting the measurement of soil C stocks is the variability with space both laterally and with depth (Goidts et al., 2009; Hiederer, 2009). This is influenced by the geology, soil type, climate, topography and land use.

Patterns in soil C variation with depth alone can be diverse and depend on any of the following:

a) the subsurface – for example it can increase where carbonate occurs in the regolith,
b) the rate of OM accumulation and oxidation (inputs /outputs) controlled by aeration properties
c) bioturbation including tillage or
d) natural phenomena – for example as occurs in the Cranbourne sand of Victoria (Leeper and Uren, 1993) and vertisols generally.
e) alluvial / colluvial processes and
f) podzolisation and eluviation

Therefore the objective to analyse a nominal depth interval, which is also well aligned with the IPCC framework, offers considerable advantages because such a single analysis can encapsulate this entire variation. In fact, an underlying driving force for producing this method was taking a soil aliquot sufficiently large to overcome problems associated with the natural variation in any dimension. Although capturing the lateral variability (across stratum scale) must depend on sampling density (influenced by real changes). Conventional soil C inventories have been based on the analyses of very small and refined (sieved) subsamples. This has placed enormous burden and cost on careful preparation to obtain representative material with its numerous impracticalities for broad scale surveys.

Size fractions in perspective

Standard soil C methods have relied on the analysis of these particle sub-fractions which was to a large extent related to expediency (covered in the earlier review). In addition it is fair to say that C separated with the finer particles may well be more decomposed (arguably older and stabilised) as has been shown by some indicators. Studying soil C tied up with the <50μm (more stable) fraction over the longer term could provide valuable information on OM dynamics and is therefore worthwhile continuing. However SOM must originate from once recent OM and sustaining this process necessitates adding more recent OM (notwithstanding priming effects). Over the timeframes (years to decades) anticipated in C sequestration monitoring and assessing efficiencies between land management practices, there may be some question marks and limits over the practically of looking for C change in the sub-fractions especially in view of instrument detection limits. A clear definition of what should be assessable under soil C stocks is still required although considerable material has been generated on the subject (McKenzie et al., 2000; Sanderman et al., 2011; IPCC 2006).

Some have called for a method by which the SOM and the contribution from root mass can be measured separately. In Canada for example, there is a growing movement toward analysing the whole soil and even including the roots in the soil C inventory (D. Angers pers. Comm., March 2013) since these are such an important part of below ground C stocks. Determining the C for the total soil core (less recent OM such as roots) was the central theme for this project bringing with it advantages of direct soil C measurement on a volumetric or gravimetric basis and obviating the need for gravel corrections.

The effect of particle-size on C measurements

Then follows the question on how conventionally obtained concentrations of C in sub-fractions interrelate and any bearing it may have for analyses obtained by SCB over the study area. This would serve to:

- provide typical C data for the area
- some measure of the spatial variation in terms of %C and
- distortion to C values because the SCB analyses all C under 2mm (not only <53µm)

To gain some insight on this aspect for the soil C within the study area, comparisons on (SPR and BPR free) size fractions were done by standard methods. Two soils at a lucerne plot were cored a metre apart at Lansdowne, via Cobbitty, NSW (located approximately S34.02.335, E150.66.454) and processed in the conventional way. After these were dried, ground and separated by dry sieving into the size sub-fractions shown on table 4-1, C concentrations were determined on maximum aliquot sizes (around 0.7g) using an elemental analyser (Elementar Vario Max, Hanau Germany with standard error on replicates within 0.03% C). The patterns exhibited by the % C data throughout these fractions indicated the commonly found decline in C concentration with depth. In particular, the data also reflected a systematic C enrichment in the finer particle sizes where the individual values (e.g. 2.34 and 1.81% in the top 10cm) showed the extent of lateral variation over a metre distance. These were sub-sampled from what were considered well mixed, representative core intervals but after the data were recalculated over the full 30cm for both sites the differences (between each site) became less extreme with 0.53 and 0.40% in the fine and total fractions respectively but still sufficiently different.

Of special interest in the context of the SCB method (which analyses the total soil fraction) was that the ratio between the % C in fine and total fractions were fairly uniform (table 4-1) for both sites and all three depths. Similarly when % C for successive depth intervals were compared (by ratios), almost equivalent values were obtained for each respective size

SPR are surface plant residues and BPR are buried plant residues

fraction. These indicated that the same (consistent) spatial C distribution patterns should result whether determined by SCB or <53µm (conventional means). It would have to be investigated separately how universal this pattern might be and the overall influence of grainsize distribution on whole core C analysis.

Table 4-1 Soil C content of size fractions at each depth and ratios of these values between fine and coarse at each depth.

Depth Intervals	% C <53µm	% C <100µm	% C <2mm	Ratio <53/< 2mm
0-10cm	1.81	1.40	1.33	1.4
11-20cm	0.79	0.68	0.57	1.4
21-30cm	0.75		0.49	1.5
1 m apart				
0-10cm	2.34	1.90	1.74	1.3
11-20cm	1.70	1.34	1.18	1.4
21-30cm	0.91		0.67	1.4

Soil Collection Methods for C Determination

The test site

Devising the most optimal soil extraction method for the SCB and testing its analytical capability was conducted at the same locality (Lansdowne, NSW). There, the soils varied from sandy loams to sandy clay loams along the N-S transect (see aerial map on figure 4-1). The area was an ideal testing ground because it consisted of a good grading of grainsizes and C content ranging from 0.2 % in the sandier parts up to several % in surface clay loams nearer the river. This meant the experimental work could include any of the anticipated difficulties associated with the analysis of soils with clay content as well as that associated with the actual sampling methodology. Theoretically, C is more readily oxidised out from sandy than clay soils because the lattice water can be problematic during analysis and where the C may be more occluded this has implications for diffusion of gases into and out of the matrix. The soils in this area had pH values around neutral (6.8 to 7.4) which may not necessarily preclude the presence of carbonates but has not been detected in thermal analyses (described in Chapter 2) of soils from this general area.

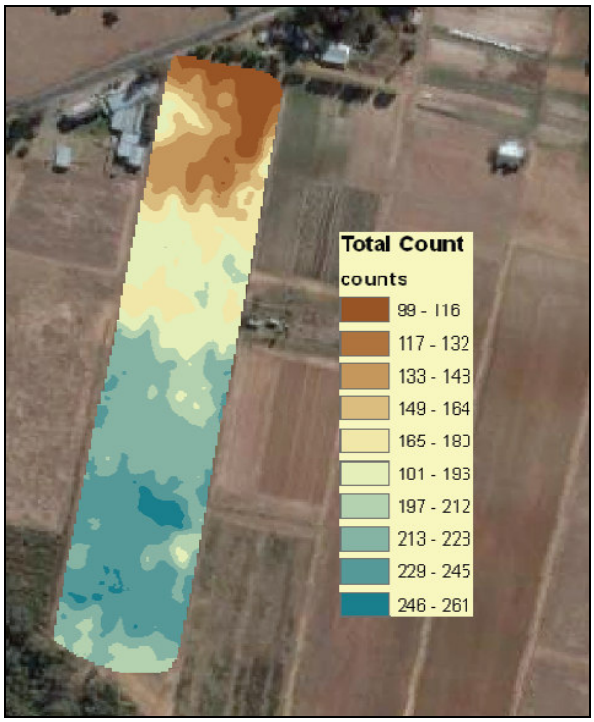
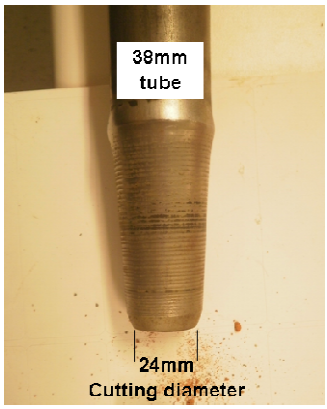


Figure 4-1 Aerial view of the test site (overlain with gamma radiometric data) at Lansdowne which was under lucerne cultivation. The sandy loam changes to sandy clay loam coinciding with higher gamma counts (blue).

Method of sampling cores

In order to develop a method that allowed C data to be expressed on a volumetric basis a method of obtaining reliable and uniform soil plugs was an important requirement. Obtaining soils from known volumes avoids issues described by Lee et al. (2009) when correcting data using bulk density. Several soil recovery tools including 50cm vibracore were tested to arrive at the most suitable soil plugs in terms of uniformity and a volume of soil that could be competently processed in the SCB. Manual soil recovery tools (e.g. Grainger product) were also tested. While these were easily deployed (very portable) and good soil data was obtained, productivity was limited for large scale surveys. Moving to a mechanised coring method of the correct volumetric dimensions increased efficiency and ease of collecting cores for the SCB. Two soil corer tubes were custom made to specification (Christie Engineering, Horsley Park, NSW) which would adapt to the existing (Gaiter or Canter mounted) hydraulically driven vibracore system (Atlas Copco). The samplers as depicted on Plates 4-1a and 4-2 were drawn down at the cutting end from the standard 38mm tubes so that a volume / mass of soil appropriate for the SCB (ca. 450cc or 600g) could be recovered up to 1 m depth.



Plates 4-1 Close-up of the corer cutting edge (a) used to recover soil plugs (b) with dimensions 24mm across and lengths to 500mm which were very suitable for the SCB.

The 24mm cutting tip in combination with this diameter tube seemed to make removal of core (as appear in 4-1b) an easy task because of the greater clearance compared to similar samplers (in the 50mm versions a paper clip had been used to ease the extrusion of tightly held core). The stronger taper from the 24mm also worked well with the sandy clay loam where soil tends to expand after it has been 'cut' from the surrounding ground. Conceivable limitations might be where soils contain stony fragments over 24mm however this tool was designed for these sandy clay loams. As with other corers, care was required to retain sandier material (as encountered at the northern most part of the trial area) which was recoverable but not as coherent as soil with greater clay content. Direct determination of C for the whole core volume circumvented the need to calculate it using bulk density data which itself was obtained as part of processing. Data from all the probes types were comparable using the calculated volumes (cross sectional area multiplied by precise depth) and masses had been recorded allowing C to be expressed volumetrically or gravimetrically. To facilitate continuity in the data it was regarded that a uniform method of sampling should become a part of the SCB method.

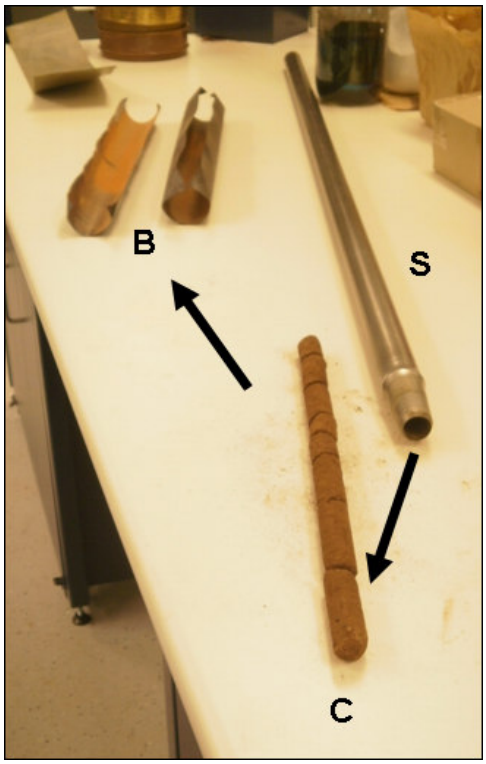


Plate 4-2 Core lengths (C) recovered in the field using the core tool (S) which are then broken up where >2mm OM (roots) are removed / dried and then transferred to stainless steel combustion boats (B) ready for loading into the SCB.

Bulk Density

Bulk density (BD) determination was based on the mass of oven-dried material recovered (only solids) over the volume of soil removed. Because insertion and retrieval of the coring tool can results in some spreading / compaction of the surrounding ground (enlarged void) it was most reliable to use the cross-sectional area of the cutting tip and multiplying by length. In addition clay loams can expand slightly after coring. Furthermore, especially when using a hydraulic hammer, some bottom fragments can drop back (in sandier ground) and any unaccounted for material would lead to error in volumetric determinations. To cross-check the depth penetration, normally gauged from graduations on the corer and total recovery (possible losses or compression) the measuring tape was extended down hole (sometimes reassembling core can assist also). These steps provided reassurance in the volume accuracies essential to determine the absolute C (kg) per m^2 or bulk densities of the soils extracted.

Results for the SCB

Accuracy and precision – Validation with conventional C analysis on homogenised samples
In order to demonstrate how accurately and reproducibly the SCB system could deliver results in comparison to the industry standard it was necessary to conduct conventional soil C analyses in parallel. These were carried out on the small amounts of soil necessary for the elemental analyser (Elementar Vario Max, Hanau Germany) obtained from the cores in one of two ways. Numerous soils (mainly from Lansdowne) which were used continuously in the development and testing stages of the SCB were well homogenised and these results have been summarised on figure 4-2 and table 4-2.

Elemental analysers are very precise instruments capable of replicating results well below 0.1% C when using similar masses of homogenous soil or standard. All soils were similarly dried in a holding oven (90 ^{o}C) prior to analysis so that values could be expressed on the standard dry basis (Rayment and Lyons, 2011) as surface water is a factor that can lead to differences if not removed or corrected. Moisture contents were variable at around 5% in the sandy northern part of the trial area to between 10 and 15% in the clay loam. In practice however, the precisions of replicate analyses for C in soils can often be closer to 0.2% reflecting the sum of instrument / process noise and any heterogeneity remaining in the sample. The accuracy of the commercial instrument had been checked against laboratory soil standards (Rayment et al., 2007) obtained through Proficiency Services Ltd. (Hamilton, NZ). These ranged in C from 0.1 to 4.02% while instrument calibration for C and N was based on L-Glutamic acid. In general, certified soil standards were inappropriate or impractical for routine use in the SCB simply because the total amount (50 gram) available in the laboratory was far less than a normal SCB loading – hence the requirement for internal standards met by the (relatively unlimited) Lansdowne soils and carefully prepared (weighed) mixtures with known C. Purely as a demonstration, an equivalent amount of soil standard as used in the elemental analyser (i.e. 4.02% of 1 gram) was analysed by SCB. In total, this resulted in a fairly minuscule amount of C for the SCB system where the output fell on the lowest limit of the calibration line (figure 3-9a, Chapter 3).

The SCB data was validated against results from the elemental analyser and the performance assessed by evaluating the respective differences between these two sets of results (see table 4-2). Unlike the conventional data, SCB determinations were in the form of a single C value derived for the entire mass of soil. The C content from the two measurements are in good agreement, with a mean absolute difference between the two methods 0.12%, RMSE =

0.16%, and bias = -0.06%. It must be emphasised these were not measures of [d]precision which tend to be much closer for a particular instrument. The variations in the two measurements do not appear to be systematic i.e. they are independent of C abundance (figure 4-2).

Note: RMSE = $[1/n \sum(\text{SCB-standard})]^{1/2}$, and Bias $=1/n \sum(\text{SCB-standard})$ where values are in % C and n is the number of observations.

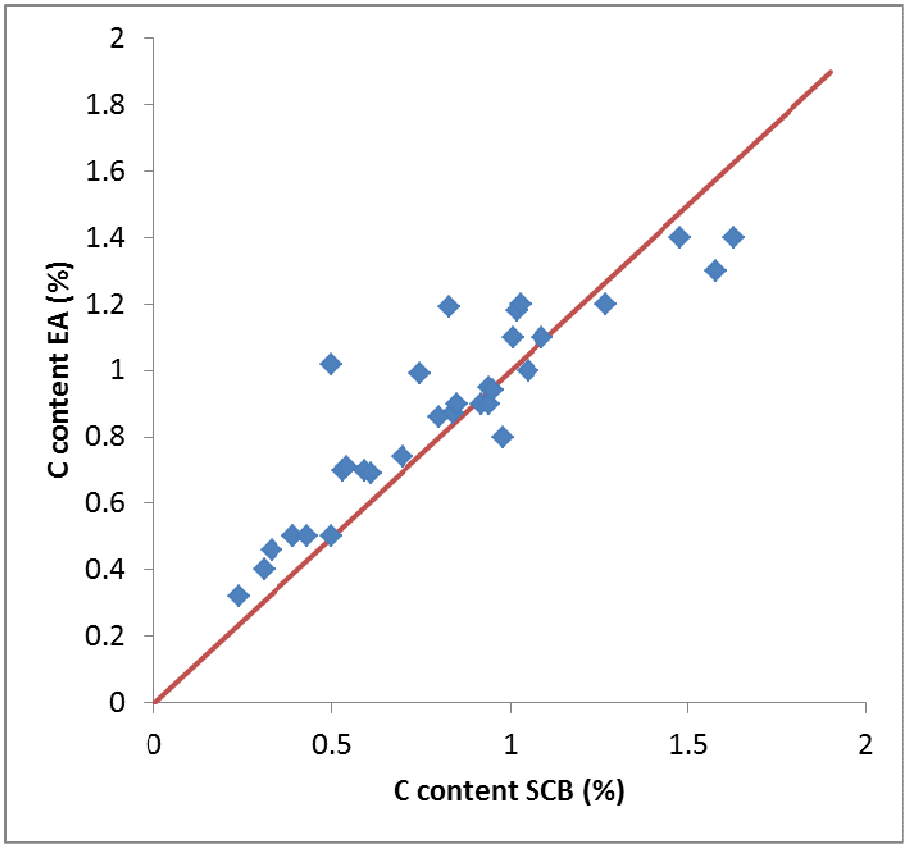


Figure 4-2 General concordance of the soil carbon analyses obtained by SCB and elemental analysis.

[d] Note: The SCB was applied to the analysis of whole cores but to assess how this instrument can repeat soil C results (as occurs with conventional methods) this required running cores of effectively the same soil. This aspect is discussed in detail in the next section on site analyses.

Table 4-2 Comparison of C analyses from the SCB with those from standard analytical methods (test soils, Lansdowne area).

SCB bulk of soil	Standard analyses	Absolute Difference (SCB-standard)
%C	%C	Δ %C
0.24	0.32	0.08
0.31	0.40	0.09
0.33	0.46	0.13
0.39	0.50	0.11
0.39	0.50	0.11
0.43	0.50	0.07
0.50	1.02	0.52
0.50	0.50	0.00
0.53	0.70	0.17
0.54	0.71	0.18
0.59	0.70	0.11
0.61	0.69	0.08
0.70	0.74	0.04
0.75	0.99	0.24
0.80	0.86	0.06
0.83	1.19	0.36
0.84	0.87	0.03
0.85	0.90	0.05
0.92	0.90	0.02
0.94	0.90	0.04
0.94	0.95	0.01
0.95	0.94	0.01
0.98	0.80	0.18
1.01	1.10	0.09
1.02	1.18	0.16
1.03	1.20	0.17
1.05	1.00	0.05
1.09	1.10	0.01
1.27	1.20	0.07
1.48	1.40	0.08
1.58	1.30	0.28
1.63	1.40	0.23

Accuracy and precision – Validation of results from whole soil cores

In the next stage of the work where whole core contents were collected along a transect in Lansdowne, duplicate or triplicate representative subsamples for each sampling interval were removed and similarly determined for C by standard analysis The results are presented on

table 4-3, arranged against each location on the transect showing replicates for whole cores (in gram C per core and % C of total core, the total masses recovered and their BD) and replicates for conventional elemental analysis. The comparison of results is graphically presented on figure 4-3.

Elemental analyses need to be conducted on small amounts of soil so aliquots were removed from the respective core intervals to capture a representative or reliable subsample of the mass to be determined. Where duplicate and triplicate EA data was obtained, the soil was taken from two/three points in the grinding vessel containing the mixed up dry core after several minutes of diminution. In the cases where only a single value appears the core had been ground (and well homogenised) for >10 min.

TABLE 4-3 Spatial data (from GPS), BD, soil mass and all carbon data at each position along transect (Lansdowne trial area). Values from the SCB represent mass C per 50cm core and %C (by mass) along with their respective standard replicate analyses (also %C mass basis) obtained using the Elementar Vario Max analyser.

Geographic location		Interval	Dry mass*	BD	Total C	Total C	Standard method replicates		
South	East	cm	g	g cm^{-3}	g / core	% (mass)	%C	%C	%C
3402127	15066210	0-50	505	1.3	2.51	0.50	0.55	0.36	0.45
3402127	15066210	0-50	448	1.1	2.23	0.50	0.50		
3402127	15066210	0-50	493	1.2	2.99	0.61	0.20	0.48	
3402173	15066202	0-50	324	1.4	1.98	0.61	0.57	0.52	
3402173	15066202	0-50	344	1.5	1.51	0.44	0.44	0.45	
3402173	15066202	0-50	309	1.3	3.02	0.98	0.91	0.90	
3402216	15066188	0-50	384	1.0	3.39	0.88	0.91	0.78	0.93
3402216	15066188	0-50	329	0.8	3.21	0.98	0.80		
3402216	15066188	0-50	341	0.8	3.53	1.03	0.81	0.78	
3402265	15066180	0-50	283	1.2	1.31	0.46	0.66	0.23	
3402265	15066180	0-50	336	1.5	1.58	0.47	0.60	0.25	
3402265	15066180	0-50	338	1.5	1.67	0.49	0.66	0.65	
3402305	15066160	0-50	488	1.2	3.35	0.69	0.65	0.65	0.70
3402305	15066160	0-50	498	1.2	3.06	0.61	0.69		
3402305	15066160	0-50	557	1.4	3.74	0.67	0.64	0.72	
3402352	15066164	0-50	263	1.1	1.90	0.73	1.02	0.32	
3402352	15066164	0-50	274	1.2	1.90	0.71	1.04	0.35	
3402352	15066164	0-50	296	1.3	2.20	0.74	0.91	0.90	
3402387	15066140	0-50	505	1.3	3.29	0.65	0.45	0.47	0.55
3402387	15066140	0-50	462	1.1	3.25	0.70	0.74		
3402387	15066140	0-50	465	1.2	3.38	0.73	0.76	0.75	
3402436	15066135	0-50	325	1.4	2.40	0.75	0.39	1.19	
3402472	15066117	0-50	413	1.0	4.06	0.98	0.76	1.06	0.91
3402472	15066117	0-50	487	1.2	4.62	0.95	0.94		
3402472	15066117	0-50	497	1.2	4.66	0.94	1.02	0.98	
3402518	15066110	0-50	317	1.4	3.20	1.00	1.74	0.64	
3402560	15066096	0-50	294	1.3	1.79	0.61	0.45	0.45	
3402560	15066096	0-50	351	1.5	1.88	0.54	0.71		
3402560	15066096	0-50	348	1.5	1.90	0.55			

*Note that a larger corer diameter was initially tested and resulted in the higher total masses of soil and gm /core.

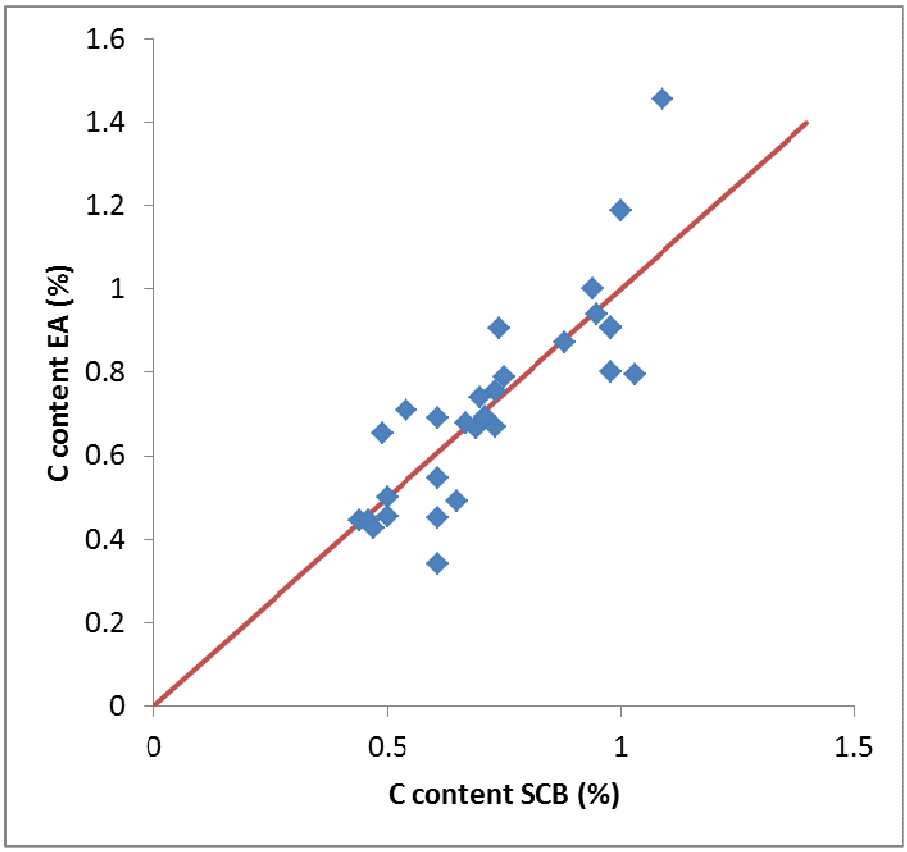


Figure 4-3 Soil analyses for cores determined by SCB and compared with representative aliquots taken prior to elemental analysis.

Table 4-3 has been set out separating data which were analysed by the EA singly, in duplicate or in triplicate. The results indicated that the differences (error) from duplicate and triplicate samples were generally higher than single value analyses from well mixed samples. The scatter or variation amongst some replicate analyses indicated most likely an issue with soil heterogeneity remaining although it must be emphasised that material for replicate analyses were obtained by taking representative subsamples prior to processing the cores by SCB (as described above). Larger variations (error) can probably be attributed to C heterogeneity emerging much more easily during conventional analyses due to the small sample sizes. Hence the analyses via SCB relate better to the replicate mean values. Statistical parameters (root mean square error or [d]RMSE and bias) have been summarised below (table 4-4) to compare the relative performance of the two analysis methods (SCB vs EA) based on the two types of soil presentations used in the SCB, i.e., homogenised soils for development and calibration (table 4-2) and whole soil core volumes as in field testing (table 4-3). The values indicated by the RMSE of 0.114% for the whole cores from transects as compared to 0.163% for homogenised soils showed that analysis of whole core volumes (i.e. one value per core) provided data of equivalent reliability. Their respective biases were 0.017 and -0.060 which is the mean value of deviation or systematic error. The (–) sign in this case indicated that the SCB produced a slightly higher result relative to the standard method but are within accepted normal analytical precision.

Table 4-4 Comparing the results from the two systems (C content in %) on a statistical basis.

Soil sample	n	RMSE	BIAS
Cores	28	0.114	0.017
Homogenised	32	0.163	-0.060

Reviewing the overall data in the tables (4-2 and 4-3) showed that the results between the two systems typically concurred within 0.15% C. The fact that differences between the systems were very low for analyses of the whole core and well worked soils or where replicate data were averaged, provided some indication that the SCB had the capacity to 'smooth' the natural variation in soil C. The preconception that such an approach incorporates the heterogeneity into one C value, effectively integrating the spatial (or depth) variation seems to have been well vindicated.

[d] Bias =1/n $\sum$(SCB-standard) where values are in % C and n are the number of comparisons.

Determining soil C stocks by analysing whole cores – Proof of concept

So far it has been shown that the SCB can provide reliable C data and that it has the capacity to average or integrate otherwise variable point analyses. It is then obvious that unless soil is homogenously distributed in the sample (where conventional analyses should agree well with the SCB) that point analyses can be problematic and necessitate multiple processing / analysis (adding to time and cost). This supports the case for processing larger quantities of soil to obtain a better picture of C in the landscape. It can be emphasised at this point that while C changes with depth are incorporated in single analyses, those occurring between sample sites must be interpolated. To demonstrate how large scale C determination could be a useful attribute for quantifying soil C inventories, the whole cores extracted from each of the sites in the test area and determined according to the SCB method have been analysed on a site-specific and transect basis.

