THE USE OF A RAPID INCINERATION FIELD TEST FOR DETERMINING SOIL ORGANIC CARBON IN THE SOUTHERN CAPE REGION

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THE USE OF A RAPID INCINERATION FIELD TEST FOR DETERMINING SOIL ORGANIC CARBON IN THE SOUTHERN CAPE REGION

By

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DECLARATION

I, Albert Arthur Ackhurst, student number: 211251038, hereby declare that the dissertation for Master of Technology: Agricultural Sciences is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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Signature

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ABSTRACT

Knowledge of soil organic carbon levels is important both for agricultural effectiveness and soil carbon sequestration accounting, especially against the backdrop of increased climate change impacts and pressure on food production landscapes. However, current methods for soil carbon determination are expensive, energy intensive, time consuming and potentially hazardous leading to a call for alternative methods, which should be cheap, fast, simple, accurate, safe and usable where resources and soil analysis laboratories are limited. To this end the student invented a novel rapid incineration field test (RIFT) for determining soil organic carbon and tested its validity in this study. This method incorporates principles found in dry combustion as well as loss-on-ignition and quantifying organic carbon through gravimetric analysis.

In order to illustrate effectiveness and accuracy it was necessary to correlate RIFT with a reference method, in this instance dry combustion with a Leco device as well as another commonly used indirect method namely the Walkley-Black wet chemical oxidation method. Samples from eleven soil forms were collected from the Southern Cape region and they were subjected to the three testing methods. It was found that RIFT is indeed as effective and in 72% of the soil forms even more effective than Walkley-Black. Furthermore it was ascertained whether the accuracy of RIFT can be improved by correcting for clay content. The correlation of RIFT with clay % was not very significant and clay % as a variable was therefore not used in this study to obtain further refinement of RIFT predictions. Another finding was also that RIFT illustrated less variability than both the Leco and Walkley-Black methods. Lastly it was ascertained that the RIFT device and methodology is indeed cost effective, energy efficient, fast and safe in terms of the need to use potentially hazardous chemicals.

Key Words: Soil organic carbon, Rapid Incineration Field Test, dry combustion, loss-on-ignition, wet chemical oxidation, southern Cape, correction matrix tool, cost effective.

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CHAPTER 1 INTRODUCTION

1.1. Background

Healthy soil is considered pivotal in the sustainability of food and agricultural systems (Bot & Benites, 2005; Chan, 2008). However, researchers agree that soils have many uses (Brady & Weil, 2008; Kettler, 2011) ranging from plant growth medium for food, fuel and fibres, the regulation, flow and filtering of water resources, providing habitat for soil biodiversity, recycling of atmospheric gases as well as natural materials, wastes and nutrients, to providing a substrate and raw materials for engineering and construction.

The organic matter portion of soils is considered valuable to agriculture as firstly a "revolving nutrient fund" wherein the stable organic fraction (humus) adsorbs and holds nutrients in a plant available form and secondly, as an agent to improve soil structure, hold water, maintain tilth and minimize erosion (Bot & Benites, 2005). The humus fraction is dark in colour and consists of non-humic and humic substances: it is relatively stable and has a major effect on various soil properties and processes (Du Preez *et al*., 2011). Nevertheless, organic matter is a complex mixture of substances, components of which are collectively referred to as soil organic matter (SOM) and include:

- Living biomass or intact organisms,
- Dead roots and other recognisable plant residues or detritus and,
- Non-living/non-tissue colloidal mixtures or humus.

However, Brady & Weil (2008) describe SOM through its component qualities being physical qualities, chemical qualities and biological qualities. The physical qualities encompass aggregate formation, increased porosity, aeration, increased water holding capacity and resilience. The chemical qualities of SOM serve as a source of N, P and S, can lead to increased cation exchange capacity (CEC) and act as a sink for carbon. The biological component of SOM provides a habitat for microbes, maintains soil biodiversity and contributes to soil resilience.

Since the element carbon (C) plays a prominent role in the chemical structure of all organic substances it is often referred to as Soil Organic Carbon (SOC) in respect to the SOM and in this regard SOM generally contains 50% C by weight (Brady & Weil, 2008). It is therefore imperative to understand and quantify the role of SOM and SOC in soil. According to the Soil Science Society of America (2013) there is a requirement for better SOC accounting methods that are scientifically defensible. Traditional methods of directly measuring organic soil C are generally slow and expensive. This led to a call for new techniques to quantify organic soil C in units suitable for C trading (Walcott *et al*., 2009; Chatterjee *et al*., 2009), and the new techniques would also be required to determine how it changes across a landscape. Conant *et al*. (2011) add that it should be rapid, accurate, and inexpensive in order to detect and quantify change in the ecosystem dynamics of C.

Farmers and producers may in future not only farm their soil judiciously but also farm with C (Konare *et al*., 2010) obtaining carbon credits for sequestering C in their soils. Soil is increasingly recognized for its role in ecosystem services such as food production and climate regulation and demand for up-to-date and relevant soil information is increasing (Sanchez *et al*., 2009) especially for finer scale soil maps for Africa and also to establish better ground truth infrastructure for soils in sub-Saharan Africa. According to the Intergovernmental Panel on Climate Change's (IPCC, 2007) fourth assessment report, emissions of CO_2 from fossil fuel use and from the effects of change in land use on plant and soil carbon are the primary sources of increased atmospheric CO_2.

In the South African context it has been surmised by Mills & Fey (2003) and Du Preez *et al*. (2011) that serious threats are posed to sustainable agriculture because of degradation of soil. This is on account of land use practices not only related to soil erosion but also to the qualitative value of soil and this, they believe, prompts the need for specific soil protection strategies and policies.

Soil degradation has historically raised some serious debate, and it is an important issue in the modern era (Lal, 1997). Furthermore climate change has emerged as one of the greatest challenges of our time. Fossil fuel burning and deforestation are principal anthropogenic sources of rising atmospheric CO_2 and other green-house gases and consequential global warming (Singh *et al.,* 2010).

Auerbach *et al*. (2013) recognize this and echo Brady & Weil (2008) in that the world's soil organic matter exceeds the worlds vegetation in terms of C by a factor of two to three times and that poor ploughing coupled with deforestation depletes soil carbon by about 2Pg (billion tonnes) per year and so contributes to elevated levels of atmospheric C and the associated changes in climate. However, well managed land use practices like for instance organic farming (Auerbach *et al*., 2013), soil management and enhancement through reduced tillage, mulching and composting as well as crop management such as crop rotation and agroforestry (Bronick & Lal, 2005), could reverse this depletion. Organic farming can be described as whole farm management, where feeding the soil feeds the plant and where optimum nutrient cycling is achieved through plant and animal management, enabling soil to sequester more C than is removed, thus helping agriculture to mitigate climate change (Auerbach *et al.,* 2013).

The IPCC's fourth assessment report (IPCC, 2007) also reiterates that the improvement of agricultural practices on carbon-depleted soils has created a carbon sink. They further report that since the introduction of conservation tillage in the USA, SOM stocks were increased by about 1.4 million tonnes C over the last 30 years.

Therefore increased C sequestration can help reverse the soil C depletion and decline in soil fertility and thereby contribute to food security. Approaches similar to the Clean Development Mechanism (CDM) under the Kyoto Protocol could provide opportunities for development aid based on activities in developing countries that sequester atmospheric C (McCarty *et al.,* 2010). However, for that to be a prospect soil carbon baseline values for whole regions (and countries) need to be undertaken. Du Preez *et al.* (2011), propose a South African countrywide baseline study to quantify organic matter contents within and between soil forms for future reference. In that regard McCarty *et al.* (2010) and Konare *et al.* (2010) found that loss on ignition (LOI) approaches can perform well for analysis of surface soils in areas such as the Sahel, provided that they are properly calibrated using elemental analysis (for instance dry combustion).

This study proposes the use of a new high temperature combustion (incineration) process based on some principles of both LOI and dry combustion and determining whether it could be useful for SOC baseline determinations at the country level. This could be useful especially in countries where the more costly and complex modern technologies are not readily available.

1.2. Statement of the problem

Although it is obvious that soil carbon content is important for both the sake of agricultural effectiveness and soil carbon sequestration accounting (Conant *et al*., 2011), it is also evident that current mechanisms of soil carbon determination are expensive, energy intensive, time consuming and in some cases constitute a pollution risk (Chatterjee *et al.*, 2009). There is also an increased requirement for methods that accurately measure soil C but which are also suitable for use by resource limited soil analysis laboratories (Konare *et al*., 2010; McCarty *et al*., 2010).

Traditional and current methods mostly entail destructive *ex-situ* and non-destructive *in-situ* techniques. The *ex-situ* destructive techniques centre around drying the collected sample and using heat to combust, or chemically treating the sample in order to determine the organic carbon content. This is done by quantifying that which was lost on ignition through sensitive combustion analysers, or evolved through intense heat or wet chemical oxidation like the Walkley-Black (WB) method which is one of the best known (Kimble *et al*., 2002; Konen *et al*., 2002; Rowell & Coetzee, 2003; Gehl & Rice, 2006 and McCarty *et al*., 2010).

The *in-situ* techniques are based on remote sensing imagery, inelastic neutron scattering (INS) and spectroscopic measurements like near infrared, mid infrared and laser-induced breakdown spectroscopy (LIBS) in the field (Gehl & Rice, 2006). A further distinction is that dry combustion is considered a direct elemental analysis whereas methods such as dichromate oxidation (e.g. WB), LOI, and diffused reflectance infrared spectroscopy are considered indirect methods (McCarty *et al*., 2010). Schumacher (2002) adds that as the complexity of these methods for the determination of organic carbon increases, the required level of operator skill and the accuracy of the technique escalate. He also reiterates the limiting issues around high cost, throughput rate, health risk and pollutants. Mikhailova *et al*. (2003) and Salehi *et al*. (2011) echo the high risk and pollution concerns related to the correct disposal of used chemicals during the wet oxidation procedures. Konare *et al*. (2010) claim that in Sub-Saharan Africa and developing countries WB and dry combustion techniques are not readily available because of a host of limitations. Some of these limitations include high cost of combustion analysers, lack of maintenance infrastructure, hazards posed by use of dangerous chemicals, and the need for highly skilled operators.