Site locations (on systematic grid with random starting point) were nominally spaced at 100m intervals along the N-S transects (see table 4-3). To gain a measure of site-specific replication (for whole soil cores) and importantly the level of uncertainty for soil C estimates over a number of locations, three cores were removed at each site in a small triangular configuration (within approximately 20cm of one another). Triplicate sampling was intended to enable not only an evaluation of the uncertainties but also to assist in the recognition of aberrations in soil C data which can arise for a number of reasons. These can result from either technical malfunctions during the analytical stages, poor sampling (although these should be minimised), but also real anomalies from the soil itself such as a buried tree stem or some other C 'nugget'. Obviously such data should be removed during numerical evaluation for C stocks. At each test site the corer was positioned and GPS coordinates were recorded. The full 50cm soil plug was retracted with the corer, carefully removed and transferred to labelled [d]bag before shifting to the next position.

Note, the results for whole core analyses from each sampling location over the study area (table 4-3) were also used for the cross validation analysis above where data were correlated with standard analytical methods (figures 4-2, 4-3 and table 4-4). The results presented / discussed here for all the sites along the transect have been expressed as gram C per whole core interval (50cm) (the immediate value obtained from the SCB) and % C of total dry core mass (divide % by 100 to convert to kg/kg). Overall the soil C values from the cores were quite coherent (per site and trend down transect) except in two cases (site coordinates 3402173, 15066202 and 3402216, 15066188) where contamination was suspected from most

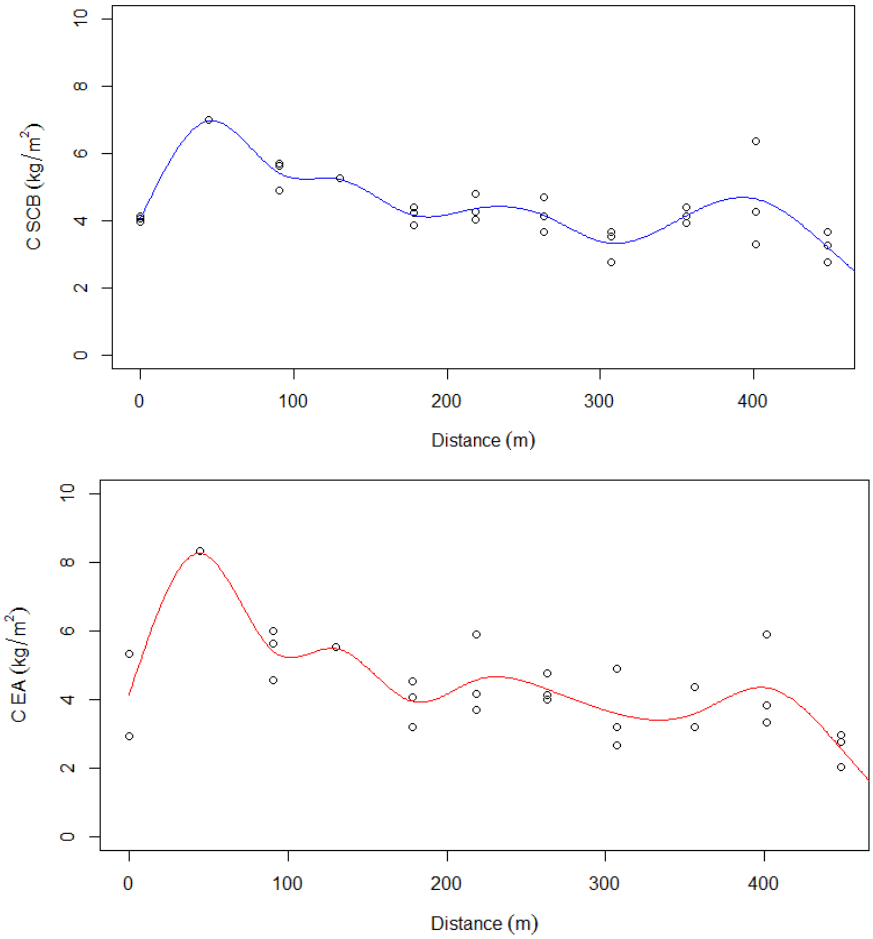
[d] Sampling bags were composed of cellulose, a rich source of C, however contamination was regarded as highly remote.

likely an old buried post and grease on the corer respectively. In the former, the low BD (0.8 g cm^{-1}) immediately alerted to an anomalous sampling point. Generally, total C per core were within 0.2 gram of their mean for each site where the total amounts ranged from 0.7 to 4.7 gram C per 50cm core for the sampled positions over the entire transect. On a gravimetric basis (the ranges were 0.2 to 1.09% C per 50cm core) the site reproducibility was typically around 0.0003 kg/kg. All the data acquired for a section of this transect have been plotted below (expressed as kg m^{-2}, figure 4-4) to show graphically that the SCB provides 'smoother' soil C stocks (top figure) in comparison to the standard method where results appear more variable (bottom figure). A clearer statistical picture is obtained through the box plots (figure 4-5) comparing the methods over the transect. On a gravimetric basis, there is a slightly higher mean for the SCB, but the SCB has a lower standard deviation. Similarly for C stock (as kg m^{-2}), both EA and SCB produce similar mean values across transect (mean = 4.3, median = 4.1 kg m^{-2}), however the values obtained by SCB has a smaller variance (std. deviation = 0.99, interquartile range = 1.08 kg m^{-2}) when compared to values obtained by EA (std. deviation = 1.36, interquartile range = 2.71 kg m^{-2}).

Table 4-5 Summary of statistical data for C stocks comparing SCB and conventional method on Transect 1 (n = 28).

	%C	%C	C kg m^{-2}	C kg m^{-2}
	EA	SCB	EA	SCB
Mean	0.695	0.712	4.250	4.305
Std. dev	0.206	0.188	1.357	0.990

Also note that means for each site have also been presented as spatial plots in following discussion on figures 3-6a and b to better visualise distribution, trend and the impact that errors may have.



Replicate C stock analyses (kg m^{-2}) for whole cores using the soil carbon bench. Triplicate cores were extracted within 20cm of one another at each site which were in metres along transect (x-axis).

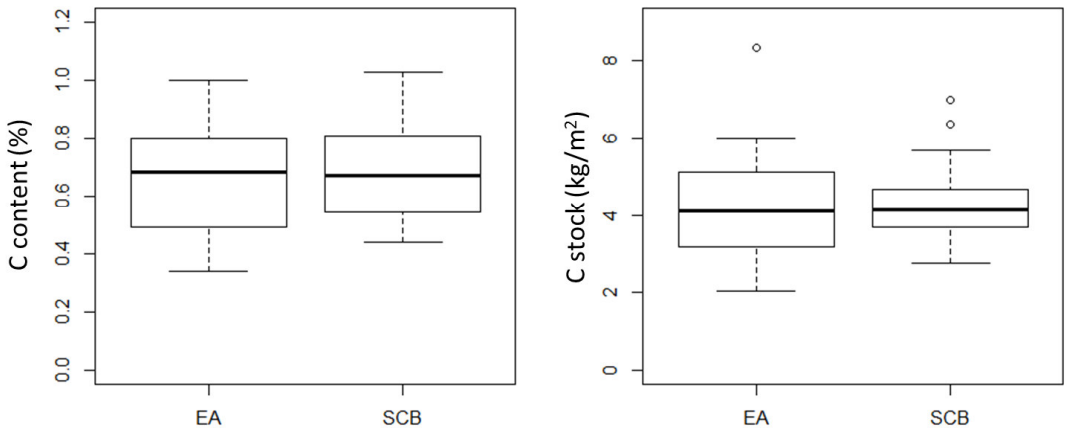


Figure 4-5 A box plot comparison of C content measured by Elemental Analysis (EA) and Soil Carbon Bench (SCB) on a gravimetric basis (left) and volumetric basis (right).

Level of uncertainty in C determinations and C stocks by SCB

Uncertainties in C stock determinations are one of the major issues hampering advancement of soil as a sequestration and C offset medium. The problems lie in the propagation and addition of errors associated with sampling procedures, C analysis, natural spatial variability in soil C and grainsize variations (Goits et al., 2009). Bulk density also has a strong influence when C stocks are calculated (Lee et al., 2009) and can lead to increased uncertainties for example, as arises in gravelly soils after applying BD corrections (Schrumpf et al., 2011). The ability to directly produce C data from a volumetric basis overcomes the need to apply BD corrections and addresses such concerns. Intuitively the greater the sample size (not only in numbers of points but soil masses or volumes), the greater the level of certainty or confidence should flow through to the final data. The SCB is designed around this idea and the field trials have been used to demonstrate the proof of concept. Carbon content can naturally be expected to vary between each sampling point influenced by changes in soils and vegetation. The magnitude of this variation should have a bearing on sampling density and therefore acquisition costs but for now it is the data extracted from individual sites that provide the most basic measure of certainty (constitutes the minimum error). Therefore maximising the confidence in soil C data at each sampling point is of fundamental importance. This can be referred to as site-specific or within-site uncertainty. To put this into perspective, commonly encountered analytical errors (for example as

reviewed by Girard and Klassen, 2001) due to the instrument plus heterogeneity in the soil can alone lead to significant uncertainty (0.001 kg C per kg). This can be relatively substantial, especially in many agricultural soils in Australia (see Web, 2002), where the C content can be as low as 0.2% (C stock ca. 13t ha^{-1} for 50cm depth) and the uncertainty translating into ± 6t ha^{-1}. Therefore the aim should be to carry through any benefit of a low site-specific uncertainty to the broader area or strata being assessed. The bulking of soils from across the field (not carried out in this work programme) offers another effective way to transfer lower uncertainties to a wider scale.

To quantify the variation at each site and hence derive a measure of within-site uncertainty or error that incorporates both sampling and analysis, the spread of replicate determinations were expressed using the standard error of the mean (SEM) which is given by SEM = $SD/(n^{1/2})$, where SD is the standard deviation and n the number of replicates per site. The mean value of 0.00018 kg/kg (excluded aberrations) could be considered the typical site specific value for this parcel of land translating to an equivalent in C stocks of ± 0.7t ha^{-1} (30cm depth basis). This still remains under the 2t recommended as a maximum figure under any market based instrument or less than the 20% uncertainty described for usually employed methods (Dalal et al., 2009). However current CSIRO estimates (April, 2013) give a range for realistic soil C gains as 0.3 to 2 tonne per ha per year (previously to 0.6t ha^{-1} according to Sanderman et al., 2010). The smaller changes would be difficult to detect on a yearly basis and in fact when calculated out (30cm depth basis), the lower gains (<0.0001 kg/kg) would be below the detection limit of most analytical instrumentation.

Spatial analysis

The instrument output, as generated from the SCB data system, directly presented the total number of grams C for the respective cores removed from the landscape. Mean values for each site indicated the steady trend down the catena shown on figure 4-6a. These output values could be used as an immediate measure of the mass C per volume and also unit area (m^2) for a nominal sampling depth, in this case it was 50cm. These values were then readily converted to gravimetric data since total mass of cores (both field moist and dry) were recorded. Gravimetric data (for this survey at least) appeared to be somewhat 'tighter' for each site (see table 4-3) due to a normalisation effect provided by their slightly different masses and these have been used to produce the C stock distributions (to 50cm basis) on figure 4-6b. This effect is most likely because the mass of C is linked with the mass of soil rather than the volume from which it came (itself subject to variation through compaction etc.). These were useful findings making the system amenable to the guidelines proposed under IPCC 2006 and a mass coordinate system (Gifford and Roderick, 2003). The method

described here addresses the concerns about the ability to reproduce soil C stocks laterally and with depth (Walcott et al., 2009) along with the required sensitivity. Carbon densities obtained for volumes (as kg m^{-2}) from sites over the landscape should facilitate the mapping and determination of total C stocks.

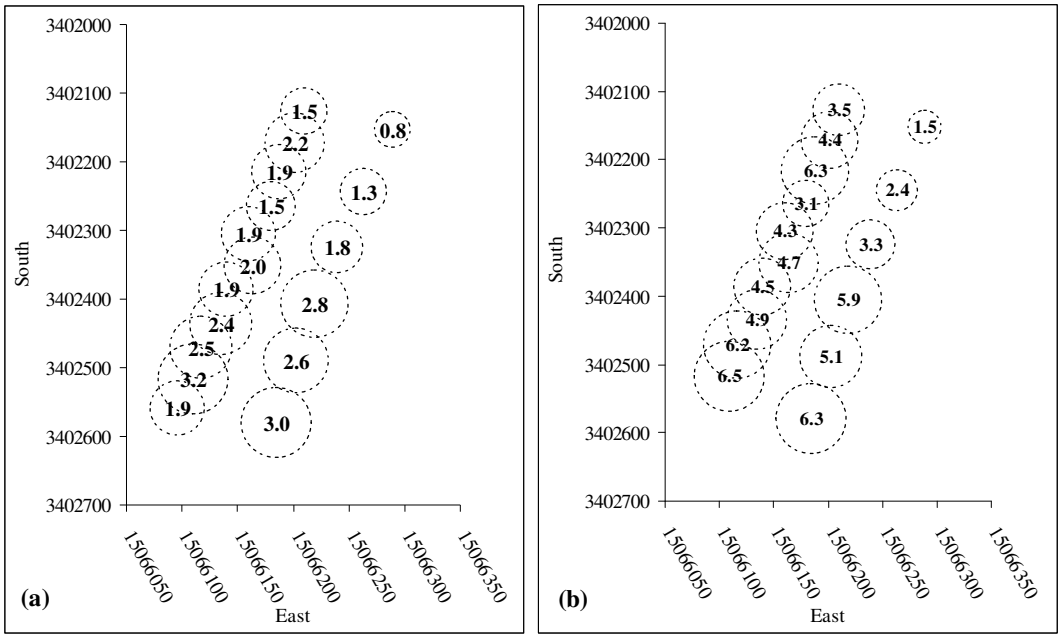


Figure 4-6 Distribution of C over the Lansdowne test field as determined by SCB (table 4-3) expressed as (a) gram per 50cm core interval (equal volume cylinder basis) and (b) kg m^{-2} from gravimetric C values.

Cost Analysis of C Determination by SCB

Lowering the cost of C analysis is equally important as improved analytical confidence. This has been achieved by transforming a fairly cost prohibitive method into a viable measurement option for determining soil C stocks. Cost has been one of the major drawbacks for on-the-ground measurement as compared to model based methods. Apart from increased certainty and confidence in soil C data at each sampling site another major improvement afforded by the SCB method is ease of application and dramatically reduced C analysis costs. These have been assessed in detail on the basis of the time – motion requirements per sample site and the costs of analysing the material.

Analysing the costs of determining soil C stocks can be best achieved by looking at the basic units of activity i.e., crushing etc., (also described by Brus et al., 1999). It is generally regarded that travel to and from sites and the extraction of soil cores comprise a significant cost component in the determination of soil C stocks but soil processing and analysis are major. For the purpose of evaluating the relative costs of soil C analysis by SCB, sampling (which includes transport and oven storage of soils) has been regarded as fairly comparable since similar amounts of soils are removed from the landscape using current methodologies. However Improvement to this component would also be feasible with the SCB since it is designed around portability and standard power requirements. Comparison of the relative cost efficiency of determining C stocks by SCB required a further breakdown (table 4-6) of the components involved in acquiring equivalent information conventionally on a pro rata basis (i.e. site-specific).

Table 4-6 Assessment of the relatives costs on a pro rata (per site) basis to obtain C stocks using SCB against the conventional method.

	SCB			**Conventional**		
Process	Time	Rate	AUD	Time	*Rate	AUD
Field collection			equivalent			equivalent
Soil prep						
drying			equivalent			equivalent
crushing	0.2 hr	$30/hr	6.00	0.5 hr	$30/hr	15.00
sizing	N/A	$30/hr		1.0 hr	$30/hr	30.00
bulking	N/A	$30/hr		0.5 hr	$30/hr	15.00
	Quantity			Quantity		
C Analysis	3 cores	$1/core	3.00	3 thimbles	$7 ea.	21.00
Totals			$9.00			$81.00

*These estimates are base costs (given in Australian currency) using in-house elemental analyser and labour. N.B. in this comparison the total amounts of soil analysed by SCB and conventionally are approximately 400 gram and 0.5gram respectively.

The running expenses that flowed onto the overall costs for soil C analysis using commercial analysers are determined mainly by consumable charges. These comprised the relatively expensive gases and reagents set as prerequisites by manufacturers. The SCB in contrast is capable of producing comparable results with welding grade O_2 at $20 per cylinder (albeit at higher consumption rates) as compared to >$900 for UHP O_2. Power consumption for the SCB was based on the current rate of $0.25 per kWh where the analysis of three cores required approx. an hour, drawing a maximum of 2.5kW. The cost of oxygen consumption was tallied as $0.30 (per hr) for the three cores.

The cost of determining C stocks over entire areas is going to hinge on the required sampling intensity adding to total sampling and analytical charges. These are largely determined by the natural variations in the soil, topography and land use enabling a basis for stratification which can reduce the number of sampling points needed. Apart from efficiencies gained through, for example bulking, sampling from small areas to constrain the variation has been another recommendation (Sanderman et al., 2011). However there have been numerous strategies (such as random sampling) and geo-statistical tools proposed to cope with the spatial uncertainty (reviewed recently by Allen et al., 2010). Testing how the SCB could contribute to and optimise these methods would need to become the next phase of research.

The SCB – utilising added efficiencies

The method by which whole cores were analysed (detailed in previous chapter) meant that each borehole or site was represented by one C content (unless split into horizons or bulked with others). When analyses with the SCB were in progress, operationally it was convenient to run core volumes successively and store the output containing C responses in a single file. Also, in this manner a portion of any transect when analysed sequentially was easily displayed graphically showing the relative amounts of C at each site or with depth as in the examples on figures 4-7 and 4-8 respectively. In the following example, seven 50cm long cores had been determined for C within 135 min.

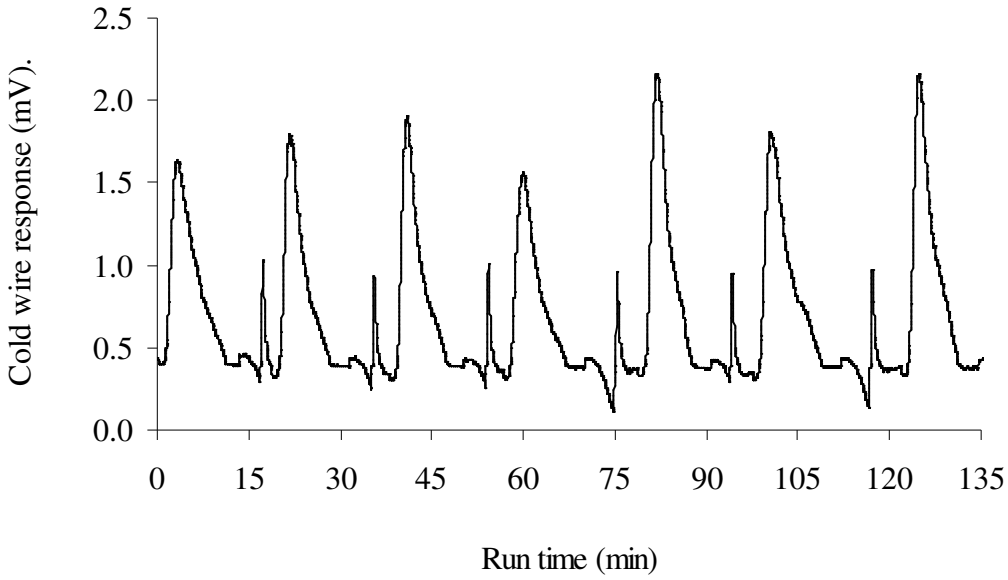


Figure 4-7 Analyses for part of transect (Lansdowne) as outputted from SCB. (Spikes between the principal peaks were atmospheric nitrogen introduced between core loadings in the current manual configuration.)

As shown peak areas were proportional to total amount of C (figures 4-7 and 4-8) but their shapes and widths reflected the rate of oxidation. This rate could be influenced by the type of OM and the mineral material surrounding the C as matrix (also the overall mass). This is because the developed method of analysis was based on monitoring the C as [d]released during the combustion cycles (Figures 4-7, 4-8 and 4-9) over which these bulk amounts of soil were oxidised. A general observation was the overall combustion characteristics (shape and distribution over run time) seemed to be reflected by the soil's physical and compostional makeup, where:

-total amount of soil influences amount of CO_2, the rate of heat penetration and gas diffusion,

-grainsize of soil influences rate of gas diffusion through matrix,

-recalcitrance of C bonds influences rate of CO_2 evolution e.g. carbohydrate vs carbonate.

[d] Responses were in near real time where offset was due to the differential rate of flows between sampling and bulk gas streams (set by total carrier flow rate and aliquot stream).

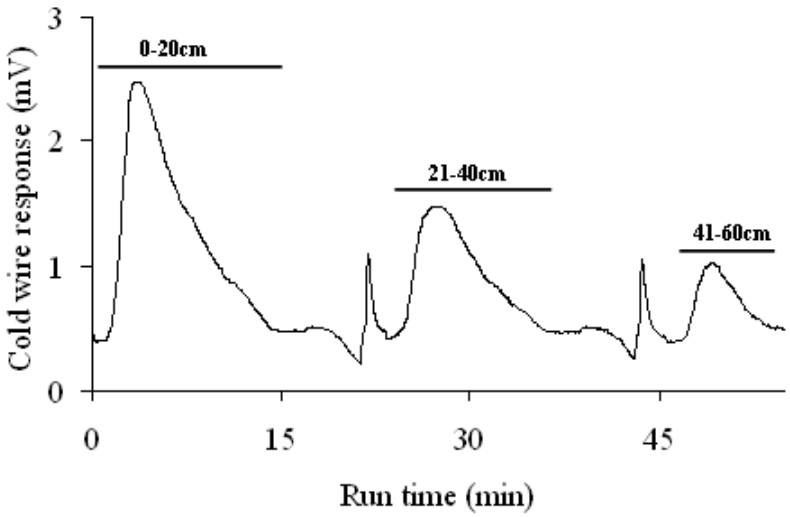


Figure 4-8 Analyses of three soil horizons (0-20, 21-40 and 41-60cm) run in succession (total mass 600 gram) showing the decreasing amounts of C (results were 1.3, 0.6 and 0.3% C respectively).

Optimising core for analysis – dried intact vs crushed and dried

To fully evaluate the advantages / disadvantages of pre-drying broken-up core and removing coarse root material as a minimal processing step, a number of cores (which were sampled in triplicate) were combusted intact and compared with their site counterparts. Comparative soil combustion experiments showed that breaking-up core maximised dehydration during the pre-drying stage that was benefited with reduced amounts of water released during C determination. Water can adversely affect the analytical system through flow retardation and interference. The detector output provided some measure of the efficiency of processing cores whole vs intact. Apart from greater moisture levels that can impede gas transfer, the duration of analyses were longer for equivalent quantities of C. This had implications for the quality of the signal output and the time/cost requirements that impact on analyses. As a result the detector responses became somewhat elongated and with perhaps more pronounced larger secondary peaks (shoulders) which was interpreted to be due to root material oxidising within the core at a slower rate compared to the mostly finely divided OM (see figure 4-9). These combustion characteristics seemed to reflect the rate of C evolution (and perhaps its form) and were reminiscent of the instrument outputs from the studies of Beyer et al. (1998) discussed in chapter 1, who sought differences related to labile and stable (i.e. humified) OM. The conclusion that roots most likely caused some of these responses was supported by the observation that gases appeared to readily permeate the core structure

to oxidise and release C from OM (see root vesicles on Plate 4-3). In some cases these contained remnant ash visible in broken core.

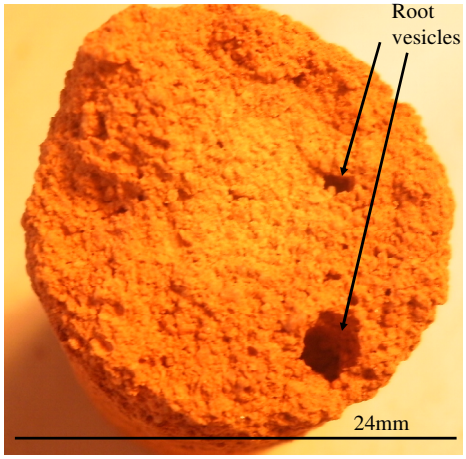


Plate 4-3 A soil core cross-section after 15 minutes combustion broken to expose the condition of the soil and any OM. Note the voids remaining where roots had oxidised and the fissile, biscuity texture.

In the 'chromatography world' some of these signal outputs would be regarded as poor (asymmetric with peak tailing) but it must be emphasised these are not 'filtered' through a separating column and are near real time responses to the release of C. In fact there may be additional value in obtaining these types of outputs if it can be shown to indicate the presence of root C.

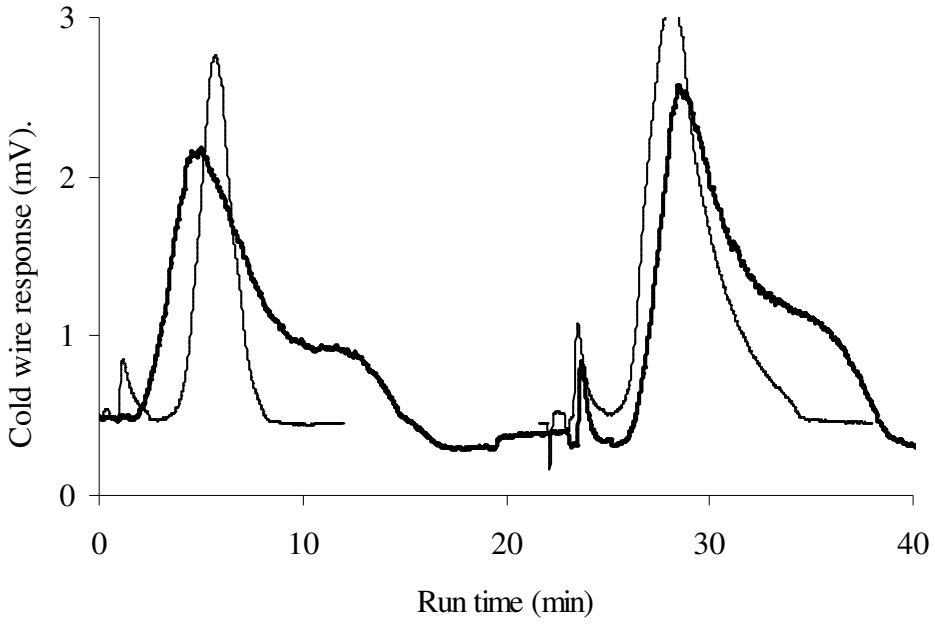


Figure 4-9 SCB recording combustion characteristic for intact core (heavy line) with approximately 0.8% C against calibration standards with 1.25 and 2.5% C (fine line).

Generally the intact cores tended to be slightly higher in dry mass compared to their respective set but not sufficiently to be evident in their bulk densities with the exception of 4c (table 4-7). In any case, heavier masses would not be surprising where water retention would have been much greater even though these were also placed in the oven for the same time periods as broken-up core (see *Best practice*). The core 2c may have had lower values through possible mass loss although the normalised C concentration (0.8%) is not consistent with this. With the exception of cores 2c and 4c the C values were fairly comparable. Overall the mass of C extracted from the cores were within 0.4 g per core length (excludes 2c and 4c). After being normalised with their masses, most cores were within 0.05% C and correlated well (figure 4-10) while again 2c and 4c varied up to about 0.2% possibly due to root material in the latter.

Table 4-7 Comparing carbon and bulk density data for whole cores oxidised from intact (c) and crushed cores (a & b).

Sites 0-50cm	Mass gram	Total C gram / core	Total C % (mass)	BD gm cm^{-3}
1a	294	1.79	0.61	1.3
1b	351	1.88	0.54	1.5
1c	**348**	**1.90**	**0.60**	**1.5**
2a	324	3.52	1.09	1.4
2b	320	3.27	1.02	1.4
2c	**289**	**2.40**	**0.80**	**1.3**
3a	314	2.63	0.84	1.4
3b	334	2.50	0.75	1.5
3c	**335**	**2.60**	**0.80**	**1.5**
4a	291	2.67	0.92	1.3
4b	353	2.94	0.83	1.5
4c	**475**	**4.60**	**1.00**	**2.1**
5a	334	1.69	0.51	1.5
5b	346	1.70	0.50	1.5
5c	**370**	**1.90**	**0.50**	**1.6**
6a	359	1.29	0.36	1.6
6b	348	1.20	0.30	1.5
6c	**365**	**1.60**	**0.40**	**1.6**
7a	317	0.75	0.24	1.4
7b	357	0.90	0.20	1.6
7c	**361**	**0.90**	**0.20**	**1.6**

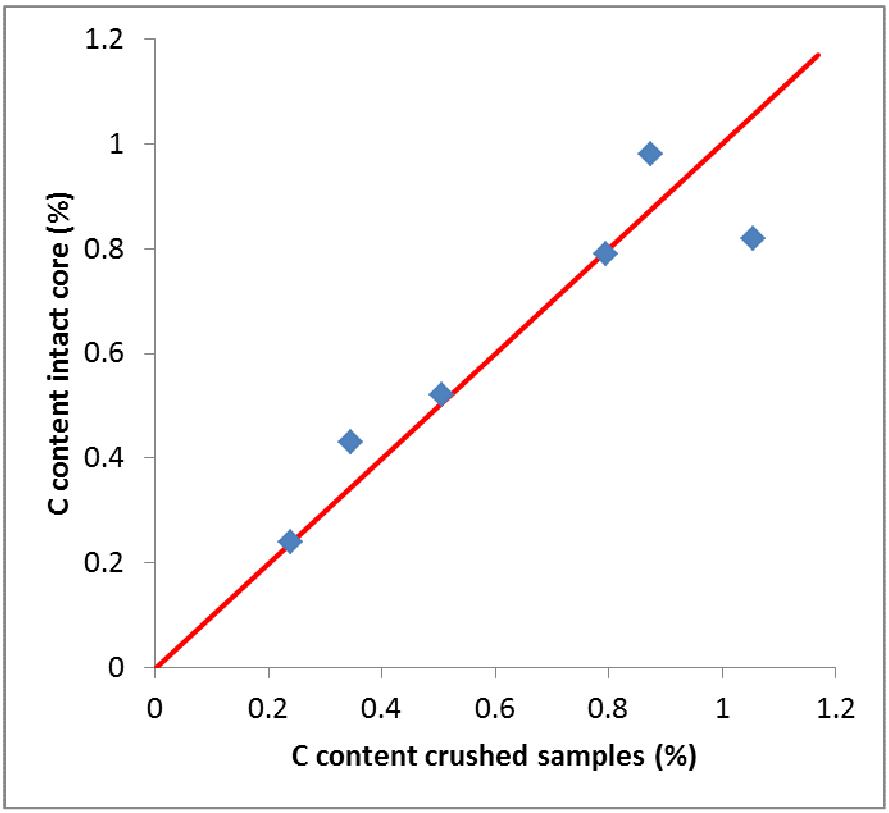


Figure 4-10 The concordance in C content from intact and crushed cores.

What all this generally means is that breaking-up core, thoroughly drying it and removing visible roots is a conservative measure that produces the most reproducible mass, BD and C data. This step was included in the cost schedule (time and labour) on table 4-6.

Best Practice Methodology for C Determination by SCB

The flow diagram in figure 4-11 outlines the steps that were used in obtaining all the soil C data and is the basis for the cost analysis. Soils were extracted from the field (point coordinates recorded) as core plugs using hydraulic hammer with 24mm tubes. Cores were then placed into paper bags to facilitate drying in transit (drying may act to lessen microbial activity i.e. degradation, due to increased aeration after exposure). Calico bags may have been better from a strength perspective as moisture weakens paper but at a higher cost. Tests to carry out integrity of sampling bags may be advisable.

Routinely soil masses were recorded on arrival to the laboratory and then placed in an oven at 90^{o}C overnight (still within bag). The soils were then removed (weighed if 90^{o}C moisture required) and broken-up either mechanically or by hand in large mortar and pestle (the latter being very effective) followed by removal of >2mm OM (i.e. recent BPR) and root material. A final drying was then carried out for min. 12 hours to maximise this on the larger surface area. This was probably the most optimal temperature because it will have halted microbial activity and it removed as much free water as possible without reaching boiling point (losses of volatile C would occur at sampling and breaking-up).

After removal from the oven the dried soil core masses were finally weighed (used for gravimetric determinations) and loaded into the SCB successively with the settings and methods as described in chapter 3. Response area data was then acquired via the detector and data system and stored as a spreadsheet with the required calibration information.

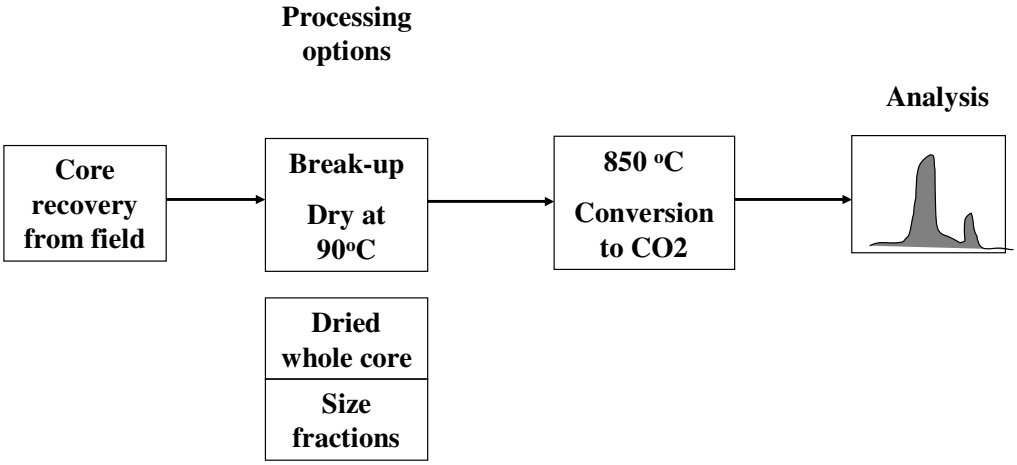


Figure 4-11 Steps required for analysis by SCB where the first option which was used throughout most of the demonstration appeared to be optimal.

In its normal operation the SCB quantifies all C from the core mass (roots can be included if required) and in so doing automatically circumvents any issues surrounding gravel corrections. However stabilised C has been associated with the silt and clay fraction (refer back to earlier review) so application of the SCB to size classes may become of importance where soil C dynamics need to be investigated between fractions on a larger scale (with some additional cost resulting from the added processing step). To test the applicability of the SCB on soil size fractions (which is most often what soil C determinations have been based on), two cores were separated into nominal size classes and analysed with the results shown on table 4-8. This exercise demonstrated the feasibility but also the advantages over current systems where material representing different size classes (all contain C but usually discarded) can be quickly determined as well as pre-separated root masses. The C distribution (% of total in table 4-8) well demonstrated the spread of C between these size classes providing an indication of the significant amounts of C residing in parts of the soil sample commonly discarded.

Table 4-8 Example of carbon distribution relative to the size fractions for two cores from the Lansdowne test site analysed by SCB.

	Size fraction (μm)		
	425 - 2000	100 - 425	<100
Silty loam			
Mass distribution (% of total)	43.1	39.3	17.6
C concentration (%)	0.43	0.42	0.85
C distribution (% of total)	35.2	32.1	32.7
Sandy loam			
Mass distribution (% of total)	36.7	53.8	9.5
C concentration (%)	0.20	0.18	0.87
C distribution (% of total)	27.6	40.3	32.1

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CHAPTER 5 - BLACK C DETERMINATION IN SOILS: HYDROGEN PYROLYSIS AND SPECTROSCOPIC CALIBRATION

Background and scope

Pyrogenic carbon also known as black carbon (BC) is composed dominantly of condensed structures such as polycyclic aromatic hydrocarbons. These chemical structures arise in soils through natural or deliberate means but report with and are analytically indistinguishable from organic matter during normal C analyses. Their different in-soil stabilities however necessitate differentiation from less stable SOM. While biochar can be distinguished from naturally occurring BC as that produced for the purpose of amendment and / or C stabilisation, both have chemical similarities in contrast to SOM but all analyse together as organic C. As the name suggests pyrogenic C is formed through the heating and incomplete combustion of OM such as biomass or hydrocarbons in conditions where there are low levels of air (oxygen). Under aerobic conditions these materials would oxidise to CO_2 but instead because of the low partial pressure of O_2 carbonisation takes place resulting in charcoal like-materials.

Why is the ability to measure BC in soils where small quantities occur so important? Within the soil environment, BC is generally more resistant resulting in very different turnover dynamics compared to other SOM as previously emphasised by Leifeld and colleagues (Leifeld et al., 2003). Therefore measuring TOC during sequestration / monitoring is insufficient because it cannot be assumed to have equivalent residence times. This also has implications for the accuracy of SOM models where relative residence times are affected by different soils, climates and land uses. The proportion of BC in the overall SOM distribution should therefore be part of any soil C inventory measurement during sequestration. However standard soil C analysis methods cannot distinguish this pool from OM as readily as it can say carbonate C. This has resulted in the range of BC analysis methods (see figure 5-1).

There are two likely sources of BC in soils as it applies to the Australian scene:

a) A prevalent history of grass and forest fires in the Australian landscape have accumulated BC in soils and may also be of ecological significance.
b) For agriculture it has been used as a fertilizer and soil conditioner but more recently has been promoted for its possible role in soil C sequestration and where intentionally produced for such purposes is referred to as biochar.

The addition of BC in crop plots to store C in this more soil-stable form and concurrently improve soil properties has become a popular idea. Farmers and horticulturalists have done

this for centuries and it was also part of ancient land practices (e.g. Amazonian Terra Preta). There may be numerous benefits resulting from the addition of biochars that include increased CEC, water holding capacity with improved structure (aeration) as well as higher fertility (increased potash). This has been used as a viable approach in horticulture, for example, where tomato waste undergoes bio-charring and the energy utilised (for warming of greenhouse). The residues containing nutrients such as P and K get recycled as fertiliser on the new crops (form of nutrient cycling). Clearly charring leads to enrichment of nutrients that are assimilated after addition of mineral fertilizers or from the soils naturally. Oxalate minerals form within the plant tissue and these become concentrated when dried and decomposed. (see Manning et al., 2005 and their relevant references). Macronutrients such as N (possibly P) can become depleted because of their volatility during charring. In forest soils (fire affected) the relative concentration of N (to C) was found to be greatest i.e. reflective of the source material, in the finest particle sizes (Adams and Attiwill, 2011).

Many Australian soils have some small component of naturally occurring BC material in the shallow horizons (refer to Skjemstad et al., 1996; Lehmann et al. 2008). This is because as previously mentioned bushfires have been a part of the Australian ecologically for millennia and allowed significant amounts of BC to accumulate in situ. Black carbon / biochars can be produced from a range of starting materials such as woody tissue, grasses (also crop residues) and animal manure. While these starting materials vary somewhat they all have in common a lignocellulose basis with comparable and overlapping compositions. The final chemistry of any BC produced is more likely to be influenced by the conditions of pyrolysis (e.g. temperature, air composition) as indicated by elemental ratios on figure 5-1. Lehmann et al. (2008) analysed 58 soil profiles in the national soil archive and found that the BC constituted an average of 33% of the total organic C, a significant contribution.

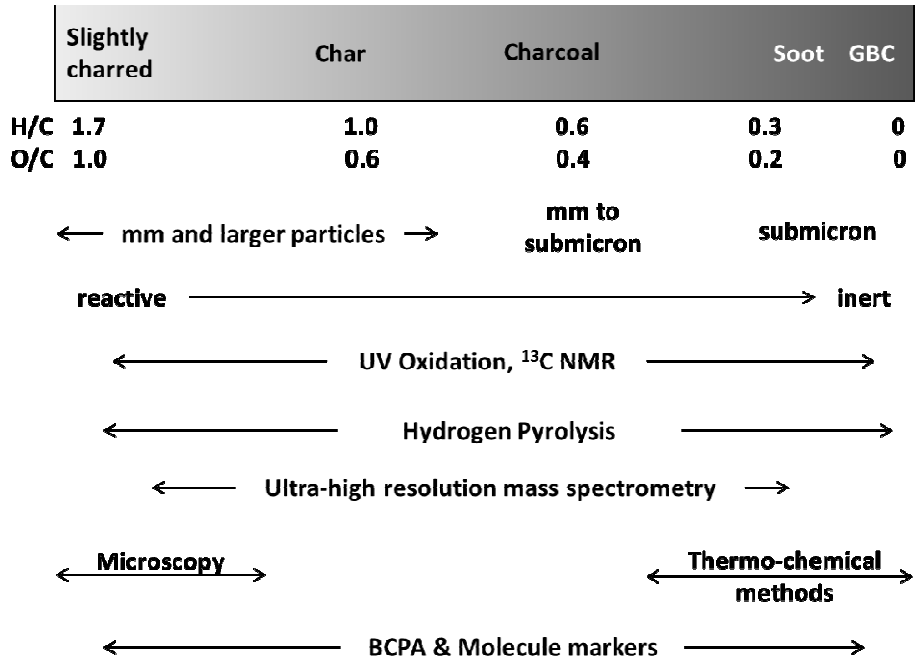

Figure 5-1. Components of black carbon ranging from slightly charred[d] to graphitised BC assessed by different methods across a continuum of recalcitrance increasing from left to right (adapted from Hammes et al., 2007 and Sohi et al., 2009).

Conventional methods for soil BC analysis

It is generally acknowledged that a simple and routine method for the determination of black carbon in soils has been lacking. BC determinations in soils remains fairly involved processes because they include combinations of physical and chemical treatments followed by C analysis and expensive characterisation methods (figure 5-1, see also Rayment and Lyons; 2011; Baldock et al. 2004). BC has also been quantified on the basis of specific molecular markers (Glaser et al., 1998) but again involves several analytical steps. However what most of the usual methods have in common is that the labile forms of C are *selectively* removed leaving BC remaining for analysis. The simplest is wet oxidation of the labile material and the most practical among these being reaction with acid catalysed H_2O_2 (Kurth et al., 2006) or NaClO (Zimmermann, 2007). However these methods probably tend to have lower efficiencies of OM removal and overestimate BC. Protocols that use a combination of pre-treatments and selective combustion can be counted as chemo-thermal oxidation (CTO) techniques and are the most commonly used to determine BC. In such procedures (see Agarwal and Bucheli, 2011), after removal of carbonates, if present, the OM is heated to 375^{o}C to remove labile C leaving the BC in the residue for elemental analysis. Although, this assumes that forms of humified OM are not present beyond 375 ^{o}C and that no

[d] Char refers to incipient alteration under pyrolysis conditions while charcoal is produced from a complete process where it is manufactured under those conditions for extended periods.

artefactual BC is produced (Bird et al., 2011). A fairly unique system developed by Jan Skjemstad uses UV photo oxidation (Skjemstad et al., 2002) on disaggregated soil where the photons generated by Hg lamp were used to degrade non BC material. Previously CSIRO (Glen Osmond) employed this method to remove labile forms prior to elemental analysis. The resistant organic carbon (ROC) soil component is now based on the physical separation after dispersion into particulate (POC) and humified (HUM) organic matter followed by ^{13}C NMR characterisation to determine the ROC (Sanderman et al., 2011). The analysis of a specific molecular marker has also been demonstrated as a quantitative measure (Glaser et al., 1998). The merits of a number of approaches used to measure BC have been summarised by Hammes et al. (2008) who also touch on thermal analysis, a dynamic method.

An easily applicable and practical method for BC determination in amended plots was described recently by Koide et al. (2011) for concentrations from a fraction of 1% to 10%. This could be regarded a quasi-LOI technique where the amount of OM pre-exiting in the soil could be quantified through a calibration set of soils providing a mass-loss baseline over and above changes due to the mineral soil fraction. It was demonstrated in this study that additional C in the soil due to amended BC was readily determined by the increased mass losses (over relevant temperature interval). It was not clear how sensitive the method might be for quantifying naturally occurring BC.

Other studies have been undertaken that assessed the effectiveness of BC estimation by PLSR and MIR spectral information using BC calibrations based on quite different determination methods. Bornemann et al. (2008) proposed a method for BC screening by MIR spectroscopy based on calibration using determined SOC and a significant BC marker called benzene polycarboxylic acids (BPCA) described in detail by Glaser et al. (1998). BCPA determination of the calibration soils was in accordance with the method of Brodowski et al. (2005). According to the models developed by the authors, prediction of BC were poorer (slopes of 0.73 and 0.85) and a larger scatter of data ($R^2 = 0.7$) than the accurate and precise POC estimations. This is consistent with the observations that quantifying BC by use of BCPA determination can lead to over or underestimation where some non-BC aromatics report as BC and conversely highly condensed BC escape detection (refer to review on BC methods by Hammes et al. 2008). Interestingly, the degree of condensation based on the proportion of mellitic acid present was also measured by Bornemann et al. (2008). When spectra were correlated with this quantity the resulting models were metrologically similar to total BC by BCPA. An analysis of the latent variables

(most important absorbance bands: 1760, 1400-1600, 770-830 cm^{-1}) indicated the dominance from aromatic C used to produce the models.

In the studies by Zimmermann et al. (2007) only carbonate free soils (and under 15% C) underwent spectral PLSR analysis. They used a SOM separation scheme to obtain the combined labile and combined stable C pools which differed from that of Sanderman et al. (2011). Soils were wet sieved but also involved density separation and mild oxidation with NaOCl. The latter was used to render OM free of labile C so that only resistant soil C (rSOC) remained. Models developed for these different fractions based on their determined C produced reasonable predictions where rSOC (closest equivalent to ROC) provided an accuracy and precision of 0.991 (slope) and R^2 (0.89) which was fairly comparable to the C_{POM}. Principal absorbance bands (based on latent variables) for the OM fractions showed that the 2800 to 3200cm^{-1} region which is prominent with regard to labile OM had no influence for rSOC prediction by PLSR.

The recent method of calibrating MIR for resistant organic carbon (ROC) by CSIRO Land and Water (Glen Osmond, S.A.) has been very relevant to this study (Baldock et al., 2009) and is a method component of the [d]National Soil Carbon Research Programme (SCaRP). The models so derived were used here to compare the results obtained for the spectral analysis of soils which had been predetermined for BC by hydrogen pyrolysis (HyPy) at the JCU facility (James Cook University, Cairns campus) and also with soils of known biochar blends. The CSIRO ROC method has evolved with the development of physical and chemical soil separation techniques followed by ROC determination by two main approaches. The subdivisions have been designated particulate organic carbon (POC), humified organic carbon (HUM) in the finer fraction and resistant organic carbon (ROC) in both fractions. Previously photo-oxidation was an important component in calibrating PLSR for BC by Janik et al. (2007, and earlier work). In a principle similar to that employed by Zimmermann (2007) it has allowed non-BC to be removed (by UV light) from the <53µm fraction prior to analysis which is due to BC. Predictions obtained by this preparation / calibration process were more accurate (regression approaching 1:1) than by the BPCA method and reasonably similar to the wet oxidation procedure demonstrated by Zimmermann (2007) even though the data set probably encompassed a broader range of soils (origins). Perhaps not surprisingly the principal absorbance regions were consistent with those found by the other authors. However since the earlier work it had been recognised that while most

[d] SCaRP is a current programme coordinated by the CSIRO Land and Water (Glen Osmond S.A.) intended to investigate the variation in total soil carbon and the C pool structure based on these fractions within the top 30cm over a range of soils and land uses across Australia.

BC is collected in the fine fractions sufficient is also contained in the >53μm (POC) portions which have led to certain methodological modifications (see Sanderman et al., 2011). As a result NMR spectroscopic results which are used to quantify BC are now bulked together for a more accurate total ROC. Note that BC can be determined by ^{13}C NMR because it is dominated by aryl-C occurring as fused rings which have a strong influence over the 130 ppm chemical shift during these analyses. While the magnitude of this response (being primarily due to BC) provides some measure of its proportion in the sample it is also contributed to from SOM making UV photo oxidation a fairly useful way to screen for BC. For routine use this necessitated access to such equipment whereas FTIR is rapid and much more readily available.

Aims of the experimental work for this study

To undertake any proper evaluation of C stocks and its dynamics there is clearly a need to be able to measure BC as a separate quantity where residence times are expected to be quite different. Infrared spectral techniques hold the promise of delivering soil information much more efficiently than any other method. To their advantage they are non-destructive, can be used proximally and spectral analysis systems can be 'trained' to provide more detailed information about forms of C present in the soil which includes carbonate and BC. In this way it has the potential to be a major contributor in supporting more accurate SOM dynamics models. A major part of the efficiency is that the amount of time and effort (cost) in soil preparation is relatively low (particle separation usually not required), is very fast but it is heavily reliant on calibration sets. Furthermore, soil is composed of a variety of mineral and organic components that influence IR spectral absorption and then there are different soil and char types as well. This means that considerable attention needs to be given to calibration over a broad range of relevant soils and BC concentrations. Some laboratories use the previously discussed separation and selective oxidation techniques prior to spectral analysis including physical particle sizing, photo- and chemical oxidation. In addition to being laborious there have been questions surrounding the accuracy of BC determinations by especially wet chemical means which can then lead to propagation of error and uncertainty into spectral based models.

The aim was to investigate and compare two soil BC determination techniques as calibration methods for MIR spectroscopy and MIR based prediction models. These were physical size separation followed by NMR (CSIRO ROC) and the selective retention of BC by hydropyrolysis (JCU facility). In addition biochars were blended into soils, analysed and studied using MIR spectroscopy.

Why Hydrogen pyrolysis?

BCs have overall a more stable chemistry but they comprise variable proportions of biodegradable components that are comparable to SOM in terms of their C sequestration potential (years rather than decades). How much of any BC falls within this portion? Studies by Kanaly and Harayama (2000) have shown that poly-aromatic structures with ring sizes 6 or less constitute this less stable portion in contrast to recalcitrant BC with greater degrees of condensation. However there has been no universally accepted benchmark for BC determination. [d]Hydrogenation techniques (including hydrous pyrolysis) have been used for some time in petroleum research applications to evaluate source rock and hydrocarbon generation potentials. Importantly, ring sizes > 7 remain intact up to HyPy temperatures of 575 ^{o}C (plateau occurs at 525 to 575 ^{o}C) where the high pressure flow-through reaction limits any alteration to the structure or the condensation of more aromatic rings (Love et al., 1995; Meredith et al., 2004). Below this temperature all labile OM has combined with H and been swept away while beyond this plateau BC is cracked to gas. Its limit of detection appears only to be constrained by the sensitivity of the elemental analyser. The effectiveness of HyPy (HyPy facility pictured on Plate 5-1) in removing non-aromatic OM has also been demonstrated by pre and post hydropyrolysis studies using ^{13}C NMR (Ascough et al., 2010).

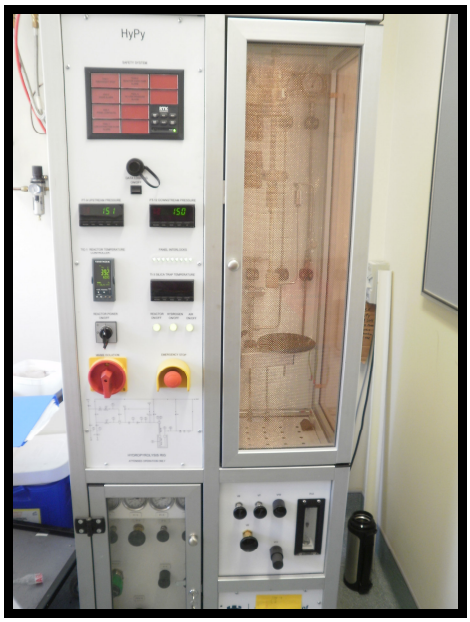

Plate 5-1 HyPy apparatus used for extracting non BC organic material at the JCU facility.

[d] The process enables the formation of solvent soluble hydrocarbons and biomarkers allowing the geochemist to characterise prospective sediments.

According to Bird and Ascough (2012) this recently embraced technique for BC research could provide the analytical standard that defines BC for determinative and preparative purposes. The latter relates to obtaining pure BC for isotope and dating studies which must be free of all recent C (these methods are described by Ascough et al., 2009; 2010).

Material and Methods

Soils investigated by HyPy

A small set (15) of various soils were selected for the determination of [d]native black carbon (NBC) by HyPy. This term has been applied to distinguish BC that is naturally occurring or pre-existing in soils most likely a consequence of local fires over time. The aim of this component was a) gaining a better understanding of the HyPy process and b) to test IR spectral prediction models based on HyPy results. The soils included three standards where one was suspected of containing NBC and three forest soils from a fire prone district expected to host some NBC. However one of the soil standards had been blended with biochar to serve as a type of control. Along with the remaining samples which were from agricultural areas in NSW, these were taken to the HyPy facility at JCU and processed for BC determination.

Preparation of soils containing a range of BC (0.25 to 20% by mass)

The aim of this experimental component was to produce a set of synthetic soils with unequivocally known concentrations of biochar to serve as a sound starting point for TGA study and the calibration of MIR spectra. This was a readily accessible way to obtain accurate BC concentrations without the uncertainties associated with CTO and extraction methods as indicated by the large variations from comparative studies (Schmidt et al., 2001). The latter may be attributable to the numerous handling steps and under or over efficiencies of removing labile C (also mentioned by Bornemann).

Two sets of BC blends were prepared by mixing carefully weighed proportions into the same batch of sandy loam (Lansdowne, NSW; S3402164, E15066212) and homogenised in a mortar and pestle. The rates of addition were 0.25, 0.5, 1.0, 2.0, 5.0, 7.5, 10 and 20% by mass and were in keeping with realistic ranges applied in field experiments where soils are amended with biochars (see Koide et al., 2011; Rondon et al., 2007). Note that any pre-existing charred C which may have been present in the original soil was not accounted for.

[d] This term has been applied to distinguish BC that is naturally occurring or pre-existing in soils most likely a consequence of local fires and not the result of deliberate soil amendment or sequestration. These types of BC have significance for ecological and dating studies.

Biochars from two different types of dry land shrubs were obtained from RCRA, Qld (who produce commercial charcoal), and these were from:

a) Mulga (Acacia aneura, 1.2g/cm^3 air dried)
b) Gidgee (Acacia cambagei, 1.3g/cm^3 air dried).

The two biochars differed in their ignition characteristics and ash residues.

Analytical

Soil pre-treatment consisted of grinding in a puck mill to pass 100µm appropriate for FTIR and other analyses and storage of soils was under the same conditions after drying (40^oC oven).

Routine carbon and nitrogen determinations were carried out on the biochar blends with the Elementar Vario Max elemental analyser on subsamples (3-400mg). Accurate C analyses were also required in conjunction with HyPy treatments to determine NBC and these were conducted at JCU on a Costech (Milan, Italy) elemental analyser with a zero blank auto sampler.