It therefore appears that new approaches to basic soil carbon determination are essential and that the method should be cheap, fast, simple, accurate and safe. This study investigates the potential of such a method and tests it with a rapid incineration device invented by the student as a prototype that responds to the need for rapid and affordable basic or routine soil carbon determinations.

To this end the student proposes the use of a novel rapid incineration field test (RIFT) for determining SOC. The RIFT method incorporates principles found in dry combustion and detection of C through automated analysis as well as LOI and quantifying C content through gravimetric analysis. Rapid incineration requires the direct application of intense heat, above 1000 °C, from a physical blue flame under pressure typically delivered through a butane torch lighter.

1.3. Objectives, hypothesis and key questions

The main objective of the study is to explore the RIFT as an alternative method for the determination of SOC. Subsidiary objectives include the following:

a) To test the RIFT alongside accepted methods like dry combustion and wet chemical oxidation;
b) To compare the accuracy of RIFT to the other methods statistically;
c) To investigate whether additional factors can be used to calibrate the RIFT in order to improve accuracy.

The hypothesis is that the RIFT technique for determining SOC levels is of sufficient accuracy and reliability to be used for routine soil analysis.

To validate the hypothesis the following key research questions need to be answered:

a) To what extent do the results of the RIFT and WB methods correlate with dry combustion (the reference method), and how accurate is RIFT in terms of SOC assessment compared to WB?
b) How variable are the three methods compared to one other?
c) Can clay, as a contributing variable significantly affect the accuracy of RIFT SOC determinations?

1.4. Delimitations of the study

The research is of an exploratory nature and aims to investigate whether the proposed RIFT method is worth pursuing with more in depth research and validation of the associated devices.

The study is restricted to representative soil types in the particular study region. This means that if the RIFT method is found to be worth pursuing, a much fuller and wider study should be conducted to test the possible application of the method across a broad spectrum of environmental conditions. Considering the notion that soils from differing geographic locations may exhibit unique relationships of SOM to SOC, probably on account of soil texture, and differing mineralogy (Konare *et al*., 2010) as well as particular soil bulk densities (Rantoa, 2009), it was important to select representative soils of the southern Cape region which, generally exclude soils with high carbonates.

Because clay is ubiquitous in most soils, it should be expected that water loss contributes to LOI (and therefore incinerations methods) in all soils regardless of the organic-matter content (Szava-Kovats, 2009). In this instance relatively high clay contents were found in the clay loams (29 to 30%), in the loams (16 to 25%) and much lower clay contents in the sands (5 to 7%).

It is important to the RIFT methodology, that if additional factors such as clay content, carbonates, dry soil mass-to-volume ratio and other mineralogy effects are going to be used in the determination of SOC, that the input data for those factors are easily obtainable. Whatever additional factors are to be used for improving the accuracy of the assessment must be easily assessable on site or in the field laboratory, and should be low cost and rapid as well.

The RIFT methodology is grounded in the requirement or need to do relatively accurate and credible assessments of SOM (and SOC) in the field or in a field laboratory and that all data for consideration should be obtained on site. This precludes the activity of sending away soil samples to a conventional laboratory for assessments of those factors. One should be able to use rudimentary field techniques to ascertain basic clay content, whether carbonates are present, and to determine the dry soil mass to volume ratio.

CHAPTER 2 LITERATURE REVIEW

2.1. Standard testing methods for soil organic carbon

This Chapter will investigate standard as well as emergent methods for SOC determination. The general properties of soil, with specific reference to South African soils, will also be discussed and the effect of rapid pyrolysis on soil properties will be investigated. Commonly used methods for measuring SOC include dry combustion, Loss-on-ignition and wet chemical oxidation, (Kimble *et al*., 2002; Konen *et al*., 2002; Rowell & Coetzee, 2003; Gehl & Rice, 2006 and McCarty *et al*., 2010).

Dry Combustion

The dry combustion (or dry oxidation) method measures total carbon by pyrolysis at high temperatures in a furnace with the collection and detection of evolved CO_2 (Schumacher, 2002). This method requires special apparatus and is not well adapted to rapid analysis of a large number of samples unless rather expensive automated and computerized carbon analysers are used, e.g. a Leco device.

Loss-on-ignition

The LOI method for the determination of organic matter is also a dry combustion technique (Chatterjee *et al*., 2009) as it involves the heated destruction of all organic matter in the soil or sediment. A known weight of sample is placed in a ceramic crucible (or similar vessel) which is then heated to between 350° and 440°C overnight (Nelson & Sommers, 1996). The sample is then cooled in a desiccator and weighed. Organic matter content is calculated as the difference between the initial and final sample weights divided by the initial sample weight times 100 and is then expressed as a percentage. All weights should be corrected for moisture/water content prior to organic matter content calculation. In a 2001 survey of United States soil analytical laboratories it was found that LOI was less expensive than both dichromate oxidation and dry combustion (McCarty *et al*., 2010). De Vos *et al.* (2005) adds that LOI is about 12 times cheaper than dry combustion techniques using sophisticated analysers both in terms of operational costs and equipment costs.

Thermogravimetric analysis (TGA)

Although also seen as a technique for measuring soil organic carbon (Miyazawa *et al*., 2000) TGA can be seen as a quasi LOI and has traditionally been used to characterise the chemical decomposition of materials (Pallasser *et al*., 2010) and can be done in oxidative and inert atmospheres using very small (e.g from 10 to 100mg) and highly refined (< 200ɥm) samples. The method has been widely used to determine the mineral fraction of materials but for soil C applications it offers mostly qualitative analysis potential and is useful to ascertain the temperatures at which the colloidal fraction denatures when variable heat is applied.

Wet chemical oxidation

The best known of the wet chemical oxidation (or chromic acid methods) is the Walkley-Black (WB) method that determines easily oxidizable C. With this method it needs to be considered that carbon in graphite and coal is not oxidized by chromic acid. It is a wet oxidation method followed by either titration with ferrous ammonium sulphate or photometric determination of chromium (Cr^{3+}) which is also known as colorimetric determination according to Rowell & Coetzee (2003). The WB method is a routine, relatively accurate, and popular method for the determination of SOM but it is time-consuming, costly and also has a high potential to cause environmental pollution because of disposal of chromium and strong acids used in this analysis (Salehi *et al*., 2011).

Both LOI and wet chemical oxidation measure SOM rather than SOC and this therefore needs to be converted first. However, the C content of SOM does vary and traditionally SOM has been estimated as 1.72 x SOC (De Vos *et al*., 2005; Périé & Ouimet, 2008) using the so called “Van Bemelen” factor. On the other hand some researchers found that through LOI analysis the ratio varies for different soil groups and depths. According to Bhatti & Bauer (2002) and Périé & Ouimet (2008) SOC rather approaches 50% SOM and rounding SOM to 2 x SOC could be seen as common practice as suggested by Brady & Weil (2008).

Of these methods dry combustion is arguably the most accurate (Konen *et al*., 2002; Rowell & Coetzee, 2003; Konare *et al*., 2010 and McCarty *et al*., 2010). However, the WB procedure is widely used because it is relatively simple, fast, and has minimal equipment needs according to Nelson & Sommers (1996). Most soil analytical laboratories world-wide have produced large datasets for estimating soil C based on either dichromate oxidation (wet chemical oxidation) or mass loss on ignition. Consequently, the methods that use wet chemical oxidation to determine SOC are often those most commonly used by soil testing laboratories (Schumacher, 2002).

LOI is considered as a quick, easy and inexpensive estimate (Heiri *et al*., 1999; Santisteban *et al*., 2004; Konare *et al*., 2010) and was also positively correlated with the Walkley-Black method by Miyazawa *et al*. (2000). Furthermore the LOI method proved to be a useful indexing method to predict SOC, since it proved to be reproducible and precise (Swanepoel & Botha, 2012). However, in a study by McCarty *et al.* (2010) the results showed that both dichromate oxidation and loss-on-ignition had substantial limitations when used to analyse very low carbon soils. Nevertheless, their threshold of “low carbon” content could have been relatively high since Rowell and Coetzee (2003) have found that wet chemical oxidation with colorimetric determination could detect very small amounts of C in a sample. It appears that LOI also overestimates organic matter content due to weight loss from the inorganic fraction, primarily hydrated clays, during the heating process and is affected by ignition temperature and sample size (Gehl & Rice, 2006; Sun *et al*., 2009; Szava-Kovats 2009).

Ball (1964) quotes early soil scientists as dismissive towards LOI because it was then considered crude and inadequate as an estimate of SOM or SOC on the grounds that further weight losses can occur on account of CO_2 in carbonates (in calcareous soils), loss of elemental or inert carbon (e.g. coal, charcoal & soot) and loss of structural water from clay minerals. However, he contends that correction factors can be instituted for samples high in carbonates, elemental C and clay, and adds that differences due to variation in clay mineralogy are unlikely to be significant for most purposes, especially in relation to normal sampling errors.

Szava-Kovats (2009) concedes that LOI may offer a crude estimate of SOC at high LOI levels but contends that although incorporation of the clay content into a regression model may improve the prediction of SOC, the added time and effort needed to measure clay content can offset the advantages offered by the LOI technique.