Hydrogen pyrolysis

Soils were previously homogenised in a mortar and pestle but no pre-treatments were carried out other than acidification with 1M HCL for 24 hrs (followed by washing) on soils containing carbonate. This process would not be required if soil pH were low (i.e. <5 indicating an absence of this anion) or the proportion of C as carbonates were known so that this could be accounted for in the final post-HyPy result. Inorganic C would endure HyPy temperatures and add as an apparent BC value.

Approximately 3-400mg soil was weighed out into suitable vials and mixed with sulphided molybdenum catalyst at a rate of 5% (soil C <10%) and at 10% (soil C >10%). The mix was then covered with methanol and stirred with spatula to obtain intimate coating of catalyst on the soil. Samples were then completely dried by placing them on a hot plate at 60 ^oC to evaporate the methanol before freeze drying overnight. After removal, about half the soil was accurately weighed and transferred to a borosilicate reaction tube and plugged with clean quartz wool both ends (to allow the passage of H_2 during pyrolysis). These were weighed again (tube plus contents) before processing in the reactor. The remaining material was used for elemental analysis to provide the pre-HyPy carbon contents of the soil (plus catalyst). Tubes were processed one after the other which involved successive loading and sealing into the reactor, leak checking, commencing the high pressure H_2 flow (5L min-1) and the programmed heating. The soils were heated (resistance furnace) from ambient to

250 ^{o}C at 300 ^{o}C min^{-1} followed by a much slower ramp (8 ^{o}C min^{-1}) to 550 ^{o}C and then held for 2 min. before gradually releasing the pressure as the system cooled to ambient. The overall process required over an hour per sample provided there were no leaks in the system. During pyrolysis any hydrogenated C [d]compounds were swept from the soil mineral matter leaving theoretically >7 aromatic ring sizes in the matrix for subsequent weighing and C (i.e. BC) determination.

Calculating NBC

NBC was calculated using the important quantities; the initial composite masses (soil + catalyst), post-HyPy composite masses and their respective analyses. This circumvented having to correct for the additional mass of catalyst present which varied a little. The gross mass differences represented the amount of labile C lost as it reacts with the H_2 gas swept through at 5ml/min along with lattice water. Pre and post-HyPy elemental analyses were used to determine the ratio of BC to TOC which were then applied to the initial elemental analyses to yield the actual amount of BC present in soil.

Infrared Spectroscopy

Soil spectra were acquired using a Bruker Tensor 37 DRIFTS spectrometer fitted with an HTS-XT microplate / detector module. Soils subsamples were firmly packed into microplate wells and uniformly levelled where one of the wells was designated for the KBr reference. The plate was then placed in the autosampler ready for FTIR analysis. The infrared beam was provided by Globar source and the beam-splitter a KBr crystal. The mercury-cadmium-telluride (MCT) detector built into the HTS-XT module was liquid-N2 cooled. Sample positioning was effected by a motorised stage holding the sample plate which is automatically relocated to perform successive spectral analyses. Absorbance spectra were acquired atmospherically corrected with a resolution setting of 4cm^{-1} using the Bruker OPUS software version 6.5. Each soil was scanned 60 times (averaged) over the frequency range 4000 to 600 cm^{-1} (2500 to 17,000 nm) against the KBr reference which was scanned between each sample. Apodization was carried out using the Blackman-Harris 3-term function. The spectra were also smoothed and normalised within the OPUS 6.5 software (Bruker) and the spectral data reprocessed (differentials) for principal component analysis (PCA).

SCaRP MIR-ROC calibration data

In order to use spectral data to predict or determine the content of a soil it must be related to other 'known' spectra which have been determined in some analytical way previously.

[d] In some research these compounds are trapped for MS fingerprinting which can be indicative of the source material.

Part of the Soil Carbon Research Project (SCaRP) by CSIRO collected soil samples from cropping, pasture and rangeland management systems located in a range of Australian agricultural regions. The samples were measured for total, organic and inorganic C in soil as well as the allocation of organic C to its component fractions (particulate, humus and resistant organic carbon).

Sydney University was part of an inter-laboratory comparison in collaboration with the coordinating soil research group at the CSIRO. The calibration set consisted of 200 finely ground soils and another 87 soils as a test set. The samples were finely ground in a puck mill for 90 sec. The resistant C (ROC) values were obtained by the CSIRO laboratory using the methods described by Sanderman et al. (2011) and their references. This involved physical separation and determination of ROC in the particulate and humified fractions with the aid of ^{13}C NMR and a molecular mixing model (Baldock et al., 2004). The samples were scanned and a model predicting ROC from the MIR spectra (MIR-ROC) was developed using the Cubist software (Minasny and McBratney, 2008).

The MIR-ROC model generated from the SCaRP data was then applied to two small test sets consisting of:

i) the soils measured by HyPy and
ii) the synthetic BC bearing soils.

The MIR-ROC model

The calibration model generated from the MIR spectra of the SCaRP data (figure 5-2a) was linear and well correlated (R^2 0.96) over the large range of ROC soil concentrations (equivalent to 0.02 to 2 % ROC). Spectral model analyses were carried out with the data mining software Cubist (Rulequest Research), see Appendix I for rules and wavelengths used.

The model separated the spectra into 8 rules or groups from high to low ROC concentrations, based on absorbance at wavenumber at 1797cm^{-1}. Each of the rules is a linear prediction of ROC based on several wavenumbers. Cubist found that absorbances at wavenumbers 2784, 1797, 1727, 1488, 1396, and 1357 cm^{-1} were the most frequently (significantly) used as predictors.

The MIR-ROC model was applied to an independent validation data set of 87 (figure 5-2b). While the predictive quality is good for most soils with a bias of -0.3 mg/kg, a small proportion of points fall away from the trend hence a determination coefficient (R^2) 0.77.

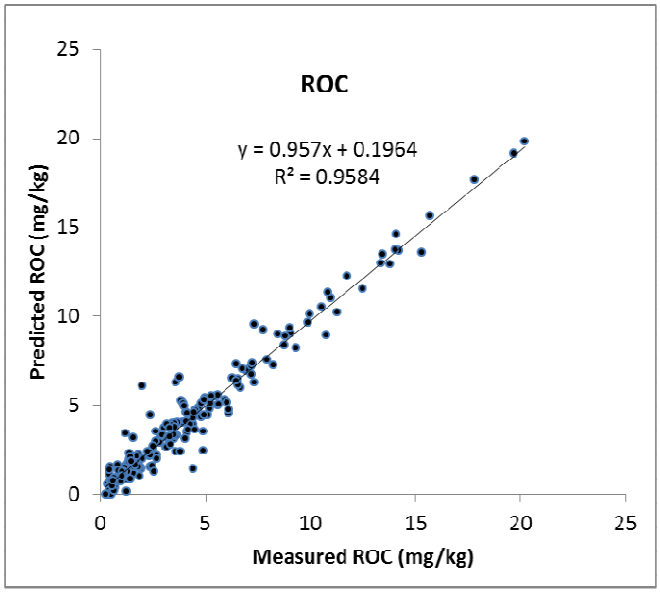

Figure 5-2a The MIR-ROC calibration model consisting of 200 soils determined for ROC by separation into POC and HUM followed by ^{13}C NMR spectroscopy. (n = 200, RMSE = 0.788 mg/kg, R^2 = 0.96).

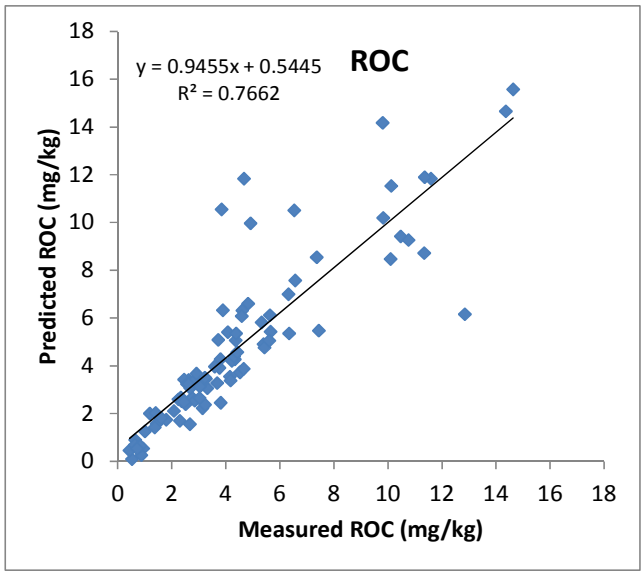

Figure 5-2b Validation of the MIR-ROC model using an independent 87 ROC measured soils. (n = 87, RMSE = 1.727 mg/kg, R^2 = 0.78)

Results and Discussion

NBC determinations by HyPy

The soils selected for HyPy were on the basis that some would have a higher probability of containing significant amounts such as those from fire prone areas. The results (table 5-1) correlated to some extent with this expectation such that most soils which were from agricultural sites were generally below 0.5% NBC down to 0.02% while the forest soils (subject to periodic wildfire) reached 1.01%. One of the samples (soil standards) had a significant amount of BC at 1.29% only exceeded by a soil biochar blend placed into the set as a cross reference (2.15% BC).

Table 5-1 NBC determinations after HyPy treatment.

TEST SOILS	Mass mg	TOC % C	Post-HyPy % C	NBC %	BC/TOC
Chromosol 2	682	0.15	0.03	0.02	0.13
Soil standard 1	413	0.60	0.08	0.06	0.11
Chromosol 1	496	0.39	0.10	0.09	0.23
Vertisol 1	507	0.90	0.12	0.11	0.12
SCL 1	453	0.70	0.14	0.11	0.16
Dermosol 2	648	0.79	0.15	0.12	0.16
Vertisol 2	563	0.82	0.19	0.19	0.23
SCL 2	464	1.30	0.26	0.23	0.17
Soil standard 2	574	2.60	0.25	0.29	0.11
Sandy forest soil 1	590	2.00	0.33	0.31	0.15
Sandy forest soil 3	544	2.60	0.63	0.56	0.21
Dermosol 1	673	4.10	0.74	0.64	0.16
Sandy forest soil 2	632	2.30	0.94	1.01	0.44
Soil standard 3	755	4.02	1.44	1.29	0.32
BAS	535	5.10	2.43	2.15	0.42

SCL refers to sandy clay loam, BAS denotes biochar amended Lansdowne derived sandy loam soil

Spectral analysis of NBC

Subsequent to HyPy analysis these 15 finely ground (100μm) soils were studied by MIR spectroscopy (according to the methods described above). A pure prediction model built from the known NBCs and their MIR data would not be particularly informative metrologically because these are based on a small set of soils (15) which tend to be heavily influenced by one or two outliers. Instead, the MIR-ROC model (as described in the previous section) was used to predict ROC concentrations and the resulting data then compared with those obtained by HyPy (table 5-2). Following on, the ROC predicted and HyPy correlation were plotted along with the SCaRP validation dataset (87 samples) used to test the model (figure 5-3), with all data expressed as % BC as obtained for HyPy. The 15 experimental soils fall generally around the trend produced by the MIR-ROC model although several do lie away from this. It demonstrated that overall the method of

determining BC by HyPy was modestly comparable and yielded results in the same quantitative region as ROC data. The effect of adding the HyPy soils to the ROC trend lowered the coefficient of correlation produced with the 87 test soils from 0.77 to 0.58 (R^2). The BC concentration obtained by HyPy was generally smaller than that obtained by ROC, possibly related to the different extraction procedures.

Table 5-2 NBC predicted by ROC model compared to measured (HyPy) values.

	Observed % BC (HyPy)	MIR Predicted % C ROC
Chromosol 2	0.019	0.000
Dermosol 2	0.115	0.127
SCL 1	0.123	0.274
SCL 2	0.227	0.335
Sandy forest soil 1	0.307	0.339
Vertisol 1	0.109	0.346
Soil standard 2	0.288	0.394
Soil standard 1	0.065	0.395
Sandy forest soil 2	1.006	0.586
Chromosol 1	0.090	0.701
BAS	2.145	0.909
Sandy forest soil 3	0.556	1.042
Soil standard 3	1.289	1.182
Dermosol 1	0.639	1.218
Vertisol 2	0.186	1.268

The most obvious outlier among this set is the biochar amended (Lansdowne) sandy loam soil (composited from 10 to30cm interval) which has been strongly under-predicted by the MIR-ROC model. This sample was composed of over 5% C, where according to HyPy 2.14% or nearly ½ was BC. It needs to be considered that biochar C is not entirely made up of black C within the definition (polyaromatised C) which may vary depending on the conditions of production i.e., heat, atmosphere, and duration (biochar blends discussed further below). Several other soils correlate poorly (e.g., Vertisol 2, Chromosol 1) and it is equally possible that the two methods used to obtain the BC values could contribute small differences. HyPy retains all the material with condensation numbers greater than 7, while the ROC method may exclude material that is not held within the HOC and POC fractions. This does not even consider the possible losses through handling but note that both methods have been developed with the aid of NMR analysis.

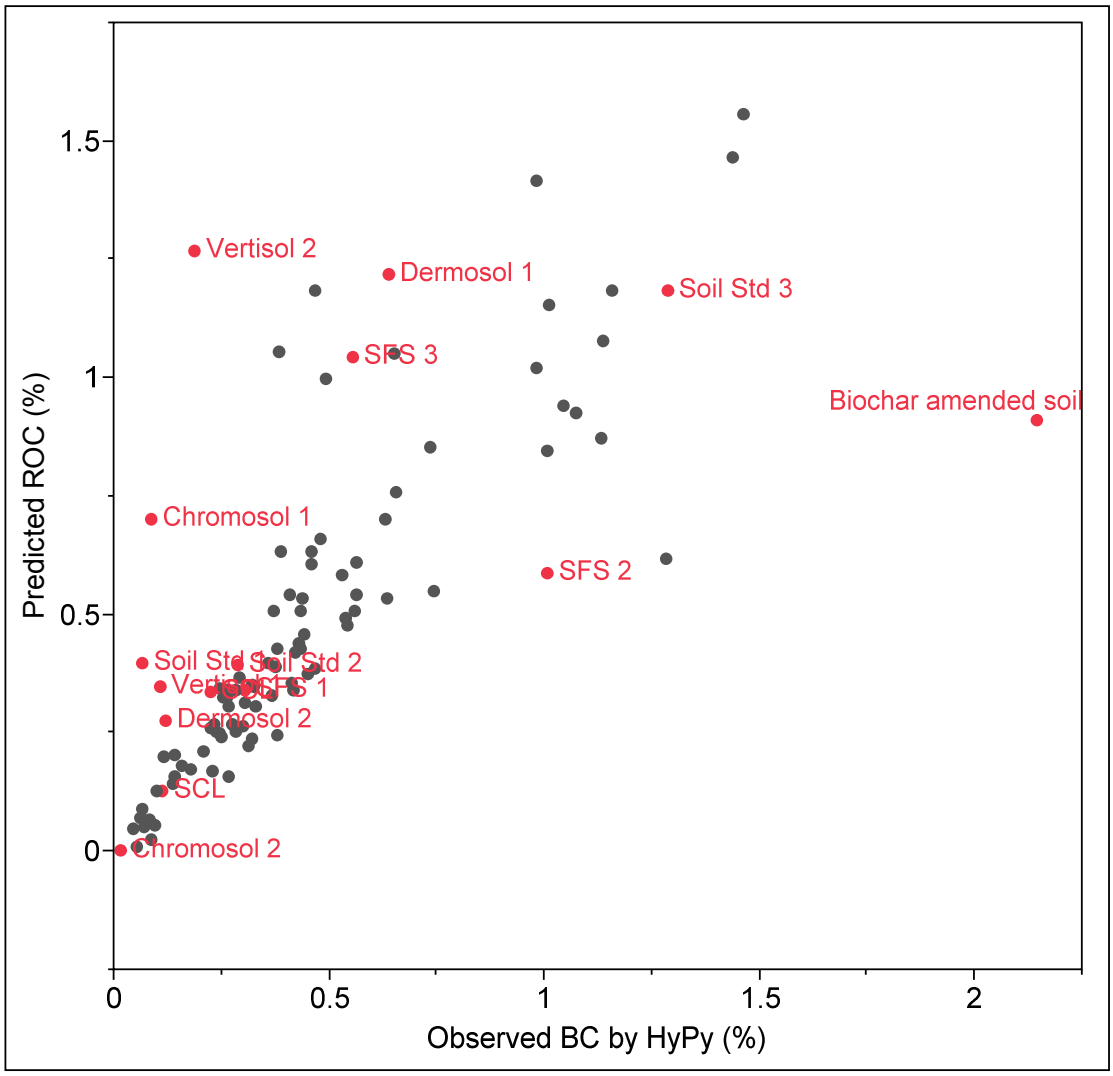

Figure 5-3 Predicting black carbon in a range of soils using the MIR-ROC model. The black points are the prediction of the SCaRP validation soils (n=87) by the same model.

When the MIR spectra of the NBC samples were projected in the principal component space of the 200 calibration soils (from SCaRP) (figure 5-4), the NBC soils are inclusive of the calibration set. This means the spectral properties of the NBC soils are well covered by the calibration set, and the difference in the BC estimates are due to the techniques (HyPy vs. ROC).

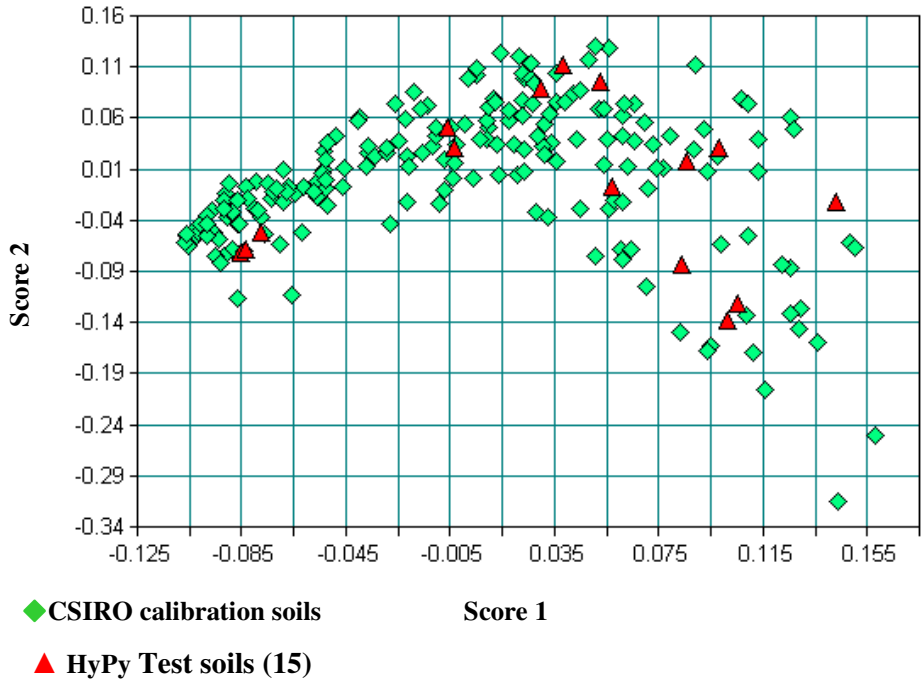

Figure 5-4 Biplot for the MIR spectra of the 15 soils (analysed by HyPy) within the field of the SCaRP samples.

When the 15 NBC soils are factorised and expressed according to their PCA scores (shown on figure 5-5) it is evident that other soil properties (including the mineral content) may exert a greater influence over the spectra than the relatively minor amounts of BC. For example, the SFS soils from Upper Tambo are located in one small area (similar scores), they all contain BC remnants after fire but are primarily the same sandy forest soil. In general it appears soils tend to group according to their region / soil type with BC secondary. This observation has been reinforced by the BC blend which was purposefully included as a cross check during the HyPy process. This blend had been made up at a rate of 16% biochar (5% C) with the soil standard-2 but plots along with this pure soil standard on the PCA, while soil standard 3 with high BC (1.29%) scores very differently (figure 5-5).

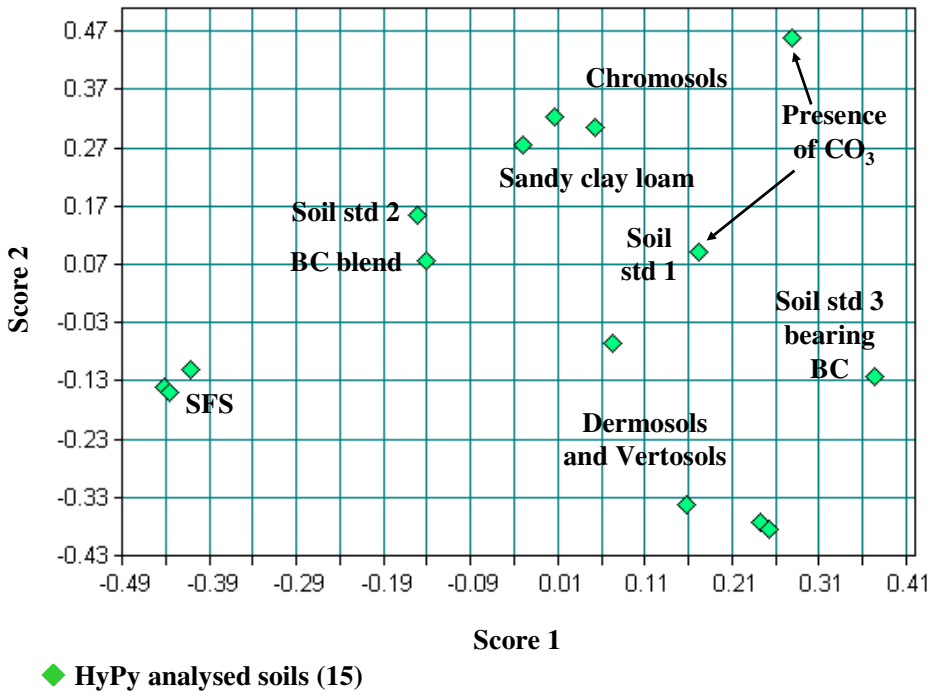

Figure 5-5 Principal component analysis (PCA) of the MIR spectra for the soils that underwent HyPy analysis. The distribution of their scores point to genetic similarities (and differences).

Furthermore, spectral analyses were carried out on untreated (only ground to under 100μm) soils to avoid influences that acid and washing may bring about in term of spectral characteristics. The level of interference due to the presence of carbonates is uncertain in these studies but according Reeves (2009) should be considered. In fact Chromosol-1 and the soil standard-1 both contained some carbonate. While HyPy required carbonates to be removed so these did not add to apparent BC, for the purpose of spectroscopy the acid treating (including washing and drying) step was removed because it appeared to alter the physical condition of the soil and avoided possible losses through handling. Note that treatment also lowers simplicity, where simplicity was a main aim for routine spectral work.

The principal component analysis goes some way to explaining any deviations resulting from MIR predictions as shown on figure 5-5. Their grouping appears to be associated with provenance as much as NBC content. Any poor predictions based on this calibration comparison may also be related to the two methods of determination. In some ways HyPy is a type of CTO relying on the removal of carbonates by wet chemistry prior to treatment followed by the removal of labile C (HyPy) to render 'pure' BC which is calculated from three sets of elemental analyses. Similarly, does ^{13}C-NMR after physical size fractionation

'see' the same BC as captured by HyPy? HyPy may likely result in slightly lower BC values because some of the more reactive BC with PAC <7 is lost. This would result in slight over-prediction on figure 5-3. The success of any BC calibration hinges on a consistent definition for BC followed by a standard and uniform preparation methodology.

Spectral analyses of blends

To further understand the spectral behaviour from BC in soils, known amounts (independent of the current determination methods described above) of soils-biochar blends were studied spectroscopically. These consisted of two biochars added precisely by mass into separate soils obtained from one batch (Lansdowne origin) and then fully homogenised. The rates used (0.25 to 20% blend, see methods), were aimed to be consistent with the possible BC concentrations encountered during field applications as well as the range used in the MIR-ROC calibration (0.02 to 2.0% C). The finely ground blends were analysed under the same conditions and MIR range as previously described. Spectra for biochar-soil blends (5 and 20%) have been shown in the examples on figure 5-6a along with end-member spectra for the unblended soil and a pure biochar and the important region expanded (figure 5-6b). The *known* quantities in these cases were the concentration of biochar C determined by the blend ratio in the soils and the C analysis of the particular biochar incorporated (ranged 0.05% to 12% BC). The Gidgee and Mulga derived chars themselves were 0.21 and 0.63% C respectively. It should be noted that the pre-existing C content (0.3%) of the soil used for the blends contained a small component of BC according to HyPy analyses (between 0.11 and 0.23%) placing them in the lowest range of mixtures (0.25 to 0.5% addition).

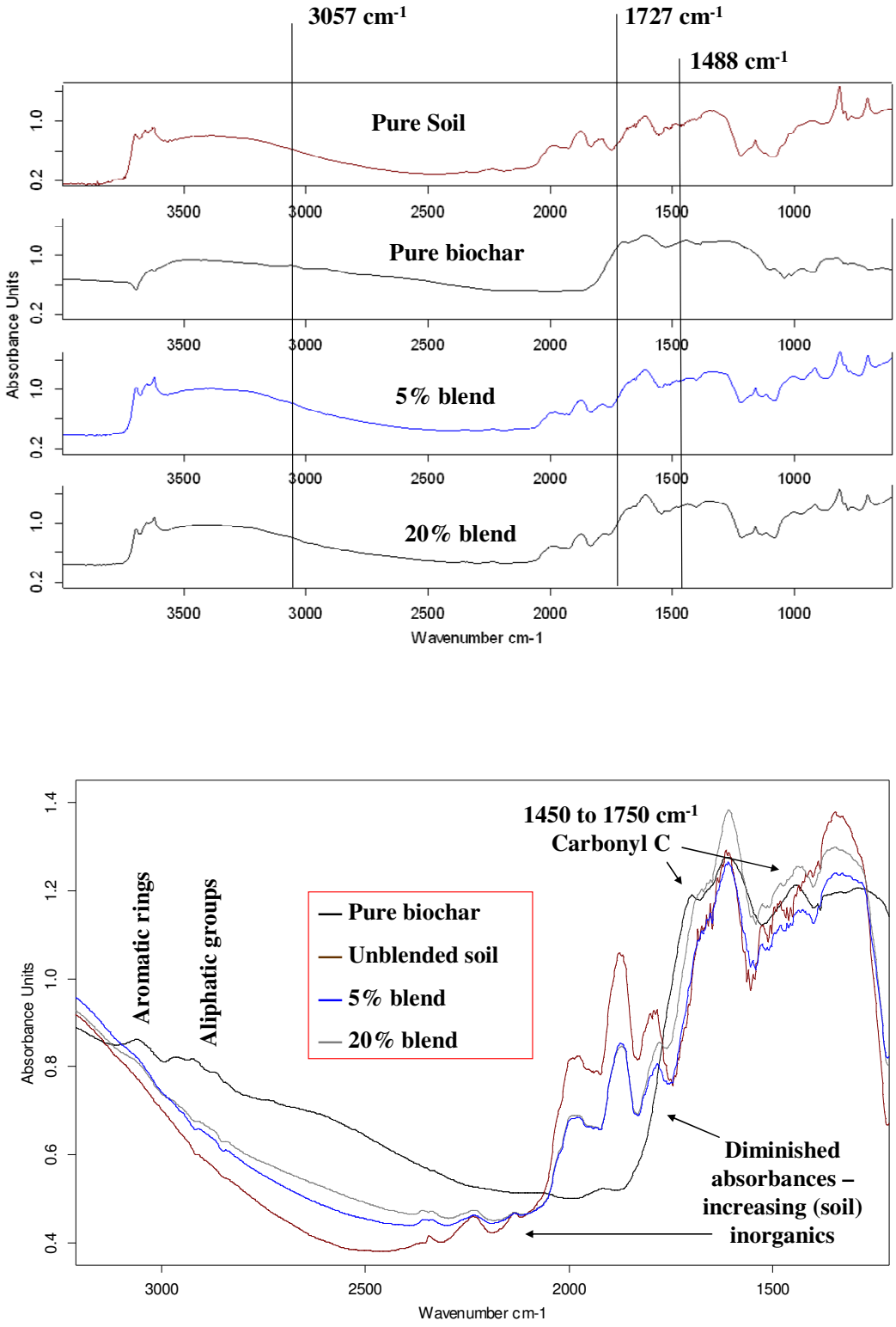

Figure 5-6 Complete FT-MIR spectra of soil-biochar blends and end member soil and pure biochar (a) and expanded region most relevant to absorbance of functional groups related to BC (b).