2.2. Emergent methods for basic soil carbon determinations

It is evident that much of the world's soil C data until recently was based on indirect *ex-situ* measurements like dichromate oxidation and LOI (MCcarty *et al*., 2010). Recently there has been an increase in the use of automated dry combustion techniques (Mikhailova *et al*., 2003; Gehl & Rice, 2006). Dry combustion has in fact become the reference method for comparisons with other methods (Konen *et al*., 2002) and most studies report good agreement in SOC measurements by different automated dry combustion techniques (Schumacher, 2002).

Because the above methods are laboratory based, time consuming and costly, most recent research efforts have focused on measuring soil C *in-situ* using a variety of methods based on remote sensing and spectroscopic measurements in the field (Gehl & Rice, 2006; Chatterjee *et al*. 2009) including laser induced breakdown spectroscopy (LIBS), inelastic neutron scattering (INS) and near and mid infrared spectroscopy (NIRS & MIRS). Since this study is focussed on basic quantitative soil C measurements and not the chemical or fractional nature of soil C, certain other more advanced analytical methods were not considered for discussion. These include but are not limited to pyrolysis molecular beam mass spectrometry (py-MBMS) that provides a powerful and rapid means of assessing the biochemical composition of SOM (Wielopolski *et al*., 2006; Plante *et al*. 2008) and eddy co-variance measurement of CO_2 fluxes (Post *et al*., 1999) as well as carbon isotope techniques that have been used to estimate retention time, C turnover rates in soils, carbon sequestration amounts and rates and even the source and history of C in SOM (Gehl & Rice, 2006).

Laser induced breakdown spectroscopy (LIBS)

This method is based on a laser pulse focused on a soil sample, creating high temperatures and electric fields that break all chemical bonds and generating white-hot plasma that is characteristic of the sample's elemental composition (Chatterjee *et al*., 2009; Izaurralde 2009). The micro-plasma emission is recorded in time and spectrally resolved by a timegated sensor to detect concentrations of elements based on their unique spectral characteristics. Calibration of the LIBS method and determination of soil bulk density allow for quantitative measurements of various major elements (C, N, P and K) for different soil types (Gehl & Rice, 2006). There is a reported high correlation (adjusted $R^2 = 0.96$) between LIBS and dry combustion C results for agricultural soils (Chatterjee *et al*., 2009; Gehl & Rice 2006) and analysis time is less than one minute per sample, providing daily sample throughput much greater than that of traditional C analytical methods. Although still in the developmental phase, field-portable LIBS instrumentation shows potential as a means to measure soil C rapidly and accurately. However, currently, calibration curves (soil C level versus LIBS peak area) must be developed for each sample set. Another challenge is the variability in the interpretation of results for samples containing fine roots and other biological substances at varying degrees of decomposition. Furthermore there is a risk imposed by this method relating to the spatial variability associated with measurement of a small point sample (sample volume is about 1 to 5 mm^3 $pulse^{-1}$).

Inelastic neutron scattering (INS)

This method is an in-situ, non-destructive technique that consists in directing fast neutrons (14 MeV) into the soil, where they interact with the nuclei of atoms including C and other atoms like H, N, O, Si, K, Ca and P (Izaurralde, 2009). Wielopolski and others (2006) found promising results from initial studies that prompted development of a field capable INS instrument that allows for non-invasive measurement of soil C to a depth of about 30 cm (Gehl & Rice, 2006). The INS system consists of a neutron generator and a shielded detector placed on the ground with a shadow shielding of iron, borated-water, and boric acid. The neutron generator, which is turned off when not in operation, generates fast neutrons that penetrate the soil and stimulate gamma rays that subsequently are detected by an array of sodium iodide detectors (Chatterjee *et al*., 2009; Wielopolski *et al*., 2006). The peak areas in the measured spectra are proportional to the soil's elemental content. The instrument can be used in either static or dynamic modes for scanning large land areas. The processes of neutron penetration, inelastic scattering, and gamma ray emission occur very rapidly, allowing for operation of the INS instrument in a scanning mode. The system cost is relatively high but no consumable costs are involved. The device is electrical but produces radiation necessitating special regulation. According to Chatterjee and others (2009) the device is well collimated and shielded without introducing any environmental hazard.

Near and mid infrared spectroscopy (NIRS & MIRS)

The use of spectral analysis for measurement of soil C based on diffusely reflected radiation of illuminated soil (Gehl & Rice, 2006) in the NIRS, 400–2500 nm, and the MIRS, 2500–25000 nm regions has advantages in that the method is rapid, non-destructive, consumes no reagents, and is highly adaptable to automated and *in situ* measurements (McCarty *et al.*, 2002; Chatterjee *et al.*, 2009). Constituents of organic matter each have unique absorptive or reflective properties due to stretching and bending vibrations of molecular bonds (e.g., C-H, C-C, C-H_2, O-H, N-H) between elements. MIRS probes the bond identities of a sample's molecules and interprets the chemical structures of molecules, offering the possibility of directly distinguishing inorganic from organic C, thus eliminating the need for acid pre-treatment to remove inorganic C (McCarty *et al.*, 2002; Izaurralde, 2009). NIRS uses a quantitative determination of components of complex organic compounds based on the absorption of the C-H, N-H, and O-H groups and has been the predominant means for quantitative determination of soil C (McCarty *et al.*, 2002). Due to differences in particle size and soil mineral absorption intensities, NIRS absorption by soil is not linearly related to the individual soil matrix components, necessitating continual calibration and quality control. According to Nocita *et al.* (2011) promising results have been obtained in previous studies in the Albany Thicket Biome of Eastern Cape Province of South Africa, but field spectroscopy (as well as remote sensing) can only provide estimations of surface SOC (0–5 mm). Furthermore its predictability is low in samples with heterogeneous particle size and high variability in moisture content. However, the technology is improving and field portable NIRS instruments are commercially available albeit rather expensive (Chatterjee *et al.*, 2009).

Lately Rodionov and others (2014) illustrated significant strides in the potential use of on-the-go field assessment of soil organic carbon using Vis–NIR diffuse reflectance spectroscopy by affixing the measuring chamber to a tractor driven sled device. Apart from the standard problems like very high cost, varying soil moisture, disturbance by gravel and continual calibration of instruments, further problems arise with these approaches, including the following:

- The soil surface needs to be pre prepared mechanically, e.g. soil tillage by plough and rotary cultivator (Rodionov *et al*., 2014) for best accuracy,
- Disturbance of soil surface and compaction of the soil by the tractor and heavy measuring device,
- Perceived slow speed at 3 km/h, slower than the average human walking speed,
- Cost of fuel (diesel) to power the tractor and its associated emissions,
- Optical quality is potentially impacted by dirt and scratches on the sapphire window which protects the contact probe (Rodionov et al., 2014),
- Potential high maintenance cost of very sophisticated instrumentation.

Remote Sensing

Characterising landscape features through remote sensing offers the possibility of spatial and temporal estimates of land cover, land management practices, cropping and tillage practices, net plant productivity, and plant residue in as far as they influence soil C dynamics. Since there is a strong relationship between soil colour and visible reflectance, the manifest reflectance of various spectral bands can be correlated with soil properties, including soil C content (Kumar, 2013; Chatterjee *et al*., 2009; Gehl & Rice, 2006).

Spectral sensors that can measure SOM usually operate with wavelengths between 0.3 µm and 1 m and are divided into the following four groups:

- Visible (0.4–0.7 µm)
- Reflective infrared (0.7–3 µm)
- Thermal infrared (8–14 µm)
- Microwave (1 mm–1m).

Wavelengths between 0.4µm to 2.5µm, are suitable for soil with >2% SOM content and research shows that predictions can be made of the SOM content from light reflectance with a linear or curvilinear relationship in the visual and infrared range (Chatterjee *et al*., 2009).

The capacity for remote sensing to characterize soil C is of particular interest due to the relative ease in periodic sampling and potential reduction in cost associated with verification and monitoring soil C sequestration. There have been limited studies on the direct measurement of soil C using remote sensing and this is probably on account of problems encountered in establishing correlations of SOC and soil reflectance because soil colour and reflectance properties are a function of many factors in addition to organic matter, including soil moisture, texture, chemical composition, parent material, and surface. Although there is a strong relationship between remotely sensed spectral data and SOC content, prediction at different spatial scales has not been achieved (Chatterjee *et al*., 2009). These problems are complicated when soils from large geographic areas are analysed and in the event that soils are not bare enough to assess reflectance remotely (e.g. croplands under conservation tillage or no-till). A further limitation of this methodology includes the effect of interference from other soil properties (e.g. Fe concentration) and consistency in image-intensity values (Gehl & Rice, 2006).

2.3. Reference methods for comparisons

Numerous comparisons have been made examining the efficiency of total organic carbon (TOC) methods (Schumacher, 2002). Perhaps the best known of these are rapid dichromate oxidation methods like WB which has until recently been the “reference” method for comparison to other methods in numerous studies. The WB procedure has on occasion served as reference method for predictions of soil carbon content through the use of general LOI (Schulte & Hoskins, 2009), the thermo-gravimetric (mass loss at 300°C) method (Miyazawa *et al*., 2000) and even laboratory and field spectroscopy (Nocita *et al*., 2011).

Konen *et al*., (2002) hold that dry combustion, also called “total carbon techniques” (Schumacher, 2002), is a reference method for LOI. However it is widely accepted that dry combustion is the most accurate reference method for comparison and even calibration for almost all other *ex-situ* as well as *in-situ* methods (Howard & Howard, 1989; McCarty *et al*., 2002; Blaisdell et al., 2003; Rowell & Coetzee 2003; Mikhailova *et al*., 2003; Gehl & Rice, 2006; Chatterjee *et al*., 2009; Konare *et al*., 2010 and McCarty *et al*., 2010). In general, the method is accurate, precise, and according to most authors relatively inexpensive (from a developed world perspective). Because equipment for this analysis is relatively commonly found in the developed world, the dry combustion technique for total carbon is often used as the standard against which other techniques should be calibrated.

Modern day *in-situ* soil C measurement techniques like reflectance spectroscopy, INS and LIBS may hold future promise to become the “reference” method in future. However, most of these methods are still under development and are still verified against dry combustion methods. Furthermore the question arises as to whether there are more accurate methods for soil C measurement and what will be the basis on which their absolute “truth” is based?

2.4. Soil properties and the nature of the colloidal fraction

Soil organic matter is material that originates from living organisms and that has been through a process of decomposition ranging from intact original tissues to a highly decomposed mix of materials termed colloidal humus (Bot & Benites, 2005). The organic portions of soil, the humus component, can be further described as consisting of humic substances and non-humic substances (Brady and Weil, 2008; Du Preez *et al.*, 2011).

According to a view by Sutton & Sposito (2005), humic substances are collections of diverse, relatively low molecular mass components, forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds. In simple terms, humic substances are organic compounds in humus not synthesized directly to sustain the life cycles of the soil biomass and they are dark-coloured, biologically refractory, heterogeneous organic compounds produced as by-products of microbial metabolism (Sposito, 2008).

The humic substances can be classified based on their insolubility in NaOH and their subsequent solubility and/or insolubility in acid solutions (pH=1). Whereas humin is insoluble in NaOH, fulvic acids are soluble in acid and humic acids are insoluble in acid. Non-humic substances contain identifiable bio-molecules that are mainly produced by microbial action and are generally less resistant to breakdown

Humic substances are derived from microbial transformations that start off when plant residues decompose. Microbes slowly break down complex compounds into simpler compounds during which some lignin gets broken down into its phenolic subunits. Microbes then metabolise the resulting simpler compounds. During this process they use some residual carbon (not lost to respiration as CO_2), most of the nitrogen, sulphur and oxygen to synthesise new cellular components and bio-molecules.

However, some of the original lignin is not broken down completely but is modified into complex residual molecules that still resemble lignin. The microbes then polymerize (link together) some of the simpler new compounds with each other and with the complex residual products to form long complex chains that resist further decomposition. These high-molecular-weight compounds interact with nitrogen-containing amino compounds giving rise to a significant component of resistant humus.

The presence of colloidal clay stimulates the complex polymerization of humic substances. Clay minerals have a high specific surface area and carry a charge, binding and thereby chemically stabilizing organic matter while contributing to the storage and long-term stability of organic matter in soils (Konare *et al*., 2010). One year after plant residues are added to the soil most of the carbon has returned to the atmosphere but one fifth to a third can remain in the soil as live biomass (~5%), humic fractions of the soil (~20%) and non-humic fractions of the soil (~5%). The proportion remaining from root residues tends to be somewhat higher than that remaining from incorporated leaf litter (Brady and Weil, 2008).

Humic substances comprise about 60 to 80% of the soil organic matter consisting of huge molecules with variable rather than specific structures and composition. Humic substances are characterised by aromatic, ring-type structures that include polyphenols (numerous linked phenols) and comparable polyquinones, which are even more complex. Humic substances are generally dark coloured, amorphous substances with molecular weights between 2000 and 300,000 g/mol. Because of their complexity they are the organic materials most resistant to microbial attack.

Thus, according to Brady & Weil (2008) the discerning factors of humic substances can be expressed as follows:

1. Humin = Darkest/heavy molecular weight/insoluble/most resistant to microbial attack

2. Humic acid = Medium colour & weight/ alkali soluble & acid non-soluble/ intermediate resistance
3. Fulvic acid = Lightest colour & weight/ all soluble/ most susceptible to microbial attack

The high molecular complexity has molecular structures that absorb almost all visible wavelengths of light giving it the characteristically darker to black colour. All three are relatively stable in soil but the half-life of fulvic acid is 10 to 50 years whilst the half-life of humic acid could be centuries.

Non-humic substances

This part of soil organic matter is the active, or easily decomposed, fraction (Bot & Benites, 2005). Non-humic substances comprise about 20% to 30% of humus (Brady & Weil, 2008). It is less complex and less resistant to microbial attack than humic substances. They comprise specific bio-molecules (unlike the humics) and some are microbially modified plant compounds while other compounds are synthesised by microbes as by-products of decomposition. Included in this group are polysaccharides which are polymers with sugar-like structures and general formula $C(H_2O)$ with variable molecular numbers for C and H_2O. They are important in enhancing soil aggregate stability. Even simpler compounds are part of the non-humic substances like organic acids and some protein-like compounds but in low quantities. However, they may influence the availability of plant nutrients like N and Fe and may directly affect plant growth.

Colloidal characteristics of clays and humus

The clay and humus particles in soils are referred to collectively as the colloidal fraction (Brady & Weil, 2008) because of their extreme small size and colloid like behaviour; they are less than 1 µm, but some soil scientists use 2µm to include the upper threshold for clay particle size.

The smaller size of soil colloid particles provides for up to 1000 times larger external surface area exposed for adsorption, catalysis, precipitation, microbial colonization and other surface related phenomena than for instance sand particles of the same mass. Some silicate clays also possess extensive internal surface area between the plate-like layers of the clay crystal units. The total surface area of soil colloids ranges from 10 m^2/g for clays with only external surfaces, to up to 800 m^2/g for clays with extensive internal surfaces (Brady & Weil, 2008).

The surfaces of most soil colloids carry negative electrostatic charges but some mineral colloids in very acid soils may have a net electropositive charge. Surface charge is variable between different soil colloid types and can be influenced by changes in chemical conditions such as pH. These charges however attract and repulse substances in the soil solution as well as adjacent colloidal particles and these reactions similarly influence the chemical or physical behaviour of soils. The occurrence of these attractions and repulsions between the mostly negative colloid surfaces and the mostly positively charged ions (cations like Al^{3+}, Ca^{2+},Mg^{2+}, K^+ and Na^+ and a few others) form the basis for the so called cation exchange process. In moist soils the cations exist in a hydrated state wherein the cation is surrounded by a shell of water molecules. Therefore the hydrated cations will be loosely held electrostatically (adsorbed) to vibrate in a swarm near the colloidal surface and frequently a cation may break loose to join the soil solution. When this happens a cation with a similar charge will take its place simultaneously as it moves from the soil solution to the swarm of cations. The soil solution in itself contains small but significant quantities of soluble organic and inorganic substances including plant nutrients like calcium, potassium, nitrogen and phosphorus (Brady & Weil, 2008).

Soil colloids differ in their particular composition, structure and properties (Brady and Weil, 2008) and soil properties can also be determined by the proportion and nature of the clay fraction (Brinkman, 1985). Clays can occur as crystalline, layered structures or as non-crystalline minerals with amorphous structure (Bronick & Lal, 2005). Clay minerals contain structural water or water that forms part of their matrix (Schumacher, 2002). Structural water refers to all bonded H_2O molecules and hydroxyl (OH) units in a mineral that can be lost during a heating process (Sun *et al.*, 2009) and includes crystal lattice water for OH-ion and water of hydration for bonded H_2O. However, the following colloidal fractions are of importance:

Crystalline silicate clays are dominant in most soils and their structure is layered like the pages of a book, also termed “phyllosilicates”. Each layer consists of two to four sheets of closely packed and tightly bonded oxygen, silicon and aluminium atoms. Although predominantly negatively charged, silicate clay minerals differ widely with regard to their particle shape, intensity of charge, stickiness, plasticity and swelling behaviour which is influenced by adsorption and removal of structural water. Common examples are kaolinite and smectite.

Non-crystalline silicate clays, also called amorphous or paracrystalline aluminosilicates by Brinkman (1985), consist of mainly tightly bonded silicon, aluminium and oxygen atoms but not in ordered crystalline sheets. These include allophane and imoglite usually from volcanic ash and they have high amounts of both positive and negative charges and high water holding capacities with extremely high capacities to strongly adsorb phosphate and other anions, especially under low pH or acid conditions.

Iron and aluminium oxides or sesquioxides (Rowell, 1994) are found in many soils but are important in the more highly weathered soils of warm, humid regions. They consist of either iron (Fe) or aluminium (Al) atoms coordinated with oxygen atoms - the latter are often associated with hydrogen ions to make hydroxyl groups. Some, like goethite (a Fe-oxide) and gibbsite (an Al-oxide) consist of crystalline sheets. Other oxide minerals are non-crystalline, often occurring as amorphous coatings on soil particles. The oxide colloids are relatively low in plasticity and stickiness (they can however also be considered as hydroxides or oxy-hydroxides because of the presence of hydrogen ions). Their net charge ranges from slightly negative to moderately positive (Brady & Weil, 2008).

Organic (humus) colloids are important in nearly all soils, especially in the upper parts of the soil profile. Humus colloids are not minerals, nor are they crystalline but rather they consist of convoluted chains and rings of carbon atoms bonded to hydrogen, oxygen and nitrogen. Humus particles are often among the smallest of the colloids and exhibit very high capacities to adsorb water but have almost no plasticity or stickiness. Humus has high amounts of both negative and positive charge per unit mass, but the net charge is always negative and varies with pH. The negative charge on humus is extremely high in neutral to alkaline soils (Brady & Weil, 2008).

2.5. The soils of South Africa

A very comprehensive account of the soils of South Africa was provided by van der Merwe in the 1940's (Fey, 2010b) describing soil groups and sub-groups of South Africa. Since then a classification system has evolved based on simplicity and the use of a binomial categorisation namely a higher or general level of soil forms, and a lower more specific level of soil families (Soil Classification Working Group, 1991). The classification of South African soils has nevertheless evolved further, and is currently well established, with 73 soil forms that can be placed into 14 groups (organic, humic, vertic, melanic, silicic, calcic, duplex, podzolic, plinthic, oxidic, gleyic, cumulic, lithic and anthropic) which are identified by means of an eliminative key based on the presence of defined diagnostic horizons or materials.