According to the ROC model about 14% of the C in these blended soils was classified as BC. The moisture contents for the gidgee and mulga biochars were 3.9 and 4.4% respectively and after mixing with the sandy Lansdowne soil dropped to 2%. The presence of significant moisture in the chars could otherwise account for discrepancies between C % in blends and their ROC predictions but these are minor amounts. More importantly with regard to the blends, both were produced (carbonised) at relatively low temperatures at the RCRA facility (200-250 ^{o}C, Karen Siepen pers. comm.) instead of the 450 to 600 ^{o}C commonly the case. The result is lower amounts of highly condensed C and is the main reason for the low predicted BC in the biochar. The naturally occurring BC values are in the same order of magnitude as the prediction model remembering that these come about as the result of wildfires which reach higher carbonisation temperatures. Over 480 ^{o}C char becomes almost fully aromatic (Adams and Attiwill, 2011) however the temperature of the organic layer near the soil surface can be considerably less such as soils under grassland (Gonzalez-Perez et al., 2004 and references therein). Therefore the organic residues in biochars or naturally produced BC (NBCs above) vary and are likely to be complex mixtures of SOM i.e. combinations of alkyl characteristics (e.g. absorbances at 2930, 2850 cm^{-1}) and unsaturated C structures (aryl groups). The latter can be expected to be increased under higher temperature / lower O_2 and spectrally indicated by absorbance >3000 cm^{-1}, in particular, aromatic groups absorbing near 3057 cm^{-1} (seen in the pure biochar on figures 5-6 a and b). Although this absorbance band was not recognised as a significant factor (according to the CUBIST analysis) in these studies compared to carbonyl (C=O) vibrations of esters and C-H bending (1779, 1727, 1488 cm^{-1}). Some other patterns in these spectra were also visually quite evident, namely, the diminished absorbances with increasing BC as indicated on figure 5-6b (approx. 2250 to 1800 cm^{-1}) related to the lower influence of soil spectral properties.

These soil-biochar spectra were then used as a test set with the MIR-ROC model. The ROC predictions (figure 5-7) obtained for the blends using the model indicated a good linearity (R^2 = 0.96) although were systematically underestimated. The ROC model predicted much lower BC values by about a factor of 7.

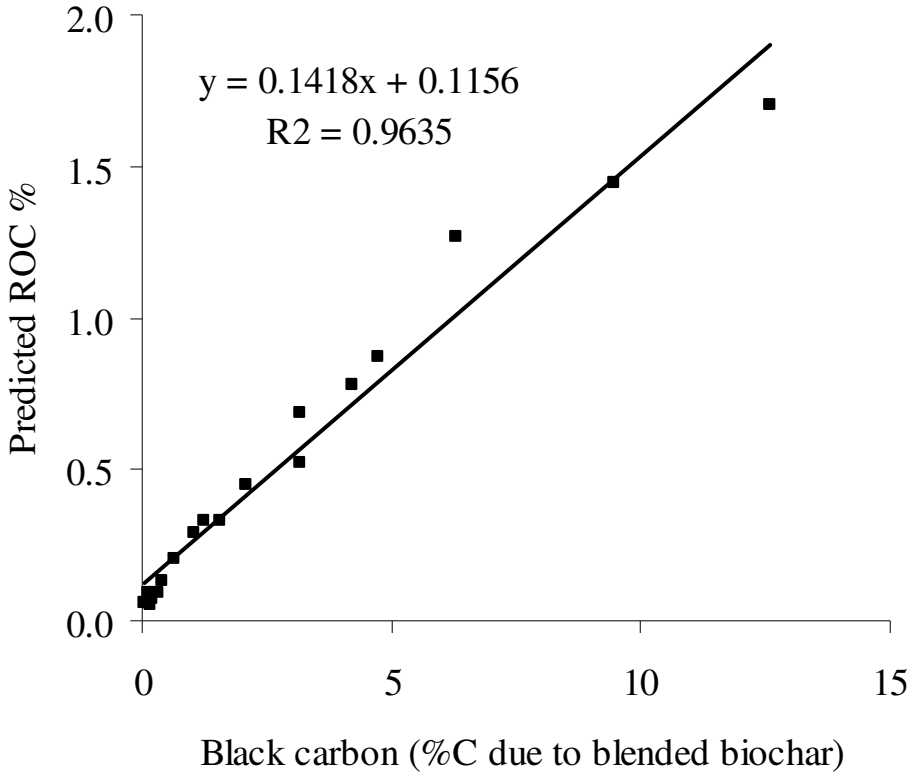

Figure 5-7 Using the MIR- ROC model to predict the amount of BC in the biochar blended soils.

Alternatively constructing a PLSR model generated from the spectra against its pre-determined BC values conveys a different story. The correlation obtained directly using the OPUS 6.5 software is shown on figure 5-8 (6 factors were used). The problem here however is that it is built from a small number of increasingly large BC concentrations and predicted values can become 'overfitted' or forced. The same would apply to a model based on the blend ratio as being the known quantity where any such calibration may otherwise be quite useful when monitoring the gross amount of biochar in amended soil unconstrained by a specific definition of BC. Principal component analysis indicated that the two biochars increasingly trended apart which can be due to either the %C present or the biochar itself.

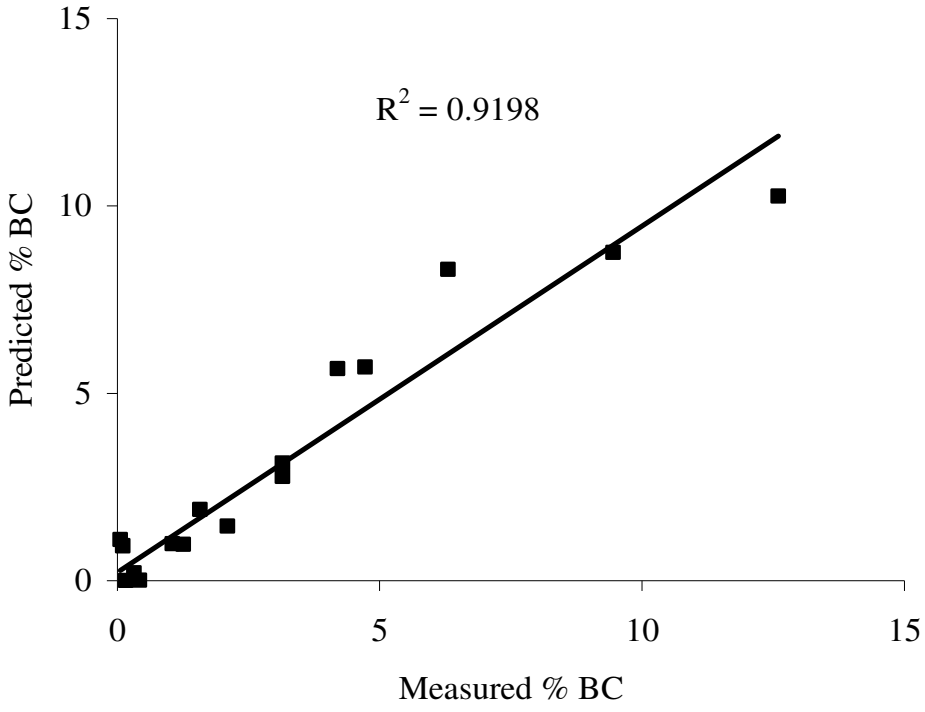

Figure 5-8 Prediction model generated (using spectroscopy software) from the spectral information and their known BC.

The disparity between the predicted and actual %BC is most likely again related to the core determination method used. Here, the BC was taken to be all of the biochar C blended into the soil (background BC from soil was regarded negligible). However during the charcoaling process not all the C is necessarily fully carbonised leaving variable amounts of only partially affected lignocellulose within its structure. In such cases the resultant chemical composition would be expected to escape the HyPy process (this includes aromatic rings with low condensation) or alternatively not be analysed as BC by ^{13}C-NMR. The end result must be under-prediction hence why the MIR-ROC model indicated that only 14% of the biochar C is resistant.

An important observation related to all the approaches used here is that good prediction models can be achieved for particular calibration method but does not mean these equate between methods or measure the same C. This comes back to the point about defining what is BC across all calibration (determination) methods.

C and N compositions of biochar soil blends

The determination of C and N are routine soil analyses where ratios (ranging from 10 to 20) can often distinguish forest, pasture and crop soils as a consequence of biological inputs. These were similarly completed for the soil-biochar blends. Charring of organic materials such as wood can be expected to lower the N content due to volatility and the process of carbonisation (as occurs through fire) where C increases at the expense of H and O. The same sorts of changes occur during coal maturation (see Van Krevelen, 1963). As a result charcoals contain up to 60 or 70% C as compared to about 58% in OM. The source and form of N in BC presumably is what remains (after wildfire) from partial oxidation with possible alteration to heterocyclic N forms (De la Rosa et al., 2008). Accompanying this are C/N ratios that can reach several hundred among charcoals and values of this magnitude should exert some influence over the overall bulk soil composition with respect to C/N. Where significant amounts are present this ratio could be used to indicate the presence / quantity of BC or alert to another C form distinct from humified OM such as carbonate.

The results for the C and N analyses have been plotted as C/N against the % of char added on figure 5-9. Two separate trends were produced corresponding to each char type. The nomogram allows a reasonable estimation of the amount of biochar in the soil over about 1% for Gidgee derived material and to a lesser extent the Mulga derived. Other information can also be gleaned from these plots namely, the C content of the biochar, the C/N ratio in the soil OM and the pre-existing % C. It should be emphasised that the presence of carbonate C (if left untreated) could increase ratios substantially and distort any such interpretation. Low levels of biochar or residues from forest fires are unlikely to be betrayed from C/N alone.

While these analyses can in themselves be quite a useful tool for BC estimation in soils where concentrations are higher (e.g., amended plots) it also highlights that as BC increases so do other factors that may affect spectral analysis.

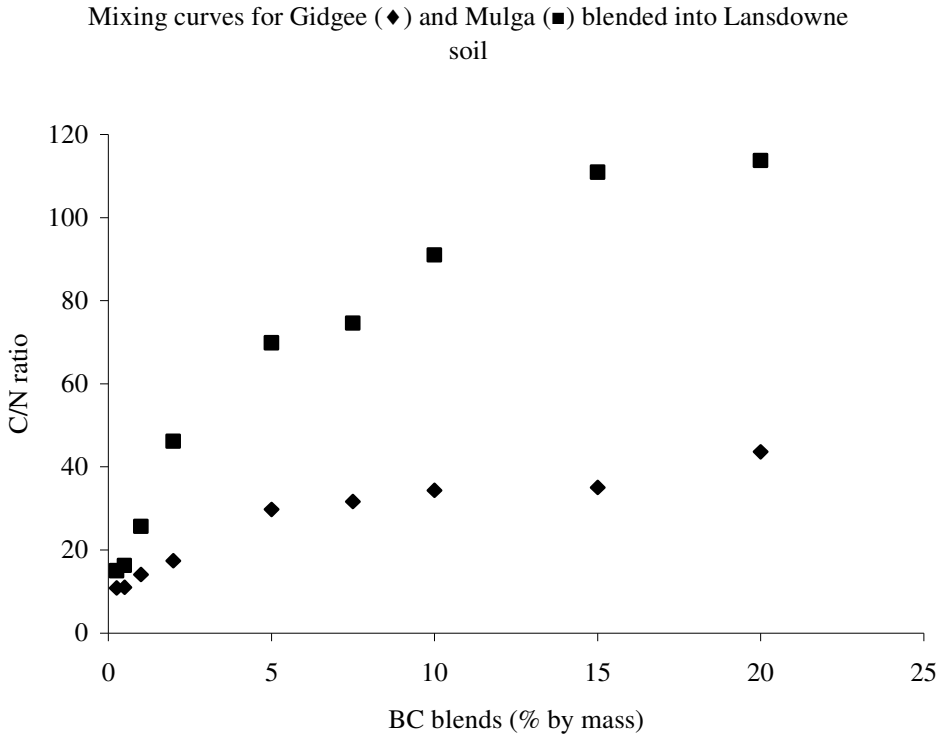

Figure 5-9 Result of elemental analyses (C/N) for biochar blends (in same batch of sandy loam).

Conclusions

1. A significant proportion of biochar C did not appear to fall under the category of BC as defined by other methods. Experiments on biochar added to soils at varying rates were estimated for their BC content by MIR spectra using a prediction model based on physical separation / ^{13}C-NMR (ROC method). This clearly showed that biochar comprised > 50% of relatively labile C (possibly C structures with ring sizes <7). These results highlighted that what is included as BC may vary according to the means of separations / analysis used and emphasised the importance of developing a standard and uniform method of determination.

2. Hydrogen pyrolysis was tested as an alternative means of determining naturally occurring BC (fire remnants) and as a potential method to calibrate MIR spectra for rapid soil BC analyses. These were based on a small number of samples (15) and from quite diverse provenance. Their spectra were predicted using the ROC model (as above) and while these plotted only generally they conformed to the overall trend produced by the model. As a possible calibration bench mark for future MIR spectral calibration these comparisons (with HyPy processed data) were very encouraging. MIR calibration on a larger set of soils is

expected to yield better correlations and could be used to build prediction models equal to ROC.

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APPENDIX I Equations used in Cubist and principal absorbances

Details of the CUBIST rules used to identify principal absorbance bands and generation of a quantitative prediction model. The 1797cm^{-1} was mostly used to separate BC concentration, followed 1488, 1396, 2784, 1727, and 1357 as useful predictors. WN refers to first derivative absorbance at the particular wavenumber.

ROC (mg/kg)	Equations
Rule 1: [127 cases, mean 2.336, range 0.24 to 6.69, est err 0.547] if 1797 <= 0.00868	ROC = -1.81 + 543 1797WN + 178 1511WN + 155 1643WN - 278 2082WN - 154 1357WN + 66 1843WN - 160 1488WN + 152 1465WN - 150 1573WN - 64 1396WN - 99 1504WN + 81 1727WN + 226 2784WN - 19 1080WN + 53 3802WN - 50 1458WN - 98 2260WN
Rule 2: [33 cases, mean 8.757, range 1.99 to 20.22, est err 0.880] if 5892 <= -4.35e-005 3972 > 7.15e-005 1797 > 0.00868	ROC = 2.757 - 3063 1365WN + 2281 1357WN + 834 1396WN + 1072 1797WN + 746 2784WN + 76 1727WN + 93 1488WN
Rule 3: [27 cases, mean 5.778, range 1.16 to 15.32, est err 0.709] if 5892 > -4.35e-005 1797 > 0.00868	ROC = -7.385 + 671 1511WN + 804 1797WN + 333 1643WN + 242 1843WN + 559 1465WN + 662 3802WN - 558 1488WN - 391 1504WN + 930 2784WN - 211 1396WN + 306 1727WN - 448 2082WN - 154 1365WN + 120 1357WN - 185 1458WN + 34 1080WN - 361 2260WN
Rule 4: [5 cases, mean 9.712, range 7.2 to 13.43, est err 0.798] if 5892 <= -4.35e-005 3972 <= 7.15e-005 1797 > 0.00868 1458 > 0.0141	ROC = 4.517 + 673 1797WN - 361 1365WN + 273 1357WN - 241 1488WN + 75 1396WN + 229 2784WN + 23 1727WN
Rule 5: [3 cases, mean 12.387, range 10.55 to 15.67, est err 0.075] if 5892 <= -4.35e-005 3972 <= 7.15e-005 1797 > 0.00868 1488 > 0.00469 1458 <= 0.0141	ROC = 6.639 + 603 1797WN - 257 1488WN - 28 1365WN + 21 1357WN + 6 1396WN
Rule 6: [8 cases, mean 6.425, range 3.59 to 8.99, est err 0.746] if 5892 <= -4.35e-005 1797 > 0.00868 1488 <= 0.00469	ROC = -2.093 + 349 1511WN + 581 1797WN + 179 1643WN + 130 1843WN - 315 1488WN + 299 1465WN + 355 3802WN - 126 1396WN - 194 1504WN + 159 1727WN + 444 2784WN - 240 2082WN - 99 1458WN + 18 1080WN - 194 2260WN

Attribute usage:

Significance	MIR Frequency
100%	1797WN
100%	1488WN
100%	1396WN
99%	2784WN
99%	1727WN
96%	1357WN
80%	1458WN
80%	3802WN
80%	2260WN
80%	2082WN
80%	1843WN
80%	1643WN
80%	1511WN
80%	1504WN
80%	1465WN
80%	1080WN
63%	1573WN
37%	5892WN
33%	1365WN
20%	3972WN

APPENDIX II Raw data for soils used in HyPy analyses

Soil	Pre-hypy SCV (g)	Post-hypy SCV (g)	Post-Hypy SV (g)	*Previous C%	Pre-hypy C%	Post-hypy C%	Pre-hypy C (mg)	Post-hypy C (mg)	BC/TOC %	BC in soil %
Chromosol 2	1.1374	1.1223	0.3092	0.15	0.19	0.03	0.62	0.08	12.55	0.02
Soil Std 1	0.9981	0.9842	0.1979	*1.02*	0.69	0.08	1.46	0.16	10.83	0.06
Chromosol 1	1.0423	1.0215	0.2366	0.39	0.38	0.10	0.98	0.22	22.98	0.09
Vertisol 1	0.9662	0.9523	0.2125	*1.20*	0.90	0.12	2.03	0.24	12.06	0.11
SCL-1	1.0409	1.0253	0.2051	0.70	0.77	0.14	1.69	0.28	16.40	0.11
Dermosol 2	1.094	1.0682	0.2332	0.79	0.87	0.15	2.25	0.35	15.52	0.12
Vertisol 2	1.112	1.0994	0.2743	0.82	0.80	0.19	2.30	0.52	22.71	0.19
SCL-2	1.0468	1.031	0.2082	1.30	1.36	0.26	3.05	0.53	17.43	0.23
Soil Std 2	1.0516	1.0279	0.2214	2.60	2.00	0.25	4.90	0.54	11.07	0.29
SFS 1	1.1468	1.1312	0.3007	1.80	2.01	0.33	6.37	0.98	15.35	0.31
SFS 3	1.0926	1.078	0.2823	2.60	2.78	0.63	8.25	1.76	21.38	0.56
Dermosol 1	1.1423	1.1001	0.2846	4.10	4.11	0.74	13.42	2.09	15.59	0.64
SFS 2	1.0839	1.0687	0.2386	2.30	2.02	0.94	5.13	2.24	43.75	1.01
Soil Std 3	1.075	1.0438	0.2038	4.02	3.88	1.44	9.12	2.92	32.07	1.29
BAS	1.0899	1.0616	0.2115	4.70	5.10	2.43	12.22	5.14	42.07	2.15

Note: SCV refers to soil+catalyst+vial; BCs were calculated based on *previous C analyses (our labs); values (in italics) were adjusted for carbonate content.

CHAPTER 6 - REVIEWING THE DETERMINATION OF SOIL C POOLS: ADDITIONAL EXPERIMENTS IN THERMAL ANALYSIS

Background and scope

Soil C is diverse and SOM itself is composed of a continuum of biologically derived substances that individually can persist for quite variable periods of time under the set of conditions (soil type, climate, etc.) that influence degradation rates (Sanderman et al., 2010). This aspect is as important as predicting soil C priming and loss minimisation because without an understanding of the processes at a component level, soil C building strategies are too uncontrolled and remain obscured by the real soil C structure. It must be emphasised that physical protection of SOM by the mineral component (while not quantified here) is another important factor capable of producing apparent changes to in-soil decomposition sensitivity (Schmidt et al., 2011).

Numerous methods of modelling the dynamics of soil C have evolved from the earliest concepts of SOM degradation studied for the past 150 years (most notably at the Rothamsted Experimental Station). Now computerised turnover simulation models (e.g. CENTURY, ROTH-C) try to predict the theoretical degradation and residence times of distinct soil C pools (Kirschbaum et al., 2001; Davidson 2006). These rely on initial values obtained for the different soil C pools to predict their respective abundances over time but may become too generic (inputs are too general) with respect to soil conditions and perhaps the type of OM. It is of some importance to be able to recognise what is occurring to these differing soil C fractions over time by a practical ability to measure their proportions rather than simply the total C abundance. As quite justifiably pointed out by Baldock et al. (2009), a method to obtain meaningful and measurable soil C pools would be more reliable than conceptual pools derived from simulation models. This point has been well demonstrated by these authors' attention to the development of a protocol to separate major pools to calibrate rapid spectral analyses for greater soil throughput. To this end, physical and chemical separation followed by various analytical methods have been seen as the effective way of dividing a soil into their broad organic C types: labile, humified and black carbon (BC). This general approach into isolating the main C groups has been adopted by others (e.g. Zimmermann et al., 2007) with several variations used in separations, all of which are time consuming and involved.

A definition for these soil C pools

Generally speaking, in addressing soil C pools in the context of monitoring stocks, from currently proposed strategies (eg. Walcott et al., 2009) there seems to be general agreement

that soil C may be categorised into four main groups (table 6-1). These are based on relative differences in residence times enabling a fairly reasonable broad definition as follows:

Table 6-1 The relative residence times for the major soil C forms and alternative terms used for the group.

Major soil C forms	Turnover dynamics	Equivalent designations
Carbonate	Millennial	Inorganic C
Black carbon	Centennial	[1]ROC, [2]Inert OM
Humus C	Decadal	[1]HUM, recalcitrant OM
Labile C	Annual/seasonal	[1]POM, POC, active pool

Note: Nomenclature used by [1]Sanderman et al. 2011 [1]Baldock et al. 2009 (CSIRO) and also [1,2]Zimmermann et al. 2007, [1]Bornemann PhD thesis 2011. The active pool includes microbial biomass.

The persistence of each C group in the soil environment is likely to be influenced by a different set of factors. For example the abundance of inorganic C is not expected to change over long periods (Allen et al., 2011 and their references) without significantly decreased pH. While inert C resulting from wildfires is relatively stable (except during subsequent fires), POM and HUM are probably mostly susceptible to alteration via increased aeration (e.g. tillage) and the affects of priming (addition of labile C-rich OM).

In Chapter 2, thermal analysis was used as a way to better understand LOI and its limitations but those experiments also provided strong indicators of this technique's ability to differentiate other forms of soil-related C. The perturbations observed in the ion currents associated with thermogravimetric (TGA) oxidation products (primarily CO_2), were interpreted to be related to a diversity of OM itself as well as other chief soil C contributors such as black carbon and carbonates.

Rather than physiochemical means, the potential for separating these same groups thermally appears to have been underexplored and is the focus of this chapter. It tries to answer two of the main hypotheses set at the outset of this research as it relates to soil C fractions and auditing with emphasis on black C, carbonate and humified OM:

1. Test the hypothesis that there is a relationship between the in-soil recalcitrance of carbon and ignition temperature.

2. Increase our understanding of the types of carbon most relevant in such studies and clarify the importance of soil size fractions during soil carbon auditing.

A review of activation energies and the basis for thermal analysis

Earlier soil researchers already recognised that differential thermogravimetry (DTG) curves were a means to characterise OM that could assist in the classification of soils (Turner and Schnitzer, 1962; Schnitzer et al., 1964; Schnizter and Hoffman, 1966). In addition they also derived rate constants for different OM based on thermal analysis data using early methods (Van Krevelen, 1951). In principle, the mass lost (degraded) for a set of heating rates provide information on rate constants at stages of the material's decomposition and from there allowed activation energies (Ea) to be calculated. Over the decades kinetic data acquired in this manner by various methods including Flynn and Wall (1966), Gill et al. (1992) have been recorded for organic and inorganic materials. In order to provide some numerical framework to discuss these four different soil C forms / pools in the context of thermal analysis and the potential to separate them on a refractory basis, published data on activation energies have been abridged for table 6-2. A brief examination of activation energy data for carbonaceous materials representative of these groups indicates that they differ in their stability which means some pools could be differentiated by thermogravimetry (TGA). For example carbonate (160 kJ mol^{-1}) should decompose at a higher temperature than charcoal (65 kJ mol^{-1}) allowing the relevant forms to be easily determined by TGA depending on the thermal behaviour of other components present.

Table 6-2 The ranges and variations in activation energies (Ea) for model materials representative of the major pools and the literature sources (obtained by TGA methods).

C form	Ea (kJ mol^{-1})	Reference/s
Cellulose	76-133	Emsley and Stevens, 1994; Xie et al., 2009; Amutio et al., 2012
Lignin	62 - 98	Amutio et al., 2012; Shen et al., 2013; Xie et al., 2009
Black C*	65	Bafghi et al., 2011
Kerogen	70	Aboulkas and El Harfi 2008
CO_3	160	Halikia et al., 2001
$CO_{3\ (in\ situ)}$	133-138	Geogiva et al., 2013

* Charcoal value used as black C varies depending on form (soot, coal etc.) Also note: cellulose may be considered under POC while lignin and black C as more resistant under soil conditions i.e. HUM and ROC respectively.

SOM can be regarded as carbohydrate in composition (Barron et al., 1980) where mainly plant debris has been oxidised and rearranged to form coiled macromolecules (Sutton and Sposito, 2011). The biomass precursor can be chemically represented by cellulose and lignin which are some of the most widespread carbonaceous materials in our environment. Their kinetic data has been considered here as way to approximate recent plant derived SOM in terms of thermal degradation since values for the latter are scant with the exception of some general information on SOM kinetics (e.g. Ruxton, 2003) and some respiration studies

(e.g. Reth et al., 2009). As an example, simulated aging of pure cellulose under air by TGA at low temperature (160 ^{o}C) was accompanied with increasing carbonyl units (Ali et al., 2001). However, although OM turnover has been correlated with activation energy where short term data has been excluded (Reichstein et al., 2005), it must be emphasised here that Ea data from TGA analysis of cellulose, lignin and BC cannot be used as a strict analogue to in-soil degradation. This is because, how the different substrates behave in soils is more complex and undoubtedly depends on the prevailing conditions such as pO_2 or presence of microbial enzymes (Sanchez-Jimenez et al., 2011). Decomposition of carbohydrates can be variable and cellulose has usually been regarded as less stable than [d]lignin although this has been challenged more recently (Schmidt et al., 2011). Whether through chemical or structural protection both these polymers are not easily broken down in the natural environment without the assistance of fungi (Kabuyah et al., 2012) and their enzymes (cellulases and lignases). In addition, fungi produce melanin, a fairly resistant pigment made of aromatic C structures, in turn, adding to the more recalcitrant lignin.

In reviewing Ea data related to the thermal decomposition for some of these 'model' materials considerable ranges have been encountered. Published Ea values for cellulose in particular, differs widely partly because of its complex decomposition / depolymerisation (pathways described by Emsley and Stevens 1994; Shafizadeh and Bradbury 1979 and many others) but also due to the varied conditions throughout all the TGA studies. Most appropriate to this study would be TGA thermal degradation of cellulose under oxidation conditions. Lignin has often been found to be more stable than cellulose in terms of TGA degradation temperature and hence Ea (Xie et al., 2009), which according to Shen et al. (2013) is because of the presence of polymerised aromatic units. These may be consistent with what can be expected in natural settings although Amutio et al. (2012) showed that the onset temperature for lignin was actually lower than cellulose resulting in a lower Ea but the bulk of the lignin degraded at temperatures higher than cellulose. Furthermore, even under oxidative TGA, there were volatilisation and oxidation phases evident (pyrolysable and non-pyrolysable fractions). This means that even where air is used (21% O_2) some of the organic (combustible) materials degrade into gases and form char to be fully oxidised at higher temperatures. Shifts can also occur in the initial stages (onset) of thermal decomposition and oxidation depending on pO2 (Tihay et al., 2011; Horrocks et al., 1985), heating rates and perhaps also substrate/matrix leading to different Ea. All these factors may provide some

[d] Lignin forms part of the plant cell (xylem) imparting strength to its structure and can be as much as one quarter of woody material. It is bound together in strong bonds making it resistant to decay.

explanation for the various Ea data available in the literature for particular types of organic material.

Activation energy data for a particular material can also vary to some degree depending on its physical condition (particle size and structure) during heating or composite thermal behaviour (contaminated or compound minerals). In some ways it offers the possibility to differentiate polymorphs of the same chemical composition.
In situ formed $CaCO_3$ appears to have a lower activation energy (Georgiva et al., 2013) which may have further importance for soil analysis by thermal means. Generally the onset temperature for carbonate thermal degradation is around 650 ^oC and may extend beyond 800 ^oC (depending on total mass and thermal disequilibria) or higher where dolomites are present (Wang and Anderson, 1998). However, $MgCO_3$ (in its native form) depending on its likely abundance in soils should be considered because of its lower degradation temperature at from around 500 ^oC (Bisutti et al., 2007) which would interfere with the clay / mineral TGA event. As with carbonates, BC is also usually indistinguishable from SOM (e.g. elemental analysis) but is not as easily corrected for as carbonate. Any advantages of acid treatment apply in such cases where the removal of carbonates other than dolomite or calcite may be necessary.

Numerous TGA studies of pure cellulosic materials (e.g. applications in bio-fuel production / plantation wastes etc.) have provided useful indicators of their thermal behaviour / characteristics. For example differentiable thermal characteristics have been shown for hemi-cellulose, cellulose and lignin and (e.g. Abdullah et al., 2010). As a result, the technique's potential to distinguish soil C types has received renewed attention over the last decade and as projected by Manning et al. (2005), SOM could be resolved into discrete dynamic C pools using the basis of programmed heating rates.

Objectives
The key goal in this study was to evaluate TGA as an alternative means to determine one or more of these soil C pools without having to resort to the more time consuming and involved physical and / or chemical fractionation as employed by the current *standard* methods. Potentially, data obtained in this way could be used in conjunction with (calibration of) rapid FTIR analysis of these subdivisions in a similar manner to those proposed by Vasques et al. (2009) or Baldock et al. (2009) and Sanderman et al. (2011) and were the approaches discussed in Chapter 5 (in conjunction with HyPy).

Materials and Methods

Soils and carbonaceous materials

Soils were obtained from mainly around NSW to include Dermosols with carbonate content, sandy loams from fire prone areas for their BC content and agricultural Vertisols and loams with variable SOM to test separation of these main pools by TGA. Two soil-biochar blends were selected (used for studies in chapter 5), to test quantification of BC in soil by TGA.

Soil treatments

A small subset of soils (6) primarily loams from Lansdowne (under lucerne) and a Vertisol under pasture from Dimby Downs were digested using wet oxidants to remove labile OM and test the TGA response. The chemicals used were 0.9M Calcium Hypochlorite ($Ca(OCl)_2$), 0.33M K- Permanganate ($KMnO_4$), 10% (3M) hydrogen peroxide (H_2O_2) and 0.1M NaOH (alkaline solution). The wet oxidants were allowed to digest for 24hrs and the alkaline solution 160hrs. Each of these reactions required periodic agitation and in the case of the H_2O_2 digestion was done using a warming plate (60 ^oC). None of these reactions was acid catalysed.

Clay separations

Clay sedimentation was carried out on the basis of Stokes law where particles finer than 2 µm remained in the surface 20cm of a soil water suspension 16hrs after agitation. Because larger quantities were required for repeated thermal analysis, settling was carried out in bucket proportions (vessel 25 cm height and width) using around 150g soil (dispersant free). After the specified time had elapsed the fine fraction was decanted carefully into a broad vessel to facilitate drying before collection of each fraction (yielding 50 mg to 2g). TGA analysis were carried out using the TA Instruments 2950 (as described in chapter 2) and a new instrument the Q5000 by TA instruments was also trialled (University of NSW, School of Chemical Engineering). The latter was capable of analysing much lower masses as obtained after settling.

TGA analysis

Thermal analyses were carried out using a TA Instruments 2950 TGA. Samples were dried (stored at 40 ^oC) and ground up to homogenise the matrix which was supposed to reduce thermal disequilibria. Soils were heated in a platinum pan (capacity to 100mg) at a rate of 10 $^oC\ min^{-1}$ from 200 to 750 ^oC under oxidative purge (40ml min^{-1} through the furnace**).** Heating from ambient conditions was at 25 $^oC\ min^{-1}$ to remove maximum free water. All the

mass changes reported here were determined using the TA Universal Analysis 2000 software.

Thermogravimetric analysis / Fourier transform infra-red (TGA/FTIR)

TGA/FTIR (Fourier Transform Infra red) was carried out with a TA Instruments 2050 TGA connected via heated (200 ^{o}C) 1/8" transfer line to a Nicolet 5700 FTIR spectrophotometer (Thermo Electronic Corp) fitted with evolved gas cell accessory and liquid N_2 cooled detector. The heating program was as with pure TGA and TGA/MS experiments and purged with a gas flow rate of 75 ml/min. Ultra high purity N_2 was used in order to extract and preserve as much functional group information as possible. FTIR spectra were averaged from 16 scans at a resolution of 4 cm^{-1} using OMNIC 7.3 software.

Results and Discussion

Separation of BC and Carbonate

The review of activation energies for possible soil constituents indicated that the more thermally resistant BC and $CaCO_3$ provided a good starting point for separation in a simple system that did not involve silicates, alumino-silicates or typical SOM. A pure gidgee biochar has been presented on figure 6-1 indicating the mass change events due to free water (below 200 ^{o}C), biochar (onset temperature ~320 ^{o}C) and $CaCO_3$ (onset temperature ~620 ^{o}C). The thermal analysis (shown by differential mass change curve) of this pure biochar yielded the following information:

1. that the biochar degraded with a sharp mass change,
2. biochar is well separated from $CaCO_3$,
3. both components can be readily determined on mass basis as well as their C contribution provided a conversion factor can be derived for each form and,
4. the residue was due to the ash content (proximate analysis) in this case 60%.

The C contribution due to carbonate is easily calculated according to the decomposition reaction:

$$CaCO_3 + (heat) \rightarrow {}^{d}CaO + CO_2$$

The CO_2 is lost in the gas stream, lowering the remaining mass by 44% but a quick rule is the amount of C due to carbonate present in the (soil) sample is 27% of the mass lost (i.e., conversion factor is 0.27).

[d] This is a calcining process where the residue (66%) CaO, sometimes improperly termed lime, has been used to amend soils and pH.

The thermogram of this biochar provided a good example of the separations achieved by TGA. In this case the $CaCO_3$ may come about through the concentration of calcium oxalate from precursor plant stems during production (not so apparent after dilution into soils).

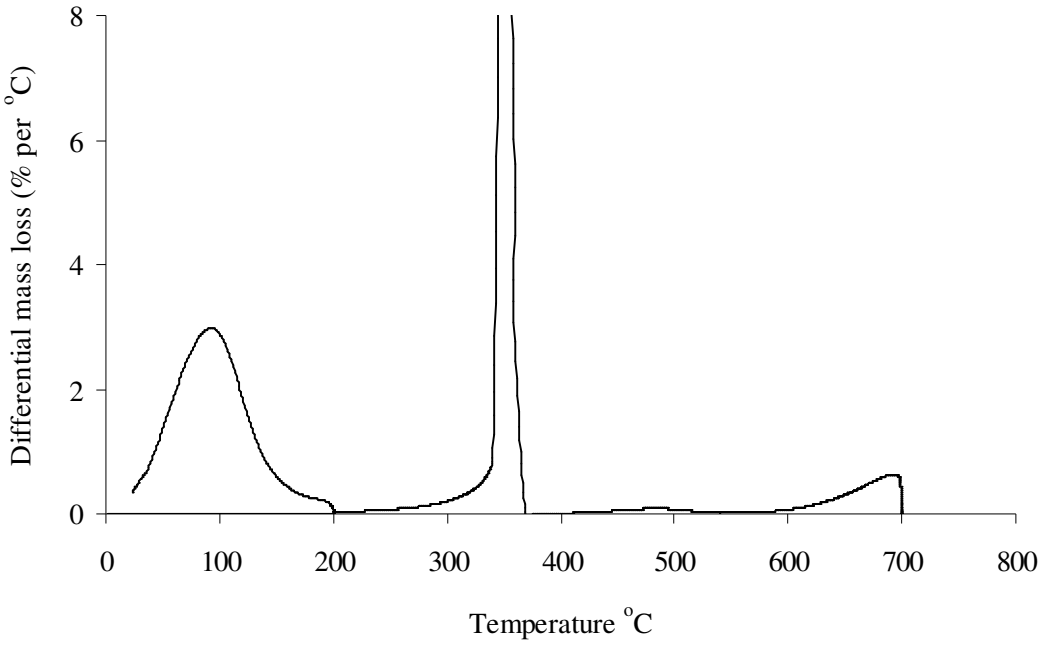

Figure 6-1 Thermogram of a gidgee derived biochar showing all mass loss events: initial moisture, biochar at 350 °C and calcium carbonate at 650 -700 °C. The biochar had maxima of 320 °C low for BC and is related to its low temperature of production (200-250 °C)

Similarly, carbonate is well separated from SOM with temperature onsets lower than BC providing for quite effective TGA differentiation / determination of SOM and carbonate. Furthermore their relative proportions can be correlated with the stable carbon isotope composition of the whole (soil) sample which quantifies the isotopic sum of all the C components present (refer to Attachment-A below as stable isotopes are not discussed further here).

Determining black carbon

However it is not always so neat and simple. Soils contain several mass change events that may mask BC notably, SOM and dehydration / dehydroxylation of minerals. To ascertain whether TGA can distinguish and determine BC, both soils with known BC and several synthetic soil blends were studied in this way. Soils (including soil standard 3) originated from various localities as well as fire-prone areas which were previously confirmed to contain low amounts of native black C (NBC) using the hydrogen pyrolysis (HyPy)

procedure described in chapter 5. Charred C is found in surface soils after wildfires and is thought to occur by depletion of polysaccharide-derived organic matter and enrichment of lignin and lipid derived compounds (Neff et al. 2005). This may well be reflected in pre- and post-wildfire differential scanning calorimetry (DSC) plots presented by De la Rosa et al. (2008) showing the relative decrease of labile and increase of more stable OM (discussed further under SOM below).

To investigate the potential to determine BC in soil by TGA alone, soils were thermally analysed under oxidative conditions according to the methods described. Examples of thermograms have been presented on figure 6-2 clearly showing the major mass change events between 20 and 50 minutes (200 to 550 ^{o}C) observed with the differential mass loss (DTG) curves. The anticipated mass losses found in earlier studies due to free water, OM between 20 and 30 minutes and the mineral event (45 mins.) were noted. However these analyses included an additional event between 33 and 43 minutes (temperature equivalent of 350 to 470 ^{o}C) suspected to be due to the presence of BC content. Although, this feature is not always evident in all thermograms and is probably abundance related. Also the two fire prone forest soils were sandy and lacked a distinctive mineral event. Note that soils were finely ground to prevent spikes during oxidative analysis.

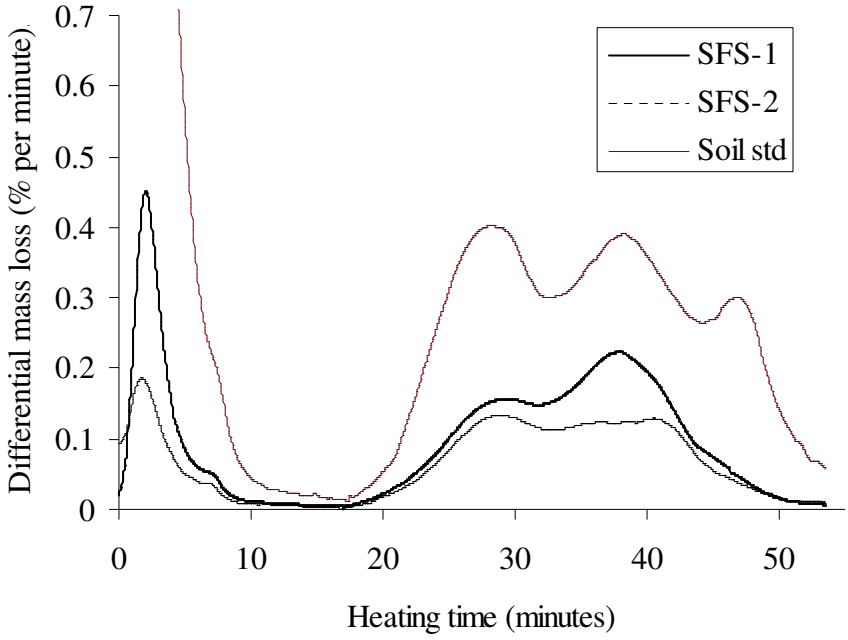

Figure 6-2 Soil thermograms containing variable amounts of SOM and BC ranges from 0.28 to 1.29% of total mass. SFS-1 and -2 refer to sandy forest soils (0-5 cm) from fire prone areas.

TGA mass loss data was then determined for the thermal 'window' (360 to 470 ^{o}C) as defined by the DTG curves in the example plots above and then compared to results obtained after HyPy (the number of soils that underwent both analyses, n=13). The following correlation on figure 6-3 was produced (R^2 = 0.91) and indicated that TGA was able to detect a significant proportion of the mass change event due to BC. The slope of ~2.4 was consistent with BC in the soils being typically composed of 40% C, fairly low for BC and closer to that of recent OM. The linear correlation function between the results obtained by TGA (converted to % BC) and determined by the HyPy method was 0.952 + 0.23 with a bias of 0.21. The small deviations from unity (including intercept of 0.5) could be attributed to slightly different C concentrations BC in individual soils (from various origins) and the likely interference from OM oxidation and mineral decomposition events flanking both ends of the BC oxidation. The latter would add mass and produce lower C concentrations.

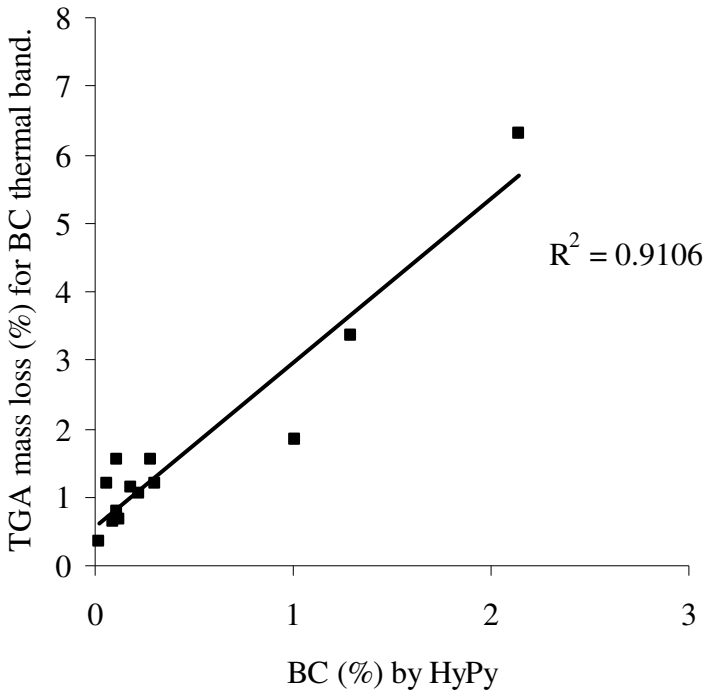

Figure 6-3 Correlating the mass losses for the TGA thermal interval (33 to 34 min.) with the values obtained by HyPy analysis for BC (n=13).

Equally the TGA analysis for that thermal region may include small amounts of less aromatised material which is lost during the HyPy process polyaromatic carbon rings (PAC) <7. As a cross check, when the mass drops for the SOM and BC in the soil standard were

combined and corrected with the factor 0.58, the result was 4.35% C as compared to the value from dry combustion of 4.02% C.

Since adjacent thermal events can interfere with BC mass during quantitative determination, another approach using TGA analysis was to disregard the mineral event and quantify relative changes. To do this, two blends (2 and 5% by mass) of Mulga biochar (negligible ash and no carbonate content) were heated at higher rates (interactive heating to 20 ^{o}C min^{-1}). This resulted in separation from labile material below 340 ^{o}C (see figure 6-4) but without any resolution between BC and the mineral event (i.e. they were completely concordant).

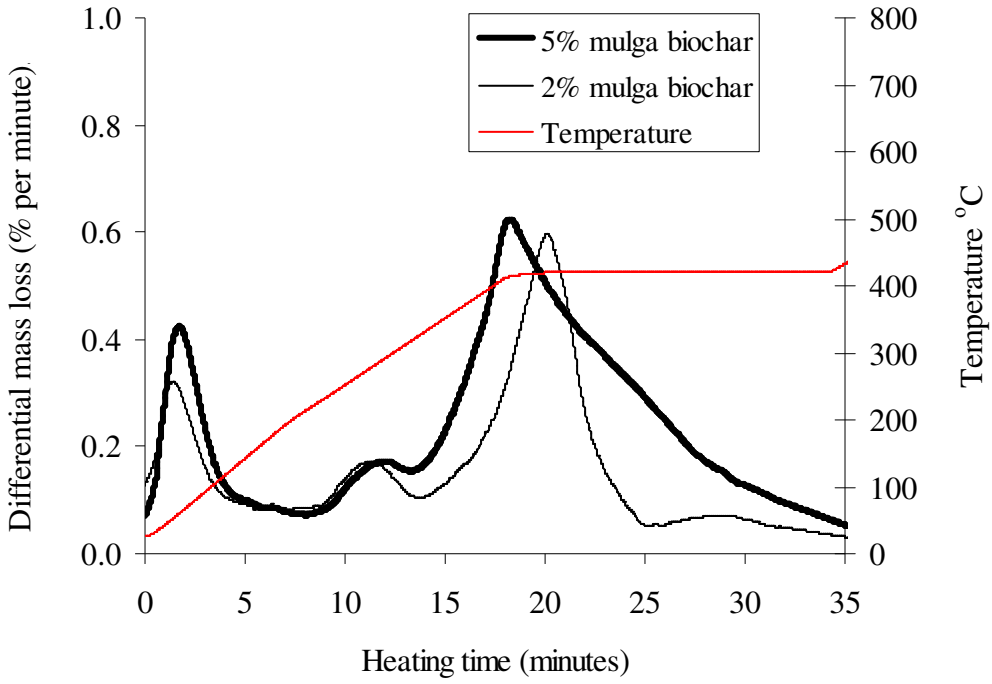

Figure 6-4 Mugla biochar blends (2 and 5% by mass ground into Lansdowne sandy loam) heated at 20 ^{o}C min^{-1}.

The mass changes (table 6-3) for the OM region were both the same (0.73) and using the conventional conversion factor (0.58) resulted in a consistent C analysis (0.42%) for the aliquot of Lansdowne soil used to blend the chars. The relative areas under the peaks on the thermal analysis relate to mass losses of 3.60 and 6.64 for the addition rates of 2 and 5% respectively. After the amount of BC was accounted for, the comparable residuals recorded (1.60 and 1.64) were within 0.04% and are representative of the losses (inorganic) from the mineral component 1.62 +/-0.2 mass %. This method may be fairly applicable where biochars have been amended and soils can be calibrated along the lines of Koide et al. (2011)

who used LOI but used unamended soils from around the trial plot to standardise the baseline.

Table 6-3 Mass losses recorded at the OM and BC regions after adding biochar to Lansdowne sandy loam at 2% and 5% by mass.

Rate of addition	TGA mass losses		
	OM	BC	Residual
2% biochar	0.73	3.60	1.60
5% biochar	0.73	6.64	1.64

In the Koide et al. (2011) approach, the background soil was weighed after LOI heating in order to correct for mass losses from substances (OM and water) other than BC. Our TGA approach has the advantage of not requiring calibration soils and is likely to be more precise because it has the capacity to separate components thermally.

Analysis of the mass change events and comparison with HyPy data has demonstrated that BC can be identified and semi-quantitatively determined. Determinations are likely to be improved by increasing resolution of components through changed heating ramps (currently 10 ^{o}C min^{-1}) and screening C only signals to overcome the problem of overlap with the mineral event. In contrast, Differential scanning calorimetry techniques used previously to monitor BC in soils (e.g. De la Rosa et al., 2008) were fairly qualitative where small amounts of char (exothermic) occur concurrently where clay minerals release OH and water (endothermic) resulting in some cancellation.

The SOM carbon Pools

The often discussed organo-mineral complex developed through the aggregation of soil minerals and organic particles probably carry the more stable SOM as found in the fine particle size fractions. That the fine fraction hosts older and more humified OM has been reasonably supported by other evidence such as C/N ratios (Magid et al., 2002) and isotope data (Bird et al., 2003) which also indicated trends with depth. The work in Chapter 2 (where TGA was discussed in the context of LOI), indicated that lesser amounts of carbonaceous material (i.e., OM) is released during TGA analysis concurrent with the mass changes from clay minerals during loss of lattice water. This observation may hold an important key to unscrambling recent and aged organic matter that may equate to or constitute designations such as POM and HUM.

Simple thermograms alone provided sufficient information from their variations in mass change to suggest separate stages of OM release not necessarily related to BC in soils (considered also in relation to catalysis below). Such patterns have been observed for different but related soils such as the cropped and pastured Vertisols (figure 6-5). Similarly differences have been observed in thermograms between slaking and stable soils where the latter was marked by variable mass changes during the transition into the mineral event. Biologically mediated stabilisation of SOM may also be recognisable by TGA (refer to Attachment-B where some observations about composted materials have been included for its intrinsic interest.

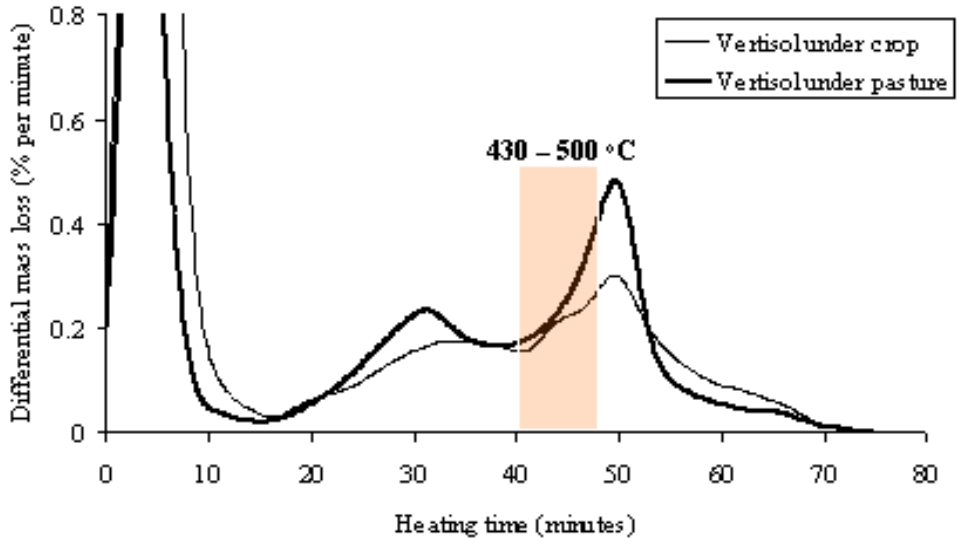

Figure 6-5 Thermogram of a cropped and pastured Vertisol (Liverpool Plains, NSW)

Chemical and physical effects on SOM indicated by thermal analysis

To gain deeper insights into the possible POC / HUM makeup of SOM and any relationship with the mineral events through TGA analysis, two approaches were used that involved chemical and physical treatments. Firstly several chemical extraction methods were applied to loam soils and a Vertisol to gauge changes in TGA behaviour to directly measure the effects of the mild oxidation i.e., to detect whether OM was disappearing from one or the other thermal mass loss region. Several different chemical reagents were used in case of artefacts due to the particular chemical (note analyses were carried out after washing with de-ionised water and drying).

The results for the mass changes along with the proportions have been tabulated below (6-4) for each thermal region corresponding to the main OM event (MOM) and the OM and mineral composite referred to as the OMC broadly over 430 to 590 ^{o}C. All the treatments removed similar proportions of OM represented by the 200 to 430 ^{o}C TGA region. However changes to the OMC were a little more variable with a 60-70% decrease when $KMnO_4$ was used, no appreciable change after NaOH extraction and a slight increase after Hypochlorite but quite marked after peroxide. Some of the increases may be accounted for by the relative loss due to the OM but not in the latter with no obvious explanation. Overall this approach may introduce other uncertainties related to the chemicals used, for example hypochlorite residue ($CaCl_2$) is very soluble but hygroscopic. Equally, the exchanging of cations cannot be discounted in producing mass changes in the OMC. SOM within the OMC are expected to be minor compared to MOM and the losses from the OMC when $KMnO_4$ was applied were in the same range or higher. This is evident in their thermograms as shown by the example on figure 6-6 below.

Table 6-4 Effects of various soil chemical treatments on respective mass change events.

	TGA masses (%)		Fraction remaining after treatment		
Soil and treatment	200 - 430 ^{o}C	430 - 590 ^{o}C	200 - 430 ^{o}C	430 - 590 ^{o}C	Effect to OMC
SCL-1 before treatment	3.1	3.1			
0.9M $Ca(OCl)_2$	1.9	3.2	0.6	>1.0	slight increase
0.1M NaOH	1.7	3.0	0.5	1.0	no sig. change
0.33M $KMnO_4$	2.4	1.8	0.8	0.6	decreased
SCL-2 before treatment	1.4	2.9			
0.33 $KMnO_4$	1.1	2.1	0.8	0.7	decreased
SLC-3 before treatment	2.3	2.3			
3M H_2O_2	1.7	1.9	0.7	0.8	decreased
Vertisol before treatment	3.3	4.8			
3M H_2O_2	2.8	5.6	0.8	1.2	increased

SLC refers to a sandy clay loam taken from lucerne cultivation, (Lansdowne via Camden, NSW) where SCL-1 was sampled from 0-10 cm; SCL-2 from10-20 cm and SCL-3 at surface; the surface Vertisol was from pasture land Liverpool plains, NSW.