The classification is based on three differentiating principles or categories based on a) soils with special topsoil characters, b) soils with special subsoil characters with an orthic topsoil and, c) young soils with an orthic topsoil but weakly developed subsoil. Each of these categories are associated with distinct soil groups as follows (Fey 2010a, 2010b):

a) <u>Soils with special topsoil characters in the following soil groups:</u>

1. **Organic** – Wetland or montane peat (organic O horizon) with soil forms:
2. **Humic** - Humus enrichment; free drainage; low base status; humid climate
3. **Vertic** - Swelling, cracking clay; basic parent material; semi-arid to sub-humid climate
4. **Melanic** - Dark, structured clay; high base status; semi-arid to sub-humid climate.

b) <u>Soils with special subsoil characters with an orthic topsoil:</u>

5. **Silicic** - Cementation by amorphous silica or sepiolite; arid climate
6. **Calcic** - Carbonate or gypsum enrichment; arid climate
7. **Duplex** - Marked textural contrast through clay enrichment
8. **Podzolic** - Metal humate enrichment; siliceous parent material (podzol B horizon)
9. **Plinthic** - Absolute iron enrichment; localised, hydromorphic segregation with mottling or cementation

10. **Oxidic** - Residual iron enrichment through weathering; uniform colour (red apedal, yellow-brown apedal or red structured B horizons)
11. **Gleyic** – Protracted reduction on an aquatic subsoil or wetland (G horizon).

c) <u>Young soils with an orthic topsoil but weakly developed subsoil:</u>

12. **Cumulic** – Incipient soil formation in colluvial, alluvial or aeolian sediment
13. **Lithic** – Incipient soil formation on weathering rock or saprolite
14. **Anthropic** – Material disturbed by humans (Unconsolidated horizon).

Although a systematic country-wide study on the status and spatial distribution of organic matter in soils does not exist yet (Du Preez *et al*., 2011), there is useful information to be gleaned from many years of soil surveys that were eventually captured and compiled into a database by the Agricultural Research Council – Institute for Soil, Climate and Water (South Africa). This land type survey data was used to produce a generalised organic carbon map for virgin top soils in South Africa and it was found that it correlated well with the long term annual rainfall map. However, apart from precipitation, SOM levels, and therefore SOC, are also determined by other ecological conditions like for instance temperature regime, vegetation and soil type (Scholes & Walker, 1993).

Furthermore it is well known that SOM influences the physical, chemical and biological properties of soils which then influence plant production and environmental quality (Scholes *et al*., 2003; Brady & Weil, 2008). The South African Soil Classification System (Soil Classification Working Group, 1991) incorporates topsoil organic C levels as a diagnostic property differentiating between the Organic, Humic and Orthic A-horizon types. Organic horizons, typically associated with wetlands, contain more than 10% organic C due to the anaerobic conditions inhibiting organic material mineralisation. Humic horizons are associated with cool, freely drained high rainfall conditions giving rise to intensely weathered, low base status soils containing 1.8% to 10% organic C. The Orthic group of topsoils include a range of soils which develop in diverse landscape types with organic C levels less than 1.8% (Soil Classification Working Group, 1991).

2.6. Effect of rapid pyrolysis by incineration on soil properties

Pyrolysis, ignition and combustion techniques imply the heated destruction of organic matter and the inorganic fraction like carbonates and clay minerals at high temperatures. Most organic matter is oxidized at a moderate to high temperature (Konen *et al*., 2002) between 100 to 400°C (Konare *et al*., 2010) and according to Rowell (1994) it is burnt off at about 325°C depending on the time period of exposure.

According to Sun *et al*. (2009), no known published studies reliably quantify the range of structural water in the soil but through the use of thermo-gravimetric methods it can be discerned at which temperatures certain components of the soil structure are oxidised (Pallasser *et al*., 2010). In a test of several Brazilian soils (mostly tropical soils) with variable chemical and physical properties (Miyazawa *et al*., 2000) the first loss occurred below 100^{o}C mainly due to water volatilization. In the second instance between 200 and 280^{o}C, organic matter was lost, mostly carboxylic and phenolic functional groups of the humic and fulvic acids and hydrocarbon compounds (this can also be ascribed to the release of low molecular weight organic compounds). Thirdly between 270 and 370^{o}C the mass loss was mainly due to further carbon oxidation of the organic matter and in the fourth instance from 380 – 530^{o}C, the mass loss was mainly due to de-hydroxylation of the metallic hydroxides Al, Fe, Mn and others. Ben-Dor & Banin (1989) also report similar results but thermal reactions such as de-hydroxylation of phyllosilicates are stated to occur from 200 to 700°C and the decarboxylation of carbonates between 700 to 1000^{o}C.

The main concerns with dry combustion techniques are that clay minerals will lose some structural water or hydroxyl groups at the temperatures traditionally used to combust the samples (Konen *et al*., 2002; Schumacher, 2002; Sun *et al*., 2009). Rowell (1994) observed that soils containing appreciable amounts of clay and sesquioxides lose structural water between 105 and 500°C. The structural water loss will increase the total sample weight loss leading to an overestimation in organic matter content (Howard & Howard, 1989; Schumacher, 2002; Santisteban, 2004; De Vos *et al.,* 2005; Sun *et al*., 2009) and similarly Ball (1964) realised early that an increase of clay content percentage in the sample leads to an increase in the LOI values.

The higher the LOI values (likelihood of higher SOM) the less is the influence of structural water loss on the measurement (Sun *et al.*, 2009). Furthermore in the analysis of a range of soil types representing a range of SOM values, Sun *et al.* (2009) found that in a few instances (about 30%) most of the LOI was attributed to structural water. However it was obvious that this was only the case in soils with relatively low levels of SOM. Wang & Wang (2011) contend that lattice water in clays can cause the C content to be overestimated by as much as 5% with LOI.

One possible means to avoid overestimation due to structural water is through the pre-treatment of the sample via removal of the mineral matter using acids (Konen *et al.*, 2002), like hydrochloric acid (HCl) and hydrofluoric acid (HF). However, the use of HCI may dissolve part of the organic matter leading to an underestimation of the organic matter content and potentially necessitate the use of a correction factor (Schumacher, 2002).

The American Society for Testing and Materials (ASTM method D 2974) allows for ashing the sample at 750°C for peats and other organic soils, such as organic clays, silts, and mucks. This is presumably based on the assumption that no carbonates and very little mineral matter are present in the sample that could influence the resultant organic matter content (Schumacher, 2002).

Another primary interferent in the determination of organic carbon is the presence of inorganic carbonates in the soil or sediment (Rowell, 1994; Heiri *et al.*, 2001; Konen *et al.*, 2002; Schumacher, 2002; Konare *et al.*, 2010). Calcium carbonate loses CO_2 to form calcium oxide from about 700°C (Ben-Dor & Banin, 1989 and Rowell, 1994) and evolves completely around 950°C (Heiri *et al.*, 2001; Konare *et al.*, 2010). If the samples are left untreated, depending upon the method used, the carbonate minerals (e.g., calcite and dolomite) will be destroyed along with the organic matter and in the process additional CO_2 will be evolved and measured. The additional CO_2 will lead to falsely elevated organic carbon content. None of the methods available for the determination of SOC are capable of distinguishing between inorganic or organic derived CO_2. Schumacher (2002) suggests that screening tests should be performed using a 1 to 4 NHCl drop test followed by observation for effervescence to determine if carbonates are present. Alternately, the pH of the sample may be determined and if the pH is below 7.4, then the sample should be free of all carbonates.

The presence of carbonates however, is not a problem in this study due to the region's high rainfall and general low soil pH but where present, they should be removed using a 2N H_2SO_4 - 5% $FeSO_4$ solution followed by sample drying prior to sample analysis. However as with the use of acids to remove structural water before combustion, Wright *et al*. (2008) propose that if acids are used to remove carbonates it may lead to damage of instrumentation (combustion analysers), interfere with analysis and also destroy organic matter present in the samples.

Besides soil carbonates, soils may contain organic compounds such as coal and charcoal (Black C, elemental C, inert C) that can interfere with the determination of SOC (De Vos *et al*., 2005; Chatterjee *et al*., 2009; Konare *et al*., 2010) but for the purpose of this study such soils were not collected.

CHAPTER 3 MATERIALS & METHODS

3.1. Study area

Location

The study area for this investigation is in the southern Cape between the town of George and Sedgefield village in the Western Cape Province of South Africa (See Figure 1). To the east of George is the village of Wilderness and between Wilderness and Sedgefield is a series of lakes known as the Wilderness lakes.

Figure 1: The study area in the southern Cape in the context of the Southern African region.

Climate

The climate of the southern Cape can be considered temperate with an all season rainfall, high frequency of cloud, frequent light rain and changeable weather (Schafer, 1991). Rainfall varies from 500 to 1400 mm throughout the year but with maximum precipitation during autumn and spring (South African National Parks, 2014). The mean annual rainfall for the whole region is 994 mm but it varies considerably, increasing with the increase in altitude from the sea to the mountains (Schafer, 1991). The summers are warm with temperatures around 22 to 25˚C and the winters are mild with average temperatures of 18 to 21 ˚C.

The Outeniqua mountain range to the north influences the climate significantly by acting as a barrier to the inland penetration of the weather system and gives rise to orographic precipitation (Schafer, 1991; South African National Parks, 2014). Topography influences temperature over the north-south gradient. The effect of altitude on air temperature is a drop of about 0.6°C for each 100 m rise which can lead to a 10°C difference in temperature over this gradient (Scriba, 1984).

Topography

The main features that characterize the topography of the area are the Outeniqua mountain range to the north, the foothills, the coastal platform and the Wilderness Knysna coastal embayment (Schafer, 1991). These geomorphological features are inherent to the Southern Cape and can be explained largely as a result of the highly contorted folding of the Table Mountain Sandstone and the subsequent erosion cycle (Schafer, 1991; South African National Parks, 2014). The four regions can be described as:

- The sandy coastal lowlands, termed the “Wilderness embayment” or “littoral”,
- The coastal platform (lower and upper plateau between 150 and 300 m),
- The foothill zone, a fairly narrow region between the coastal platform and the mountains at an altitude between 350 and 550 m,
- The mountains (rising to almost 1600m)

In relation to these topographical regions four of the 11 soil sampling plots were located in the Wilderness embayment with five plots on the coastal platform and two plots on the transition to the foothills of the Outeniqua Mountains.

General geology

The mountain belt is composed of Table Mountain Group sandstones with overlying Bokkeveld Group Shales which occur within two valleys to the extreme east of the area (Schafer, 1991). These rocks of the Cape Supergroup underlie most of the area, while Pre-Cape and Cretaceous rocks and unconsolidated deposits of recent age occupy smaller areas. The Pre-Cape rocks comprise the Maalgaten Granite to the west and east of George (including the Woodville – Beervlei area), separated by a variety of sedimentary and metamorphic rocks of the Kaaimans Formation that include phyllite, quartzite, grit, hornfels and schist as found in the Saasveld and Karatara areas (South African National Parks, 2014). River catchments in the Outeniqua Mountains are comprised primarily of sandstones and quartzites of the Table Mountain Group (South African National Parks, 2014).

The coastal plateau is an old sea floor dating back to the Tertiary period (South African National Parks, 2014), which in places has been deeply incised by rivers. The plateau is underlain by pre-Cape granite, Kaaimans Group sediments (phyllites, schists, shales) and Table Mountain sandstones, mantled in places by alluvium and aeolian deposits. Remnant high-level terraces capped by silcrete and ferricrete occur in places (Schafer, 1991).

The coastal embayment dates predominantly from the Pleistocene period, though the landform is not static with some areas still in the process of formation. It is comprised predominantly of dune deposits, with Quaternary sands in which dune rock or aeolianite has been formed from the cementing of sandy ridges by calcium carbonate (South African National Parks, 2014). Four ages of aeolian depositions can be zoned (Schafer, 1991), represented by three dune cordons on land, and one underwater at sea.

The development of the barrier dunes in which the lakes are located spanned at least the last two glacial-interglacial cycles (South African National Parks, 2014). Inland coalesced dune deposits age back to the Middle Pleistocene, about 1 million years ago. The Wilderness lakes formed as a result of the cutting off of the rivers by these dunes, thus preventing them from flowing directly into the sea. The lakes and estuaries are thus transient features that are slowly silting up through natural erosion and deposition processes (South African National Parks, 2014).

Soil

The southern Cape is represented by a complex suite of soils ranging from deep sandy profiles developed in coastal aeolianites to shallow residual soils and peaty lithosols in the Outeniqua Mountains (Schafer, 1991). These soils vary in nature due to parent material, climate variation and exposure to soil forming factors since the mid tertiary giving rise to lithosols, podzols, duplex and gradational soils, gleysols and often paleosols developed in deep colluvium or tertiary sediments, superimposed with modern soils.

In the Outeniqua Mountains, soils are in general, acidic, leached, low in nutrients, and have a poor buffering capacity. In the upper river catchments soils are derived primarily from sandstones and are typically light textured, acidic, podzolised fine sandy loams. Rocky well drained soils commonly occur on north-facing slopes, whereas poorly drained or even peaty soils frequently occur on southern aspects. Dark acidic topsoils with high organic matter content frequently occur in wetter areas, particularly at high altitudes. In drier areas topsoils are frequently ash-grey in colour and low in nutrients as a consequence of podzolization processes where iron, aluminium and organic matter are stripped from the topsoil and deposited lower down in the profile (South African National Parks, 2014).

Along the coastal plateau in the Wilderness area soils are generally of a duplex nature, with clay subsoil occurring at between 300 and 500 mm depth, overlain by a thin concretionary gravel horizon. Soils in this region are often poorly drained, acidic and have low biological activity (Schafer, 1991). Topsoil crusting and compaction occur as a consequence of the fine texturing of the soils.

Soils under indigenous forests tend to have humic topsoils and are high in organic matter and biological activity. Soils on the coastal embayment are derived primarily from Pleistocene and recent coastal sands. Soils range from excessively drained Orthic on Cumulic soils on the youngest dunes, to finely textured, poorly drained podzols and duplex soils in older dunes (Schafer, 1991). Much of the floodplain of the lake systems is covered with a dark alluvium which is rich in organic matter (South African National Parks, 2014).

The high silt and fine clay fraction in the topsoil of older dunes, coupled with underlying impervious clay or rock layers and thin ironpans contribute to restricted drainage.

The selected soils for the study

The soils for this study were identified and collected by Prof Josua Louw of the Nelson Mandela Metropolitan University (NMMU) during April 2013. Soils representative of the region, with maximum variability in terms of conditions of formation, morphology and classification were selected (see Figure 2 for location of the sample plots). The 11 soil forms can be divided in the following groups:

- The Organic soil group
- The Humic soil group
- Orthic on, podzolised, duplex, Plinthic and oxidic soil groups
- A younger Orthic on Cumulic soil group.

Figure 2: Distribution map of soil samples S1 to S11 collected in the study area

The **Organic** Soil group was represented by a Champagne soil form (Plot S3) which is typically associated with wetlands and bogs where saturation with water inhibits the breakdown of organic residues. This soil was sourced on the northern banks of the Bo-Langvlei, a Cape Lowland Freshwater Wetland.

The **Humic** soils were represented by Kranskop (Plot S9) and Magwa (Plot S10) and Nomancy (Plot S5) soil forms. These soil forms are generally from a topsoil horizon showing intense weathering with high accumulation of humus (although limited by nutrient deficiency and acidity) that occur in relatively cool areas of high rainfall, free drainage and plateau topography (Soil Classification Working Group, 1991). The Kranskop and Magwa in this instance were collected from the coastal platform within Southern Afro-temperate Forest, on a southern aspect. The Nomancy form with a lithocutanic B horizon (Soil Classification Working Group, 1991) was collected in natural Southern Afro-temperate Forest on a steep slope underlain by phyllite.

Orthic on duplex soils were represented by Estcourt (Plot S7) and Klapmuts (Plot S11) soil forms, illustrating the accumulation of clay by illuviation into subsoil. Marked enrichment with clay results in strong blocky or prismatic structure with cutanic character (clay skins) defining a duplex character, with a clear to abrupt transition from the overlying horizon. Both soil forms were characterised by strongly defined E-horizons above the clay layer, which resulted from seasonal wetness combined with eluviation of clay, humus and sesquioxides (Soil Classification Working Group 1991). The Klapmuts with a pedocutanic B horizon was obtained in Garden Route Shale Fynbos in an area transformed by plantation forestry. The Estcourt soil form with a prismacutanic B horizon (Soil Classification Working Group, 1991) was located on the Saasveld Campus on the fringes of Southern Afro-temperate Forest.

Orthic on Podzolic soils occur in the event that the concentration of water soluble humic substances from decomposing vegetation exceeds the clay's capacity to adsorb it and migrates downward through the soil profile and through the complexing metals, mostly aluminium (Al) and iron (Fe), precipitating only when a solubility threshold is reached. This probably occurs along a pH gradient as well but leading to a spectacular chromatic subsoil horizon, sometimes hardened or cemented, forming below more bleached E or A horizons. Podzolisation is more likely to form underneath fynbos or evergreen forest vegetation combined with sandy substrates. In this instance a Lamotte soil form (Plot S1) with a podzol B horizon (Soil Classification Working Group, 1991) was collected from Southern Cape Dune Fynbos in the lowlands between Bo-Langvlei and Rondevlei.

Orthic on Plinthic soils are evident where a fluctuating water table gives rise to the reduction and mobilisation of iron and its migration and reprecipitation as mottles, nodules, concretions and vesicular hardpan (ferricrete). This could lead to the possibility of absolute enrichment with iron oxides. Plinthite formation is common in warm sub-humid to humid climates where a distinct dry season is evident, especially on middle to lower slopes in the landscape. Westleigh (Plot S8), with a soft plinthic B Horizon (Soil Classification Working Group, 1991) was collected in a Garden Route Shale Fynbos area transformed by plantation forestry on a flat plateau section in close proximity to the Saasveld Campus.

Orthic on Oxidic soils are the result of red and/or yellow oxides of iron accumulating through weathering and imparts a mostly uniform colour to soils in the upper well drained and aerated layers. Although the Constantia soil form is broadly considered to be within the oxidic soils since it imparts an achromic colour to the upper solum, the oxidic group is not a suitable home for it because the E horizon probably contradicts the implication of free drainage and aeration. It is defined as having an orthic A-E-yellow-brown apedal B horizon sequence (Soil Classification Working Group, 1991). The Constantia soil form (Plot S2) was obtained from Southern Cape Dune Fynbos between the lower upward slope of Bo-Langvlei and Rondevlei freshwater lakes.

Orthic on Cumulic soils represent immature soils on account of having been formed in recent, unconsolidated sediments such as colluviums (down-slope), alluvium (down-valley) deposition from flowing water, mass movements like landslides, soil creep or aeolian (deposition of sand by wind). The Tukulu (Plot S4) sample was collected from Southern Cape Dune Fynbos above the northern embankment of the Bo-Langvlei lake and the Oakleaf (Plot S6) form both with a neocutanic B horizon (Soil Classification Working Group, 1991) was obtained from Southern Afrotemperate Forest transformed by plantation forestry on the Saasveld Campus.

A summary of the collected soil groups, soil forms as well as the specific habitats and coordinates where they were collected are provided in Table 1 below.