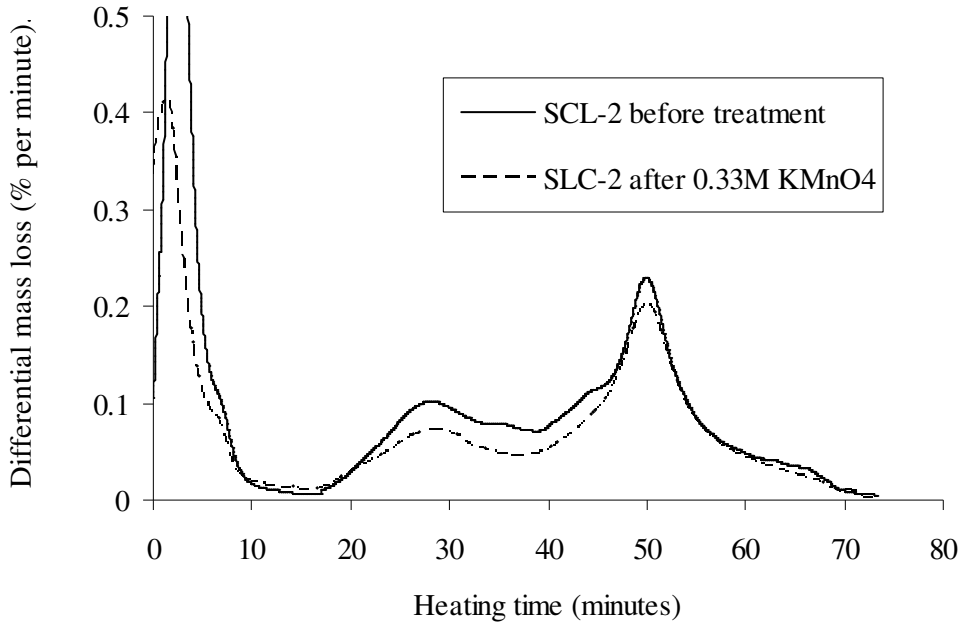

Figure 6-6 Thermogram of SCL-2 showing the effect of $KMnO_4$ treatment on both MOM and OMC.

Zimmermann (2007) used Differential scanning calorimetry to investigate (limited study) the effects of wet oxidation (6% NaOCl) and found that it did not produce any shift in SOM maxima (also not noted here) but did unmask the presence of BC. These chemical treatments may have introduced unexplained alteration effects, especially to the OMC, so the following experiments set out to analyse by TGA the settled (physically separated clay-sized) fractions along with their whole soils. The soils were Vertisols from the Narrabri area and also three loams varying in texture. As an example, the composite thermograms for the Lansdowne 2b sandy clay loam and its clay-sized fraction is shown on figure 6-7 to illustrate the dramatic increased mass events in the finer material. Such data for all the samples tested have been presented numerically on table 6-5. In this example the maxima for MOM is near 315 ^{o}C (whole soil) and 330 ^{o}C (clay fraction) but for OMC decreased from 532 to 513 ^{o}C for the settled fraction. The latter is probably a reflection of the increased amount of OM concurring with the mineral dehydration / dehydroxylation thus shifting the gross thermal maxima 20 ^{o}C lower.

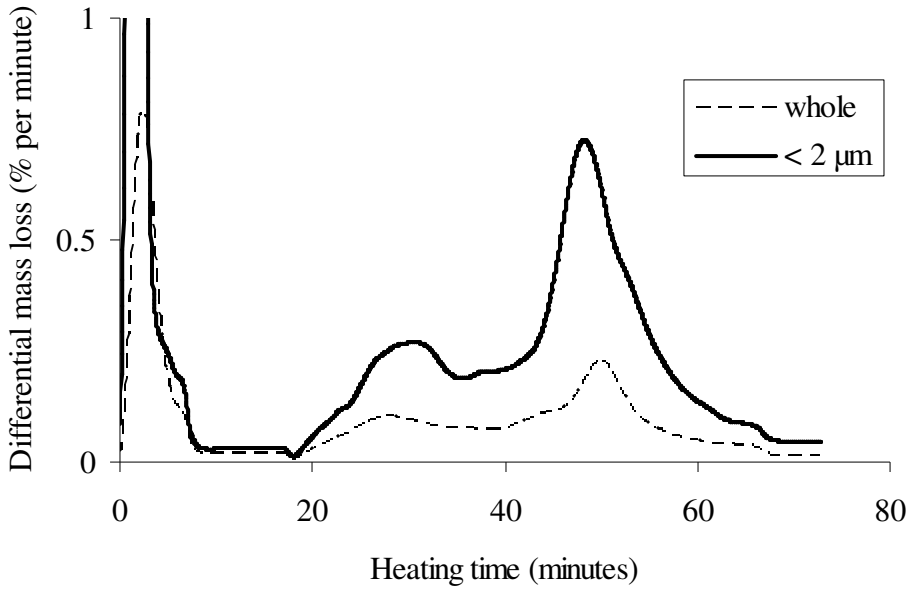

Figure 6-7 Illustration of the increased mass losses associated with the finer soil fraction (Sandy clay loam from 11-20 cm depth, table 6-5 Camden 2b).

An analysis of the relative increases after settling for the region (200 to 430 ^{o}C) associated with SOM indicated that the Vertisols and Lansdowne SCL generally doubled while two loams with lower clay content had reduced OM in the fine sediment. The combined organic and mineral (OMC) loss of mass was also more pronounced in the Vertisol clay fractions and generally exceeded that due to OM by around twofold. The data strongly implicate OM to have settled with the clay particles which included less resistant OM (indicated in the 200 to 430 ^{o}C temperature region) perhaps as a coating on minerals as suggested by [d]Bornemann (2011).

[d] Some positive correlations were found during spectral studies between POM-C and O-H vibrations (clay minerals) where it was inferred that OM may be coating minerals grains. Referenced from PhD Thesis by L.C. Bornemann, 2011)

Table 6-5 The mass losses from soils and their clay fractions at the discrete temperature regions and their relative changes.

Soil type / origin	Fraction	MOM %	Δ MOM	OMC %	Δ OMC
	(<2μm by settling)	200 to 430 °C		430 to 590 °C	
Camden 2b	whole	1.4		2.9	
Camden 2b	<2μm	3.7	2.3	8.8	5.9
Sydney Basin loam	whole	13.8		7.1	
Sydney Basin loam	<2μm	13.0	-0.8	10.2	3.1
Sydney Basin sandy loam	whole	15.8		6.3	
Sydney Basin sandy loam	<2μm	10.8	-5.0	11.6	5.3
Narrabri Vertisol 1a	whole	2.1		2.4	
Narrabri Vertisol 1a	<2μm	2.3	0.2	6.7	4.3
Narrabri Vertisol 1b	whole	0.9		1.5	
Narrabri Vertisol 1b	<2μm	2.0	1.1	6.5	5.0
Narrabri Vertisol 2a	whole	1.5		1.5	
Narrabri Vertisol 2a	<2μm	4.2	2.7	7.8	6.3
Narrabri Vertisol 2b	whole	1.4		2.2	
Narrabri Vertisol 2b	<2μm	3.0	1.6	7.2	5.0

Note: MOM and OMC refer to main organic matter and organic mineral composite respectively. It was also noted that carbonates were present (590 to 750 °C) in the loam soils but not in their clay fractions and was assumed to be related to its particle size.

Furthermore, when the differences (Δ % mass loss) for the two groups were plotted (figure 6-8), any increased mass in OMC due to settling was accompanied (linearly) by small increments of mass related to the MOM. The Sydney Basin sandy loam (excluded from chart) lay outside the trend set by all the other soils including a loam which also had a small negative Δ value but the reasons are unclear. That finer soils carry greater amounts of OM has been widely acknowledged (e.g. Spain et al., 1990; Wang et al., 2003) and is also consistent with the findings in chapter 2. However the generally correspondingly higher amounts of MOM with increasing OMC due to settling says something much more striking about the connection between the organic and mineral components (and how it is distributed).

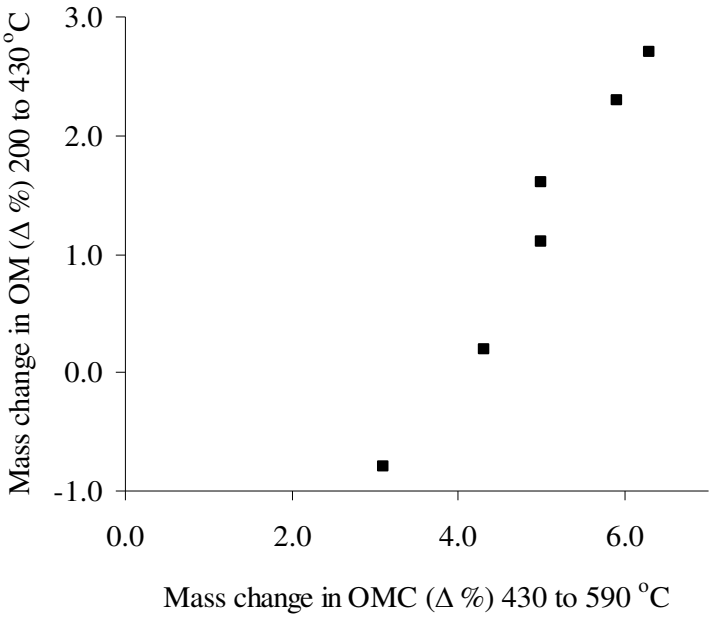

Figure 6-8 The relationship between ΔOMC (mineral) and ΔOM (organic) mass changes used to infer that OM settles with clay particles.

TGA / evolved gas analysis (EVA)

The results have left some of the important questions unanswered because these have all been mass-change studies and cannot tell us specifically about the C events over the heating cycle. That is, were the increases observed in the fine material due to more resistant OM and how much of the more resistant OM coincided with the OMC in the range (430 to 590 °C)? Also, during treatments, the TGA characteristics may have become altered – another important reason to seek out a simultaneous C-only signal. To this end a combined TGA / evolved gas analysis (EVA) method was investigated to determine whether C may be observed in the thermal regions related to the OMC. Initially TGA/FTIR was tested for another underlying reason: to try and obtain more information about the C chemistry such as dominant functional groups corresponding with OM and OMC thermal regions. The TGA in this case was a TA 2090 model.

Soils were heated at the same rates as in all the other studies only that here ultra pure N_2 was used to purge decomposition products from the reaction cell to the analytical instrument (FT-MIR). Inert gas was used because the object was not to oxidise the OM but to release meaningful OM fragments to the detection system as the mass changes were being recorded from the TGA. Such approaches have been used to deconstruct chemical building blocks and to fingerprint pyrolysis reactions (e.g. Xie et al., 2001). The FTIR instrument output

resulted in a 3-D image that recorded the range (x-axis in frequency) of relative MIR absorbances (y-axis) as the soil was heated (z-axis on time / temperature scale). Figure 6-9 presents an example of a FT-MIR absorption spectra after TGA heating of a clay loam. However despite the use of ultra pure carrier N_2, the amount of functional group information was limited to that for CO_2 and water molecules evolved from the heated soils. Water was evident by the broad absorbance around 2000 and again at 3900 cm^{-1} but that from mineral lattices is mostly obscured by carbonyl, the major absorbance at 2360 and 669 cm^{-1}. The reason for the dominant carbonyl absorbance is that as SOM is heated carboxyl and methyl groups are thermally broken away from the OM molecules. Decarboxylation and demethoxylation have been noted as significant reactions during the thermal decomposition of SOM (Miyazawa et al., 2000) and from important SOM constituents such as organic acids (Smith et al., 1998). Therefore monitoring the FT-MIR absorbances of the gases from controlled heating of SOM under reduced conditions (anoxic) had no particular advantages in terms of differentiable functional group information over aerobic experiments. The same was found for earlier MS experiments (chapter 2) where the major ions reflected CO_2 and water, consistent with the findings of Dell'Abate et al. (2003).

It was of concern that the CO_2 from decarboxylation of OM might not reflect the true pattern of decomposition for the various thermal regions. To minimise these unwanted effects such as re-deposition of gaseous C species (Amutio et al., 2012) leading to possible distortion (which may differ from soil to soil) it was advisable to use aerobic purging thereafter although, according to Cihlar and Kucerik (2010) the phenomenon may still be present.

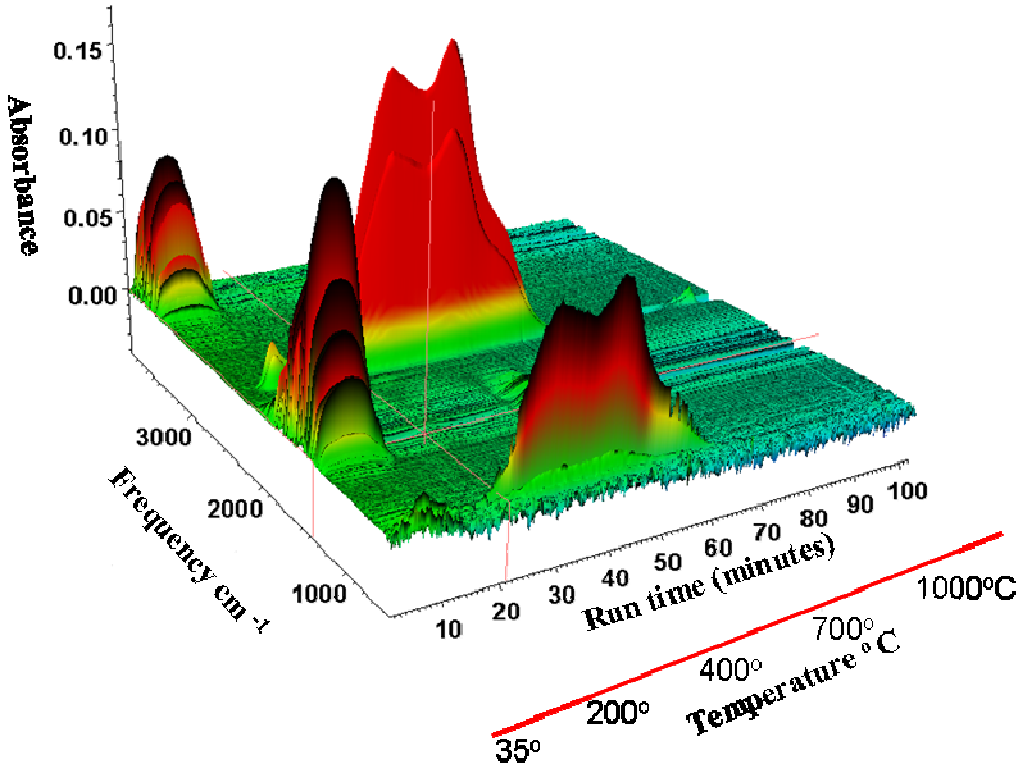

Figure 6-9 Three dimensional FT-MIR output of a Dermosol from a mixed farming area (15-30 cm depth) heated by TGA at 10 ^{o}C min^{-1}.

Mass change differences have been observed between related soils but how might these be due to OM rather than the mineral content. The objective in using the FTIR signal here was to test and compare the output of several soils using the carbonyl functional group as a proxy for C to show the relative proportion of C associated with the MOM and OMC events and that the mass losses for the latter were not just due to clay mineral decomposition. The prominent 2360 cm^{-1} signals acquired as part of 3-d outputs (above) were extracted (time / temp on x-axis) for ready comparison between soil fractions. Two sets of plots with both DTG and MIR outputs have been presented below: the fist of a clay loam with its settled (<2µm) fraction (figure 6-10) and a Dermosol sampled from 0-5 and 15-30cm depths (combined on figure 6-11).

Soil thermograms typically have maxima for the MOM and OMC around 320 and 530 ^{o}C respectively but these FT-MIR experiments showed some deviations. Firstly, the surface Dermosol and whole clay loam had FT-MIR signal patterns that were fairly amorphous resembling a continuum of organic material from the MOM to OMC (red solid traces on figures 6-10 and 6-11). A small amount of carbonate mineral was present in both of these soils which have been used as a time marker to confirm the signals (DTG and FT-MIR) both

were comparable on a time / temperature basis and that no significant lag was noted. In contrast, the deeper Dermosol and clay-sized fraction from the clay loam were more resolved (two small discrete maxima) consistent with MOM and OMC in their DTGs (see dashed red traces in same figures). The strong OMC seen by TGA (<2µm clay loam) due to increased mineral content was relatively lower according to the 'C' plot. Nonetheless both these samples indicated significant amounts of C (seen by the carbonyl absorbance) associated with the secondary TGA (OMC) event. There have been some general similarities with these patterns of C release (whole / surface soil compared to their deeper / fine counterparts) with those reported by Beyer et al. (1998).

In addition, FT-MIR revealed small amounts of carbonaceous-sourced volatiles released from below 200 °C not evident by TGA where it has been masked by free water loss. The FT-MIR signal for the fine fraction was somewhat delayed, perhaps an experimental artefact due to attenuation rather than any relative mass differences (note that evolved gas is likely to expand and slow after entering the IR cell).

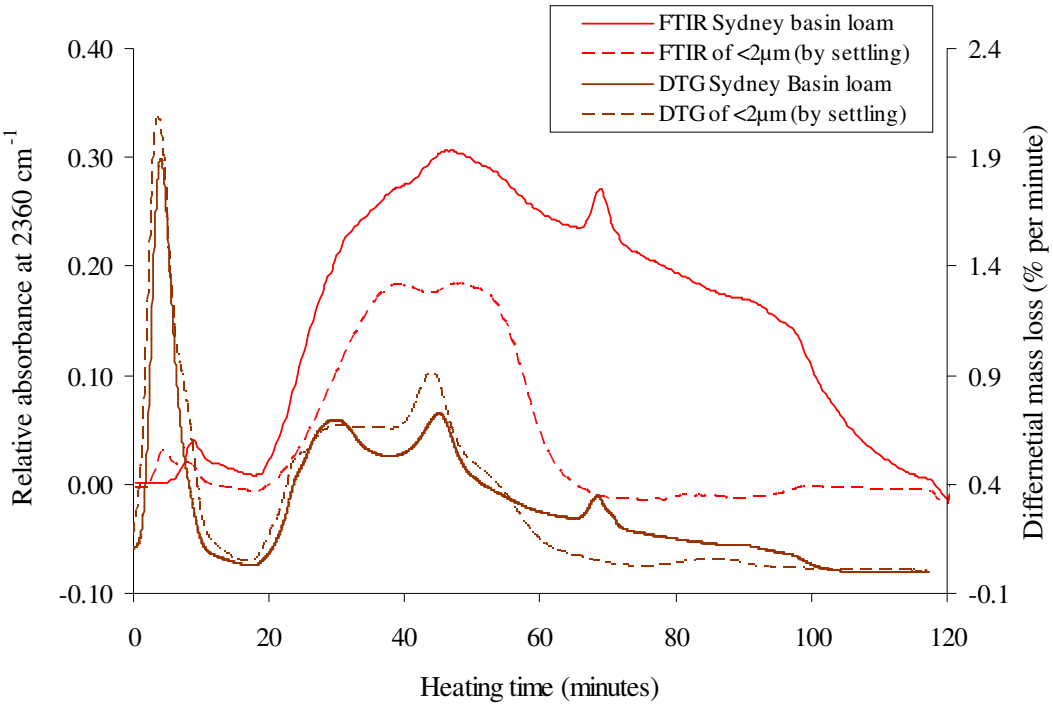

Figure 6-10 TGA / FTIR analyses of a Sydney basin loam (carbonate amended) sampled from 0-5 cm (whole soil as solid line) and its (<2µm) settled fraction (broken line). The FTIR signal recorded CO_2 at 2360 cm^{-1} (in red) while the simultaneously recorded differential mass losses (DTGs) are presented for the two fractions (in brown).

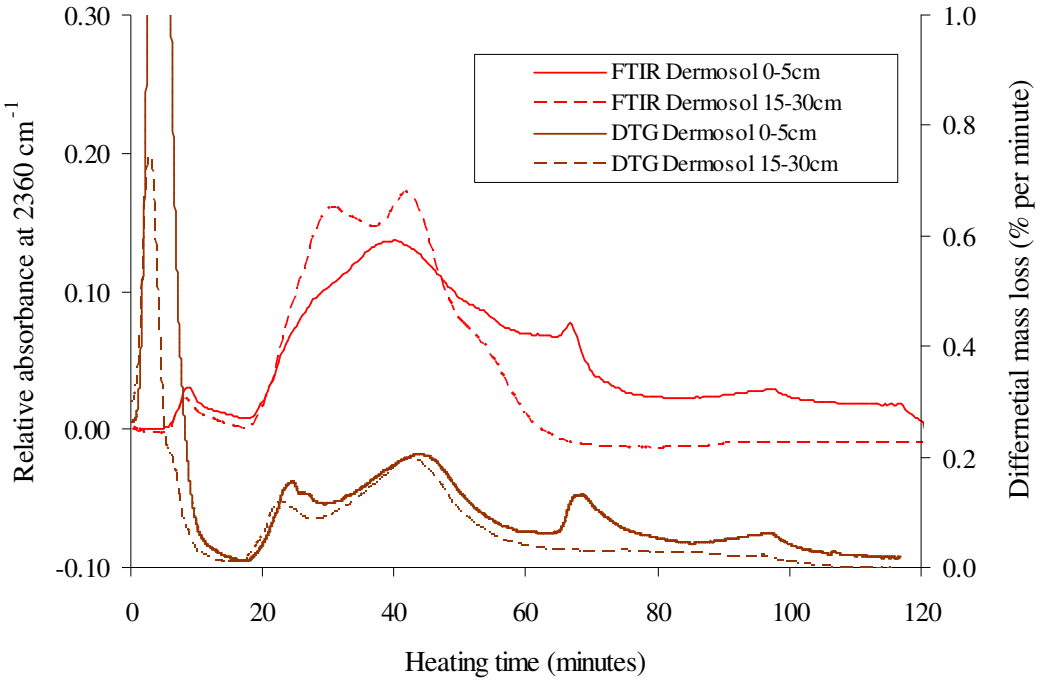

Figure 6-11 TGA / FTIR analyses of a Dermosol (under mixed farming) sampled from 0-5 cm (solid line) and 15-30 cm (dashed line). The FTIR signal for the two depths have been recorded by their CO_2 (2360 cm^{-1}) outputs (in red) and the simultaneously recorded differential mass losses (DTGs) are presented for the two depths (in brown).

Several significant observations could be drawn from these experiments:

- small quantities of carbonaceous material were volatilised below 200 ^{o}C,
- important amounts of C were released concurrent with the OMC comparable with that for the MOM event and
- both the deeper (15-30 cm) and finer (<2μm) soil material which is expected to host the older SOM, was more resolved than the surface (0-5cm) and whole soil.

Experimental artefacts or soil characteristics?

However, before any rigorous interpretive study into patterns associated with 'older' SOM be undertaken it needs to be established that artefacts associated with attenuation (quite possible due to gas diffusion in FTIR cell) or poor catalysis do not contribute to the final instrument outputs. Overcoming attenuation (tighter responses) but more importantly dealing with poor catalysis (lack of instantaneous oxidation) would potentially enable a kinetic mapping of SOM distribution and be quite useful in the study of different carbon contents, soil types, depths (ages) and grain sizes (HUM). It should also assist in the determination of more resistant OM in soils, such as BC. Specifically it should be followed up how much intermediate reactions (preferential volatilisation of parts of OM at lower

temperature) followed by final oxidation as described by Cihlar and Kucerik (2010) affect TGA (and EVA) signals and ways of solving this. This type of problem can be graphically described by the changes occurring to SOM during wildfires where the amount of oxygen can become limited due to insulation (analogous to poor oxidation during TGA). In the following observations by De la Rosa et al. (2008) using Differential scanning calorimetry, the plot shows how partial combustion results in loss of labile OM at 350 °C but adds to more resistant OM at 550 °C (where U-DSC is for the unburnt SOM and B-DSC after the fire event on figure 6-12).

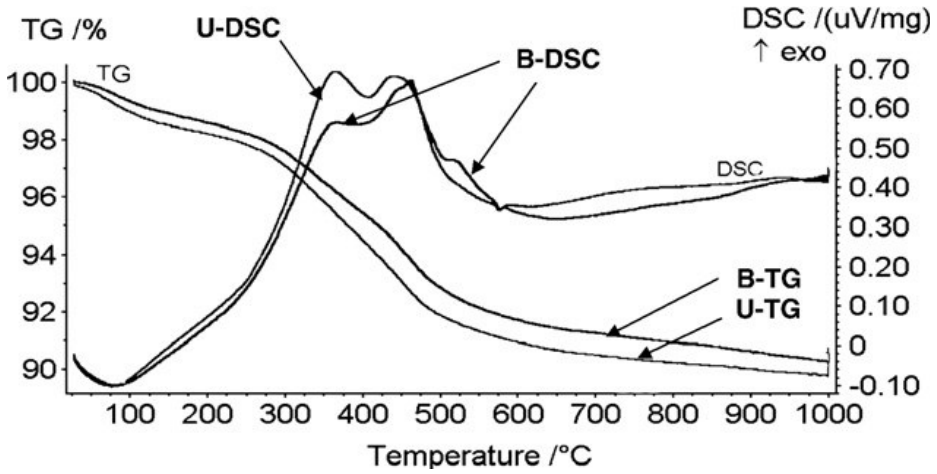

Figure 6-12 Differential scanning calorimetry of pre- and post-wildfire soil OM reproduced from De la Rosa et al. (2008).

Leifeld (2007) similarly demonstrated the effects of charring on cellulose and how these alter (increase) onset temperatures of a proportion of the material as indicated by comparative DSC curves. Differences in the stability between BC types were also noted thus enabling their thermal differentiation. It was inferred that oxidative thermal stability increased with bond energies and the degree of molecular order hence higher temperatures were indicated for aromatic materials and in the case of graphite (which is highly ordered) 700 °C.

Clarification about the quantitative oxidation of OM on heating under air may explain why the LECO CR12 has not been taken up to a greater extent as it showed considerable potential in differentiating OM types (described in literature review).

Conclusions

Black carbon and $CaCO_3$ are determinable from SOM by thermal analysis methods making it a potentially powerful tool to unravel one or more soil C pools. SOM pools (e.g. POC, HUM) may be more difficult to separate as indicated by Ea on simple 'model' materials (cellulose and lignin) but can be characterised by their individual SOM continuum. Thermal analysis of SOM could benefit from further fine tuning of experimental conditions (including quantitative oxidation) but particularly devising a better means of obtaining a pure C signal to separate mass and OM based events. The latter should provide better characterisation / distinction of SOM thermal events and possibly enable fairly reliable C determinations. This should help resolve some of the mystery surrounding the OM as a continuum with the mineral event (rather than discrete pools). This may lead to comprehensive studies that look at many more soils and environments and provide a foundation for a simpler and accessible determination to support rapid spectral methods.

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ATTACHMENT - Further observations during TGA studies

During the course of completing thermal analysis work on the numerous soils and organic materials throughout the experimental program some other potentially useful observations were noted and these have been summarised here.

Soil C pool-structure by TGA and stable carbon isotopes

CaCO3 is an important consideration during determination of soil C surveys given its widespread occurrence. Its presence may be indicated by high soil pH values (>8 and sometimes a little lower) however determination of organic C or carbonate itself usually requires acid treatment and repeated washing in between two C analyses. Such exercises are labour intensive and there is always some risk of altering the OM through the acidification and repeated washing steps. Thermogravimetric analysis (TGA) was used as a more elegant way to determine the relative amounts of inorganic C (chapters 2 and 6) in soils as it was a direct approach, without the need for pre-treatment and possible losses. In testing this simpler TGA method for measuring the relative amounts of carbonate to SOM when determining soil C stocks, a complementary set of stable isotope determinations were completed on a small number of soils also.

Materials and Methods used here

A limited set of carbonatic soils (mostly Dermosols from central NSW) were finely ground for analysis and oven dried (40 ^{o}C). TGA was conducted with a TA Instruments 2950 using the same methods described in earlier sections. Stable carbon isotope compositions were determined using a Delta V (Thermo Finnigan) continuous flow isotope ratio mass spectrometer (IRMS) interfaced via capillary to an elemental analyser upstream which was used to effect the conversion / decomposition of SOM and carbonate. Catalysts such as CuO or VaP_2O_5, as sometimes incorporated, were not employed since oxygen gas was present and furthermore is not expected to alter the carbonate-decomposition equilibrium. Results were expressed in the usual stable isotope notation (δ) as parts per thousand (‰) relative to the international standard, Pee Dee Belemnite Limestone (PDB) accordingly:

$$\delta^{13}C\ (‰) = \frac{(^{13}C/^{12}C\ \text{sample} - {}^{13}C/^{12}C\ \text{reference})}{^{13}C/^{12}C\ \text{reference}} \times 1000$$

The precision for isotope analyses are within 0.2‰ to 0.5‰. Note the absolute ^{13}C abundance relative to ^{12}C of most planetary C is around 1.1 atom %.