Soil group	Location	Coordinates	Habitat	Soil form	Plot no
Organic	Banks of the Langvlei	33° 59’02’’ 22° 40’34’’	Freshwater wetland and dune fynbos	**Champagne**	**S3**
Humic	Saasveld Campus	33° 56’31’’ 22° 31’16’’	Afro-temperate forest	**Kranskop** **Magwa**	**S9** **S10**
	Groenkop Forest	33° 57’57’’ 22° 33’23’’	Afro-temperate forest	**Nomancy**	**S5**
Orthic on Duplex soils	Saasveld Campus	33° 57’44’’ 22° 32’08’’	Transformed areas	**Estcourt**	**S7**
	Timber forestry area at the George Dam	33° 57’31’’ 22° 30’53’’	Transformed areas	**Klapmuts**	**S11**
Orthic on Podzolic	Floodplain area between freshwater lakes at Rondevlei	33° 59’41’’ 22° 41’53’’	Dune fynbos	**Lamotte**	**S1**
Orthic on Plinthic	Plantation area on Saasveld Campus	33° 56’56’’ 22° 31’19’’	Shale fynbos transformed by plantation forestry	**Westleigh**	**S8**
Orthic on Oxidic	Mid-slope on dune system between two freshwater lakes at Rondevlei	33° 59’45’’ 22° 41’52’’	Dune fynbos	**Constantia**	**S2**
Orthic on Cumulic	Edge of Langvlei wetland area	33° 59’00’’ 22° 40’36’’	Dune fynbos	**Tukulu**	**S4**
	Saasveld Campus	33° 58’09’’ 22° 32’05’’	Afro-temperate forest transformed by plantation	**Oakleaf**	**S6**

Table 1: Summary of the collected soil groups and soil forms with their coordinates

Vegetation

The natural terrestrial vegetation includes fynbos and forest and coastal vegetation (see Figure 3). Indigenous forests occur from sea level to altitudes of 1000 metres or more, but most of the high forest occurs on the coastal plateau and foothills of the mountains (SANParks, 2012).

Southern Afrotemperate forest is well represented especially to the immediate east of the NMMU Saasveld campus (Mucina & Rutherford, 2006). These warm-temperate mixed evergreen natural forests of the Garden Route occur as patches in a rugged landscape, surrounded by fire-prone fynbos vegetation.

Figure 3: The vegetation types in the immediate vicinity of the collected samples: (A) Southern Afrotemperate Forest, (B) Garden Route Shale Fynbos, (C) Cape Lowland Freshwater Wetlands, (D) Southern Cape Dune Fynbos (Mucina & Rutherford, 2006).

The forests occur in many small patches in the rugged mountains, but mainly in a few large but isolated patches on the coastal platform and in river valleys (South African National Parks, 2014). Human induced disturbances, such as exploitation, clearing, grazing and fire, must have eliminated and fragmented forest to some extent in many areas of the Garden Route since the arrival of the Europeans during the latter half of the eighteenth century. However Geldenhuys (1994) contends through compelling arguments that actual forest location pattern is determined by the fire pattern as a function of the interaction between prevailing winds during dry periods and the terrain physiography. His study has shown that forests persisted in the topographic shadow areas of the coastal platform sheltered against the gusty, hot, desiccating, north-westerly bergwinds common during autumn and winter. This concept should be considered alongside other limiting environmental factors, particularly rainfall, and the fact that the establishment and growth of forest in the Garden Route is limited in conditions where rainfall is below 500 mm.

The fynbos component of the study area is represented by Garden Route Shale Fynbos and Garden Route Granite Fynbos on the coastal plateau and foothills of the mountains as well as Southern Cape Dune Fynbos of the coastal embayment up to the coastal plateau (Mucina & Rutherford, 2006). In the low lying areas between the dunes Cape Lowland Freshwater Wetlands vegetation occurs in the immediate vicinity of the Wilderness Lakes.

3.2. Pilot study handling and incineration regime

The soil handling and incineration regime was initially established on the rudimentary RIFT device. Consistent with the literature (Heiri *et al*., 2001) it was evident early on that results for RIFT, not unlike LOI, are also influenced by sampling methods, sample size, period of exposure, heat intensity and laboratory handling. Due to the nature of the RIFT it was necessary to settle on a sample size suited for rapid incineration. The rift heat source could not penetrate the sample effectively enough if the sample was larger than 1.5 g, virtually a thimble sized crucible. Furthermore it was noticed during incineration that the sample glows red under the heat source in the upper surface but that the lower parts of the sample don't incinerate to the same extent. It was decided to introduce mixing in between consecutive incinerations. This allowed for more complete oxidation of all SOC in the sample within an average of 3 incinerations.

The results were promising and illustrated some consistency for similar samples ranging from what could clearly be discerned as dune sand, loamy soil and humus rich forest floor soils. However the samples were treated to 3 incinerations and measured (weighed) only before the onset and after the third incineration. This left significant room for uncertainty in measurements in that the observer can't be sure that the weight loss was attributed to residual hygroscopic or structural (intercrystaline) water from clays and/or the oxidation of organic matter.

A consistent level of weight loss determination was required and it was found that measuring the incremental weight loss after each incineration until no further loss within a threshold of 0.005g was registered, provided that kind of reliability. It is then assumed that the loss that occurred was the result of the consumption of most (if not all) of the organic fractions, the bound water and residual moisture. This assumption is also based on the fact that organic matter and bound water starts reducing at relatively low temperatures from as low 105 °C to 250 °C (Heiri *et al*., 2001; Chatterjee *et al*., 2009). From the above it was decided to aim at a total destruction of consumable material (SOM-like organic fractions and structural water). The next step is to convert it to SOC by multiplying the loss by an agreed factor (e.g. 0.5 x SOM) and then to ascertain whether it is necessary to correct for known quantities of clays or deduct those fractions from parallel tested quantities for those consumables. This thinking established the final incineration regime of reiterative incinerations of the same sample until all consumables appeared fully oxidised or evaporated. For most samples, to conduct full consumption or depletion of consumables occurred within 2 to 6 incinerations.

3.3. Standardisation of the RIFT method and prototype device

The RIFT principle

The RIFT method is founded upon principles evident in dry combustion which detects evolved C through an automated analyser, as well as weight loss on ignition. Rapid incineration implies the direct application of intense heat (in excess of 1000 °C) from a physical flame under pressure onto a pre weighed sample for a specific period of time and then determining the loss on incineration by gravimetric means in order to derive the oxidised SOM. The flame is typically delivered through a butane torch lighter.

Whereas the dry combustion method measures total carbon by pyrolysis at high temperatures in a furnace with the collection and detection of evolved CO2, the LOI method determines organic matter through the high temperature heated destruction of organic matter in the soil after which organic matter content is calculated through the measured difference between the initial and final sample weights as a percentage. RIFT deviates from dry combustion in that the organic matter destroyed by intense heat is determined gravimetrically rather than detecting with an analyser the characteristics of the elemental gas evolved from the dry combustion process. Furthermore RIFT deviates from LOI in that the carbon content is derived by the total or near total destruction and oxidation of SOM with intense applied heat (incineration) rather than by ambient oven heating at longer exposure and significantly lower temperatures. The overestimation of organic matter content is therefore likely to occur based on the indiscriminate high temperature oxidation and destruction of fractions of structural water, carbonates and elemental carbon. However, in many instances such fractions are very small and unlikely to influence the results significantly and in instances where the soil does contain significant levels of those fractions, it can be corrected for (Schumacher, 2002; De Vos *et al*., 2005). For the purpose of this study all the samples had a pH below 7.4 (in KCl), implying that the samples should be free of carbonates. The specific contribution of soil forming factors in the study area also will prevent accumulation of $CaCO_3$ especially the relatively high rainfall (Louw, personal communication, 2014).

A process is followed whereby reiterative incinerations lead to the near total digestion of SOM. For low carbon soils this occurs within one or two incinerations and for high carbon soils it can take up to 4 or even 6 incinerations. The initial incineration usually consumes most of the soil organic carbon and consecutive incinerations may remove remaining carbon and the remainder of structural water which may have persisted longer than the organic matter present. However, when no further loss of weight (of at least 0.005g) is detected, then the soil carbon and structural water are deemed to have been consumed in full. Once again this differs from LOI in that the organic material destruction is more profound and virtually no residual carbon, structural water or even elemental carbon remains in the sample.

The RIFT device

Initially a simple hand-held device was put together by combining general utensils based on the conceptual understanding of the dry combustion and LOI processes. From there it evolved to a more stable but still very rudimentary device on a fixed base which sufficed to help determine sample and incineration regimes. However, it became obvious that the device needed to undergo changes to compensate for mechanical and operator inconsistencies related to flame distance, flame intensity and sample integrity (e.g. flame force blowing micro particles out of the crucible). Experimentation with various designs ensued leading up to the current Prototype RIFT Device (See Figure 4).

The device has an aluminium crucible (it is actually a common thimble) with a loading capacity of around 1 to 1.5 g of dried soil sample. The crucible is fitted in an adjustable clamp bracket that is fixed on an articulated wooden block that can be set at the desired angle (in this case 45°) on a wooden frame. The incinerator is also fixed on the frame of the device in such a way that it can slide and be fixed on its own ramp (also 45°) and move forward and backward in order to fix it at an exact distance (in this instance 4 cm) and perpendicular to the crucible so that the flame heat directly impacts squarely on the surface of the sample. A further critical design aspect is that the incinerator (Butane torch) rests on a flexible but stable arm that can be flipped-up and dropped instantly in order to apply (or disengage) the heat onto the sample at a specified constant angle and distance to the sample. A further adaptation to the incinerator is a flame gauge consisting of a thin copper wire fixed to the nozzle of the incinerator with a tiny coil or an eye at the end through which to visually align the flame length.