Some brief background on stable isotopes

Isotopic fractionation is well known in biological systems. This is related to the *kinetic isotope effect* where reaction rates are governed by the physical mass of respective isotopes and their bond energies (activation energy). This has consequences for physical processes like diffusion as well as biological metabolism such as microbial alteration of lipids, in the latter case resulting in relative ^{13}C depletion (Hoefs, 1973) of the respired product. These processes are also responsible for producing small differences in isotope composition of cellular components such as proteins, lipids and carbohydrates but isotopic fractionation also accompanies the initial stages involved in photosynthesis, the extent of which depends on the metabolic cycles used by the plant. Two primary cycles (known as C3 and C4) have evolved in the plant kingdom which produces a distinctly different range of isotopic compositions in their respective biomasses. These differences can be well employed when studying SOM turnover and is the usual method by which rates are determined based on simple mixing models. However they require a gross change (re-planting crop) in vegetation type and studies are long term (decades). The final isotopic composition of SOM is the product of the plant source material (determined by its metabolic pathway) overprinted by any microbial alteration effects. Models may become complicated when vegetation from the two metabolic groups coexist (have coexisted) in the same district. On the other hand, limestones (primary $CaCO_3$) have stable carbon isotope values that cover a small, relatively enriched range from around zero ‰ to – 6 ‰ (PDB) depending on when and where the carbonate was deposited. These are expected to be fairly uniform for a particular region (pedogenic carbonates not considered) and are isotopically distinguishable from any type of organic matter.

Results[d]

This set of experimental soils was used to test the reliability of determining C pools (i.e. the proportion of SOM and carbonate) by TGA along with the stable C isotope signature of the whole soil. The stable isotope compositions ($\delta^{13}C$, ‰) and the relative amounts of organic C (stocks) and of total obtained for these soils have been summarised on table A-1 and two contrasting thermograms from this set have been combined and presented on figure A-1.

[d] These preliminary results were presented at the 11th Australasian Environmental Isotope / 4th Hydrogeology Research Conference in Cairns (July, 2011).

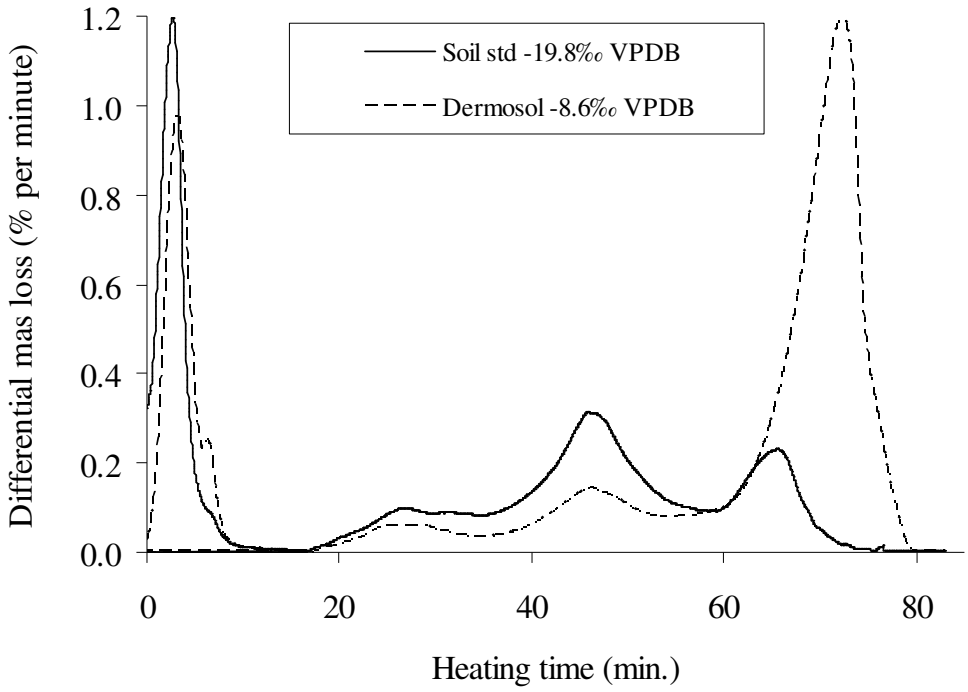

Figure A-1 Thermogram of two soils with clearly different proportions of SOM (28 mins.) and carbonate mineral content (60 – 70 mins.) reflected in their overall stable C isotope compositions.

The data from Dalal et al. (2011) re-plotted in figure A-2, reflect the increasing C stocks due to OM accumulation after wheat cropping (12 management practices) over what was previously C4 grassland. The $\delta^{13}C$ for our study (where the organic C from a number of localities ranged from 4.3 to 35 Mg ha^{-1}) related very poorly because of the influence of carbonate on the isotopic composition. This highlighted the limitations of using isotope data (not acid pre-treated) to study turnover dynamic where calcareous material is present.

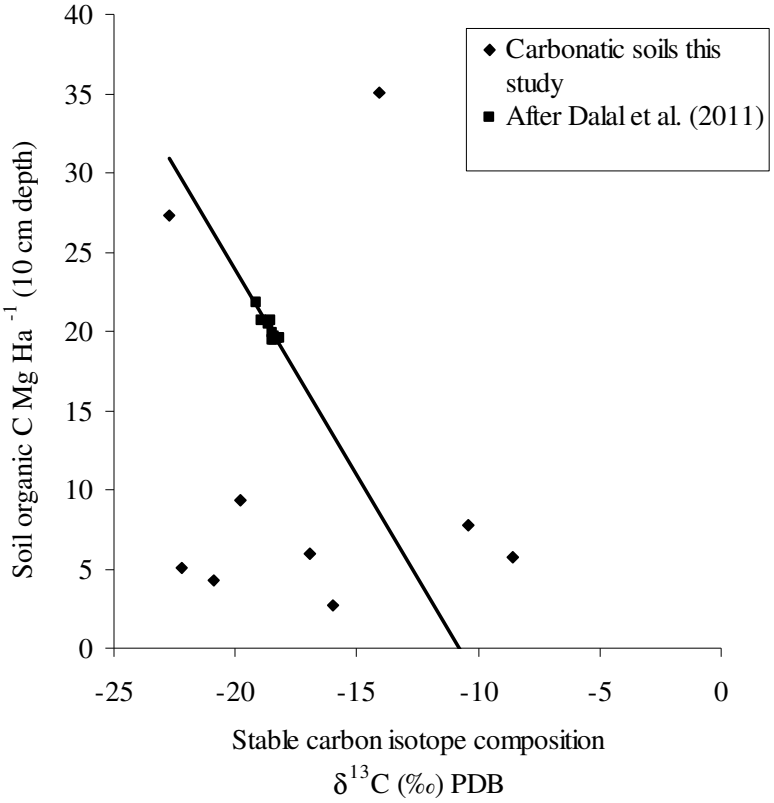

Figure A-2 Plotting the 9 carbonatic soils together with those from a stable carbon isotope study of Vertisols after changes to cropping (replotted from Dalal et al., 2011).

Table A-1 The proportion of the soils inorganic C pool and the $\delta^{13}C$ isotope composition of the whole soil.

Soil Analysed	Organic C stocks Mg ha^{-1}	Proportion organic C % of total	Total soil analysis $\delta^{13}C$ (‰)
Dermosol-4	5.7	14.5	-8.6
Chromosol-1	7.8	21.4	-10.4
Clay soil	2.7	38.2	-16.0
Sandy Calcarasol	35.1	38.6	-14.1
Soil standard	9.4	58.5	-19.8
Dermosol-2	6.0	60.5	-16.9
Dermosol-1	27.3	76.6	-22.7
Dermosol-3	5.1	86.7	-22.2
Chromosol-2	4.3	90.4	-20.9

After the compositions were replotted however, on the basis of the proportion of Mg organic C ha^{-1} / total C ha^{-1} (table A-1) the points become linearised (figure A-2). These produced a similar trend against isotope ratio (whole soils) albeit in terms of their relative organic C contents but appear to reflect the variable contribution from C3 dominant vegetation adding

to total SOM. The sum contributions towards the whole stable isotope composition were from two distinct sources, in this case C3 SOM and $CaCO_3$. What this indicated is that the presence of a carbonate isotopic signature could be used to advantage when monitoring SOM accumulation rates (e.g. during sequestration) while still maintaining the same (C3 or C4) cropping regime.

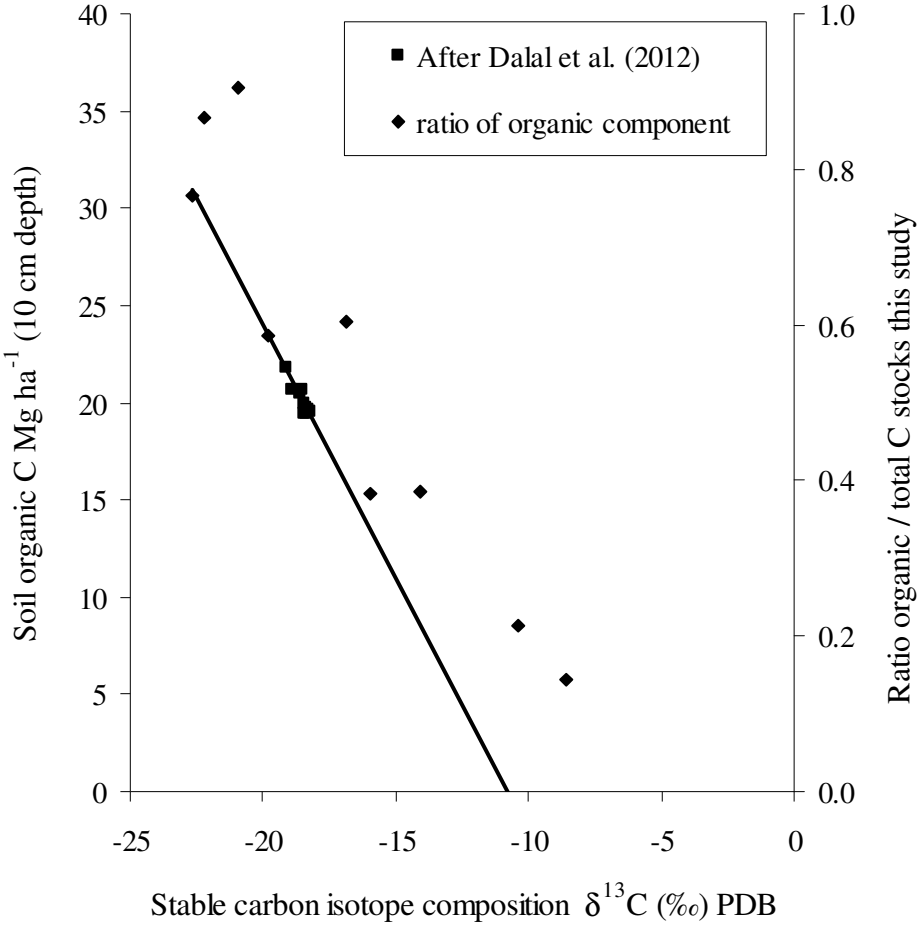

Figure A-3 The same 9 carbonatic soils replotted but as their proportion of organic C over total (equivalent to % of total) against their stable C isotope composition. This trend reflects dominance of C3 plants over these soils whereas a theoretical trend due to dominantly C4 plants would rise more steeply from the x-axis.

Conclusions and further work

The stable isotope compositions of soils (not treated in any way other than ground up) reflected the imprint of both inorganic and organic C present depending on their relative proportions (as determined in these cases by TGA). In studying C sequestration / land management methods, the ability to readily quantify both total soil C and the balance of organic to inorganic C pools quickly and robustly, is fairly important. In field applications

of this method, CO_3 and organic C contents in soils could be similarly determined based on a knowledge of end member $\delta^{13}C$ composition. The ultimate aim may be to develop this to an extent where SOM / carbonate information can be obtained from single stable C isotope analyses. The method can potentially inform on changes in relative SOM abundance due to C management or the use of amendment of crushed lime ($CaCO_3$) which may also respond to changing pH.

Possible uses for this method include:

- mapping calcareous terrain / carbonate distribution in soils,
- quantifying increased SOM stocks based on single analyses where soils are carbonatic or amended but also where vegetation types are mixed,
- detecting addition of $CaCO_3$ where SOM sequestration practices are being tested,
- detecting loss of C from the inorganic pool where decreases in pH result from management.

SOM stabilisation by earth and compost worm activity

In the course of conducting TGA experiments on SOM samples, other organic materials used for soils were also included. Worms are well known for their soil improvement and nutrient concentration capacities where they accelerate the breakdown of OM to a more nutrient balanced residue (see C/N table A-2). These take place through physical working of the ground (restructure, aeration) but also more effective SOM stabilisation than composting (Lazcano et al., 2008). Both mineral and organic matter passes through the gastro-intestinal tract with the involvement of enteric microbes modifying these substrates which also include denitrifying bacteria implicated in unwanted N_2O production (Lubbers et al., 2013). Sand grains are thought to assist in the physical grinding process also. However, strong parallels may exist between internal worm activity and external humification processes that give rise to OMC.

Results

Two composts and a vermicompost analysed both elementally and by TGA analyses have been presented on table A-2 and figure A-4 respectively. A second garden compost from the same batch had a lower C content indicating some heterogeneity but interestingly the C/N relationship was maintained. These samples were only air dried and have retained reasonable amounts of water (peaks prior to 15 mins.) Organic matter residues from commercial and backyard composts (very little mineral-related signal) were marked by principal mass

changes in the region 230 to 330 °C (20 to 30 mins. heating time) and seem to be representative of typical starting materials (lignocellulose, other carbohydrates). However the Vermicast fed on similar organic materials (kitchen waste as well as ground coffee residues, I. Wheeler, pers. comm.) also showed a smaller signal (a remnant or product?) in this region 20 – 35 minutes which is usually associated with the SOM continuum during TGA. The main mass change event for the vermicompost occurred at a much higher temperature 480 to 510 °C than found in the typical food sources (the composts) and indicates increased thermal stability (even higher than the biochars analysed/discussed in the earlier sections). This main peak for the worm casting was due to a fairly sharp mass change and therefore probably chemically fairly homogenous.

Table A-2 Carbon and nitrogen analyses of compost and vermicompost.

Organic matter type	%C	%N	C/N
Commercial compost	14.2	0.99	14.3
Garden compost	3.1	0.21	14.8
Garden compost rpt	0.7	0.05	14.2
Vermicompost	6.8	0.77	8.8

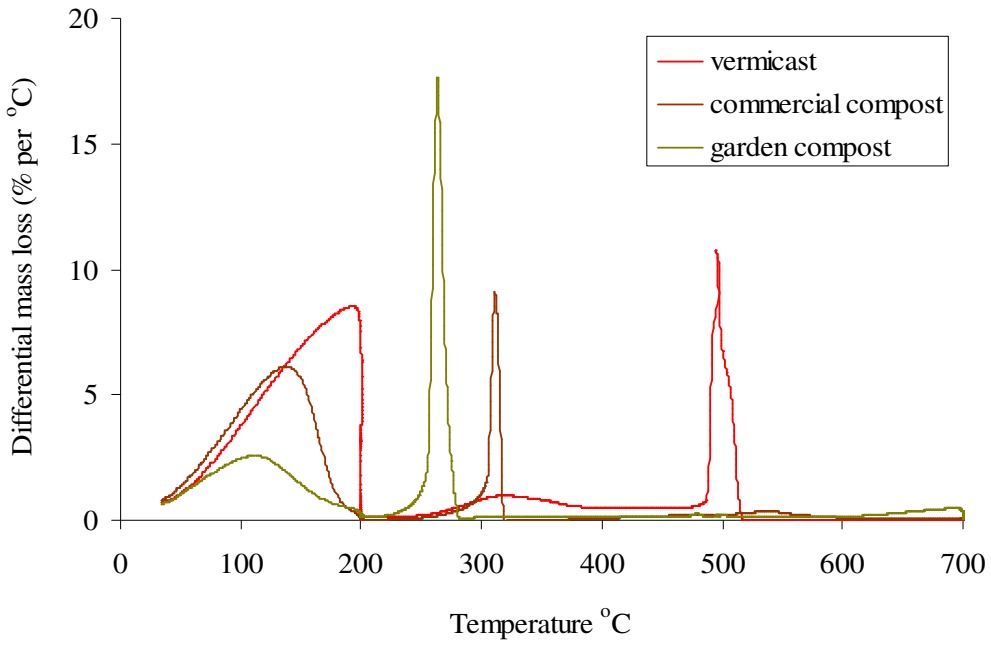

Figure A-4 TGA analyses of a Vermicast and composted OM.

Further work

These TGA analyses indicated evidence of greater stabilisation seen by kinetic characteristics and while this is based on one example only, the seeming lack of work reported along these lines would indicate a fertile area for further investigation. It is suggested that monitoring the starting materials over the weeks and months required to produce vermicast products by various analytical means (TGA, FTIR) may provide important insights on how SOM stabilisation proceeds. Monitoring the transformation of SOM by FTIR has been previously attempted using C-H / C-O ratios and how these might be affected by earthworm activity (Ellerbrock, 2009). Stable isotopes may be another useful tool in tracking changes since biological mediation is generally accompanied by fractionation.

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REVIEW OF STUDY OUTCOMES

The specific goals of this research were to advance the current tool kit that can be used in determining soil C, in particular total C stocks on the broader scale, as well as its C pool structure for its fundamental importance to understanding long term soil C sequestration.

The project enabled a new method, using the SCB apparatus, to determine C from whole cores to reduce the margin of error resulting from the natural C variability and capturing a reliable site-specific measure. The project also explored the importance of soil C fractions and its relevance to sequestration with further study into methods for the rapid analysis of one or more soil C pool/s. In particular was the application of the thermogravimetric analysis technique on SOM to advance our understanding of LOI approaches and how these C pools might be differentiated thermally.

Achievements:

- Critical analysis of the methods used across the sector and the basis of their use.
- An objective evaluation of the LOI method. Loss on ignition is a possible means to determine C stocks for larger quantities of soil and may be regarded as a viable option to current methods as well as the SCB. However these comparative thermal analytical studies indicated various restrictions associated with LOI but also its optimal use.
- Carbon analyses on large soil volumes were demonstrated by SCB.
- C stocks by analysis of whole cores were demonstrated using the SCB. Whole cores can now be reliably determined using the SCB where all C is extracted thermally under O_2 stream and quantified. Values can be directly reported on volumetric or gravimetric basis.
- Spectral calibration for black carbon based on hydrogen pyrolysis (HyPy). These analyses appeared to be an effective means to calibrate MIR soil spectra for black carbon determination in finely ground whole soils although considerable comprehensive calibration work is still required.
- Use of thermal analysis in determining the inert soil C pool. Thermal analysis has been demonstrated as an alternative method to determine black carbon and carbonates and could play a role in MIR calibration.
- Provided further inspiration for novel approaches to enquire into SOM and other pools using controlled heating methods.

Soil Carbon stocks

The SCB was a system specifically designed and developed to measure soil C from whole cores and large soil aliquots with the intent to lower sampling and analytical errors associated with the natural variability in soil C surveys. The concept was based on the quantitative extraction of all C from these soil volumes by a combustion method followed by analysis.

Carbon analyses from the SCB agreed well with those obtained using conventional methods based on calibration materials as well as soils. The sensitivity of this instrument was determined by the method detection limit (MDL) to be 0.085 g C. At the same time, the SCB has a large dynamic range allowing the analysis of soil masses from one to many hundreds of grams where the upper limit is controlled by the furnace volume rather than the detection system (Chapter 3).

It was shown that whole cores could be analysed successfully although benefits accrue from breaking and drying the core thoroughly which also enabled recent OM (roots) to be efficiently removed. Carbon stock data was determined over a trial area and results could be immediately expressed as g C per whole core interval (e.g. 50cm) but were readily transformed to mass coordinate units so that % C of total dry mass or kg m^{-2} was readily available (recording core masses was a routine part of acquisition). Analysing the entire soil C from the cores produced coherent data over the test field but with a lower variance (std. deviation = 0.97, interquartile range = 1.08 kg m^{-2}) when compared to values obtained by conventional analysis methods (std. deviation = 1.36, interquartile range = 2.71 kg m^{-2}). Total C values per core was within 0.2 g of their mean for each site where the totals ranged from 0.7 to 4.7 g C per 50 cm core for the sampled positions over the transect. On a gravimetric basis (the ranges were 0.2 to 1.09% C per 50 cm core). Based on these soils and the C compositions at the trial sites the reproducibility in C stocks were within 0.0003 kg/kg. Overall these results were extremely encouraging and prompt further testing. The relative costs of determining soil C stocks were estimated to be about 1/5 of conventional methods (based on the work to date and test site). This is owed largely to the cost savings in reduced processing time and the much lower running costs compared to the standard analytical instruments used (Chapter 4).

Demonstrated benefits of whole core analysis

Unequivocal C determinations derived from whole soil cores or sub-fractions (as averaged over space) are expected to provide a more accurate assessment of C management practices (over given time period) than point analyses which is more likely to reflect variable changes.

In addition, the determination of total soil C over an entire depth interval was able to readily yield volumetric C stocks as well as mass coordinate / gravimetric units which were all amenable to uptake by any IPCC accord (e.g. soil C stocks as TOC m^{-2} to 30cm). Rapid and cost effective acquisition of farm-scale C stocks should lead to an increased number of land holdings covered over the next five year period. This resultant enhanced knowledge of C distributions is expected to facilitate comprehensive soil C base maps and allow more effective assessment of the C management practices and relative impacts.

Areas for improvement to the SCB and recommendations for future work

The SCB has been demonstrated as an effective and viable means to determine C stocks on bulk amounts of soil or core. There are however several components that could be improved / modernised which fall into the following areas:

- Flow control devices for smoother, regulated gas rates with possible improvements to reproducibility
- Increase the efficiency of the catalyst to minimise CO production
- Automation of the sampling apparatus so soils can be introduced to heated areas without handling after initial loading
- Redesign / build sensor and electronics and reduce size where possible
- Retrofitting heat control device/s for more accurate heat setting

These design changes (e.g. lengthening the catalyst) would be expected to deliver small increases to the precision of analysis but importantly add to safety and efficiency. Increasing the catalytic function should further lower CO levels and allow high concentrations of C to be analysed (e.g. composts, biochars) possibly using a secondary heat supply. The rate of gas flow is crucial to measurement and has to date been achieved with rotameter and bubble meter technology but currently available metering methods (electronic flow controllers) may provide advantages and should be investigated. Along with the renewal of electronic components these should result in an improved baseline and quantitative determination of C from any core mass.

The SCB forms a template that could be applied to larger sample sizes (if required) using the same principles and modifications incorporating some of these further developments listed. Stainless steel may be another good option to cope with robust handing should such a system be deployed commercially or on-farm. Attention to the choice of materials is an additional consideration when using pure oxygen streams. Similarly automation of modules such as sample loading and furnace actuation should be explored appropriately as high soil throughput becomes routine.

Further application and testing of the SCB comprises the next phase of development and should evaluate its performance over different soil types and land uses (range of natural C variability). This can and should be conducted in combination with sampling schemes: systematic sampling (grid based); stratified random sampling; spatial probability sampling (on stratified areas) to test optimal sampling densities. These further applications should also test C management strategies and the minimum limits of sensitivity to change detectable by the SCB.

Soil Carbon Pools

Thermal analysis as separation tool

Methods were investigated for the determination of important C pools but in particular BC, which is important in the Australian context because of the historical prevalence of wildfire in this soil ecosystem. This form of organic C tends to be more stable in the soil hence its accumulation and persistence. The capacity to readily quantify this component would enable more immediate availability of such valuable information for soil C sequestration studies.

HyPy was tested as one alternative method to calibrate MIR soil spectra albeit, using a small number of soils but its potential was indicated by comparison with an established BC model (Chapter 5). Synthesised biochar blends were also studied spectroscopically to demonstrate that MIR could be used to directly determine addition rates in amendments (e.g. where indiscriminately applied to soils) independent of the BC content (i.e. mass % aryl C).

Many of the studies of recent years have pursued the pure chemistry and biology of SOM whether through fractioning and analysis or sophisticated imaging. A fresh look at differences in OM on an energy basis, as thermal methods (TGA and DSC) offer, may yield more practical information not only for pools but mechanistic aspects of humification or SOM stabilisation processes. Considerable time was invested in thermogravimetric analysis techniques as a means of separating the four major soil C groups (labile OM, humified OM, inert C, and carbonates). It was found that TGA can be used to successfully determine BC and carbonates but is still underdeveloped with regard to differentiating and characterising SOM (i.e. labile and humified OM). This may be largely because part of SOM is associated with some of the mineral component and when thermal decomposition proceeds this can lead to conflicting instrument responses with unclear results. This is the case irrespective whether using TGA or DSC. However these studies did provide important information for the more effective use of LOI techniques, a possible method considered to tackle broad scale

C stock determination. For example it indicated the restrictions using LOI for soils with finer grainsizes where C overestimation can commonly result at the higher temperatures (550 ^{o}C).

Conversely the correlation between thermogravimetric data associated with the oxidation of BC and results obtained from HyPy (chapter 5) strongly suggested that as an analytical tool, thermal analysis could be used for this purpose i.e., pure determination of BC (chapter 6) as well as for the calibration of spectral techniques.

Suggested further work on thermal analysis

It is anticipated that much of the ambiguities related to differentiating and attributing mass changes (e.g. due to overlapping events) can be overcome by obtaining a pure C signal as when using the more costly techniques (TGA/MS and TGA/FTIR). Additional work is expected to yield a valuable method to characterise or perhaps even separate and determine SOM types. Attempts to do this have been held back by the lack of a cost effective and direct means to acquire a concurrent C signal in order to de-convolute mass change events during heating. A more direct and cost effective means needs to be devised which would also aid BC determination and overcome some of the analytical issues associated with pure TGA or DSC analyses. Such a continuous C signal could then be easily implemented in conjunction with DTG curves to monitor both mass changes and real-time C evolution through two simultaneous signals. Related to this could also be the direct coupling of TGA to a GC to obtain C/N analyses at different stages (related to pools) of heating cycle. It is proposed some potential applications that may follow on are i) stability characteristics of SOM and to try to devise a humification index (HI) based on this and ii) study compost and vermicompost SOM stabilisation processes possibly also in conjunction with gas analyses.

Poor catalysis / oxidation has been identified as a potential issue during TGA analysis and may have diminished the information obtained through coupled FTIR (Chapter 6). This aspect should be studied in more detail which may then lead to more precise methods of TGA analysis. Another way of observing differences in functional groups for MOM, OMC etc. over a heating program (as used in TGA) would be to monitor the soil's emissions spectra. This is more likely to indicate certain functional groups (aliphatic, aromatic) and their temperature of disappearance. This does not seem to have been applied to the characterisation of SOM and may remove interference from decarboxylation of OM.

Recommended further work on Spectral calibration for BC

Spectroscopic analysis for BC using HyPy in the calibration process was limited with regard to the number of soils and the resultant predictive model. It is suggested that expansion of this approach (possibly in collaboration with JCU and CSIRO) built on a database of critical size (200 finely ground soils) should produce a much improved independent and robust prediction model. Performance comparisons could then be competently made against established models (e.g. CSIRO).

Similarly, the potential for thermal analyses to be used in this calibration process (ground truth) should be fully tested. However, before such applications can be carried out the instrument development / refinement work discussed above is strongly recommended.