The device also incorporates a primitive but very effective "drying oven" in order to establish consistency related to the moisture content and soil texture. A simple soda can was cut on two opposing sides to have two large air holes. The top of the soda can was removed with a tin opener and fitted with a thick aluminium-foil 3" tartlet pan. A tea-candle (tea light candle) was installed underneath the tartlet pan and when lit the portion of soil sample was dried for a predetermined period, mixed and the process repeated until the fine soil fraction visually behaved consistently and stopped sticking to polished metal surfaces before being tested.

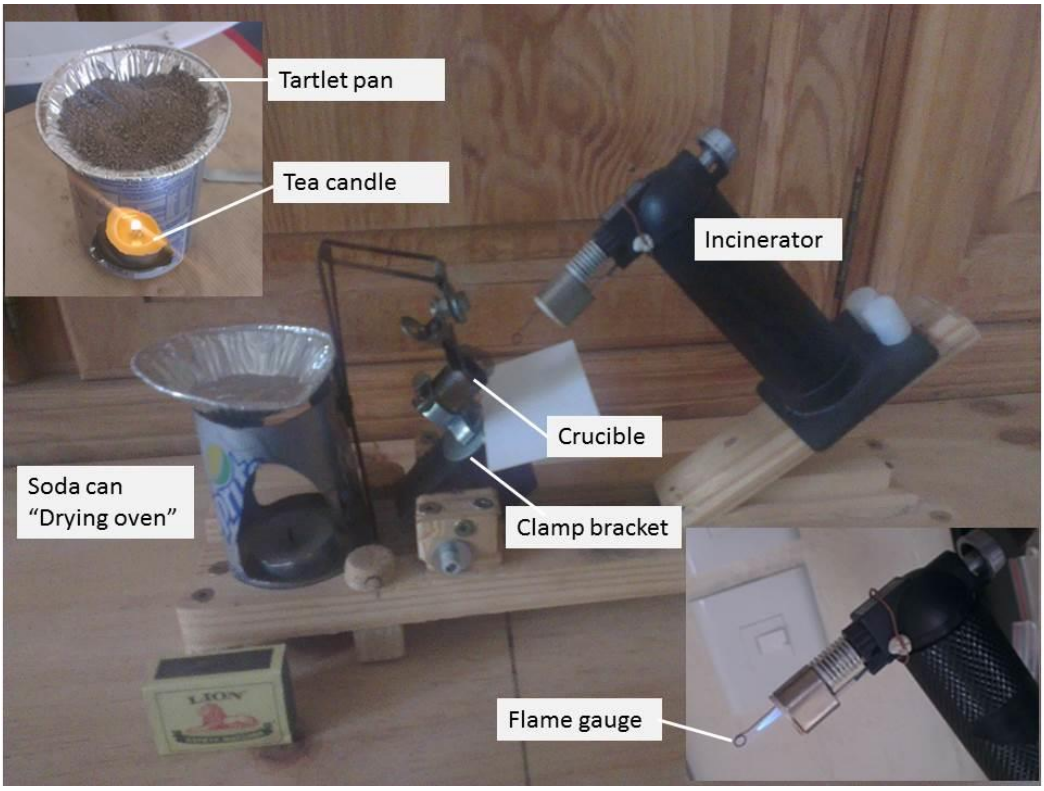


Figure 4: The development of the RIFT device from concept to prototype.

RIFT method

The method is based on the collection, homogenisation and drying of samples (around 100g is adequate) before incineration of relatively small samples and recording the resultant weight loss. The steps are as follows:

a) **Collection of samples**. Samples were collected through standard methods. A standard hand held Edelmann auger was used for sample collection. Samples were collected in the zone 5-10 cm below the soil surface. Five samples were bulked on each site, taken within a radius of 2m from a central point in the sample plot. The samples were homogenised before being bagged and tagged and sent for RIFT and conventional laboratory analysis.

b) **Pre-preparation of samples**. On receipt of the bulked and homogenised field samples they were all air dried on standard brown blotting paper until dry to the touch. Samples were then crushed in a mortar and pestle. Once samples were evenly crushed and mixed they were again homogenised.

c) **Homogenising of samples**. The samples were homogenised first by manual mixing in a mortar and then by tumbling them in a sieve drum which ensured further refinement and homogenisation. Samples were sieved with a 2mm aperture sieve drum (a wire metal mesh pen holder) to separate and remove macro fractions like stone, woody elements and large organic and root detritus. The process was repeated until all larger fractions were removed, weighed and recorded.

d) **Drying of samples**. About 100g to 150g of air dry soil was scooped into the tartlet pan and set upon the adapted soda can with a lit tea candle underneath. Samples were dried in this way for 3 minutes at a time before being stirred with a spatula (drying cycle was repeated two to three times). Once the soil appeared light and dry and ran freely from e.g. a metal spatula without sticking to the surface it was considered ready for further analysis.

e) **Handling of samples**. Homogenised soil for each of the 11 soil forms was split into the following samples for testing by the student with the RIFT methodology and for testing by Bemlab, an independent analytical laboratory:

  - 5 identical blind samples of about 5 g each for testing with dry combustion with a Leco device by Bemlab.
  - 5 identical blind samples of about 5 g each for testing with wet chemical oxidation, in this case the Walkley-Black method by Bemlab.
  - 5 identical samples of about 10 g each for testing with the RIFT method.

- 1 sample of about 50 g of each of the 11 soil forms to conduct a full range of soil characteristics and features by Bemlab.

Bemlab (Pty) Ltd is an independent analytical laboratory that provides full agricultural testing services in Somerset West in the Western Cape, South Africa. The 5 samples per testing method were linked by a random number code associated with the particular original soil form so that it was not known by the laboratory which samples were from the same batches. There were therefore 55 soil samples for each testing method that could be compared amounting to a total of 165 samples tested.

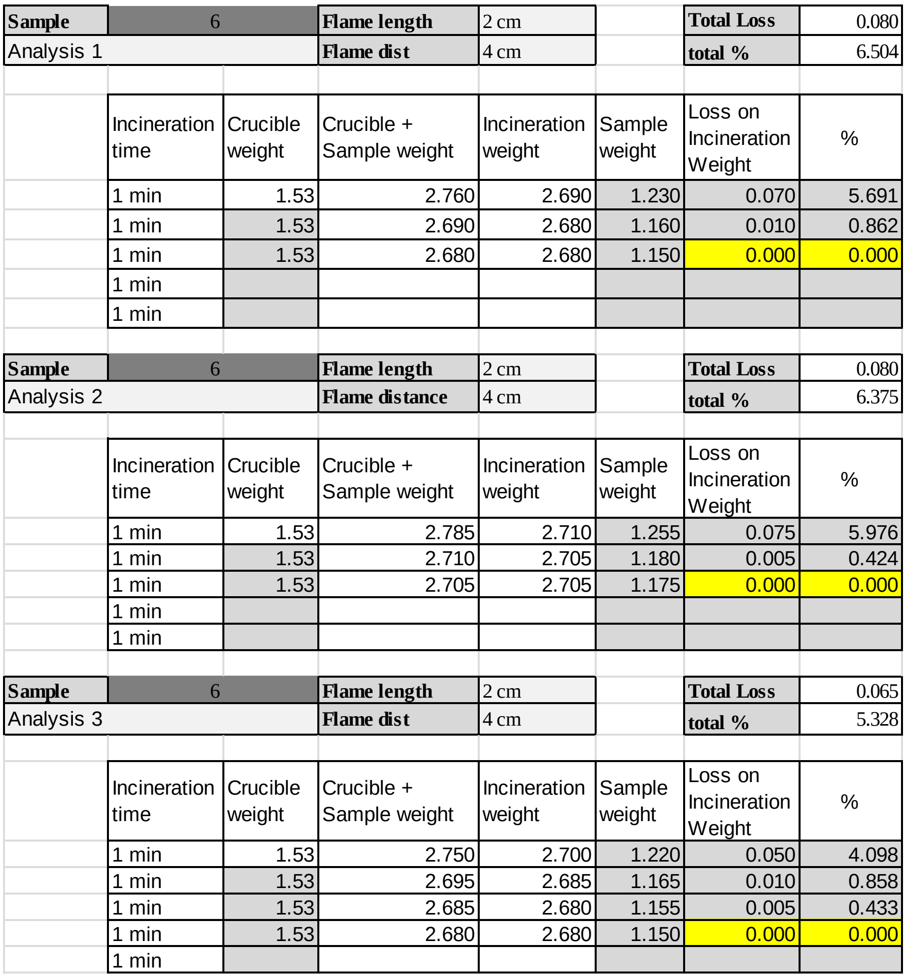
f) **Preparation of samples for analysis**. The RIFT process starts by pre-weighing the crucible on a scientific field balance (in this instance an Adam Portable Precision Balance 150 g, 0.005 g) and recording the weight before zeroing the balance. Using a thin metal spatula place about 1.2 g to 1.5 g of a specific soil sample in the crucible until about ¾ full and weigh it again. The sample weight is recorded.

g) **Incineration of samples**. The crucible containing the measured soil is placed in its holding bracket which is already set at the receiving angle. Once the crucible is secured the butane torch lighter or "incinerator" is prepared. The flexible arm of the incinerator should be flipped up so that the incinerator, when initially ignited, does not incinerate the sample. The steps for a single sample are as follows:

1. The flame is ignited and set to lean with a fully oxygenated blue flame at 2 cm in length.
2. The flexible arm is then swiftly lowered onto the sample at the same moment that a timer or stopwatch is engaged.
3. Samples are incinerated for 60 seconds at a time after which the heat source is immediately removed by disengaging the incinerator and switching off the flame.
4. The crucible is left in place to cool down for another 60 seconds.
5. The crucible is then picked up with large forceps and placed on the field balance. The weight is recorded.
6. The next step involves stirring of the sample with a stainless steel micro probe while holding the crucible with the forceps. Stir 10 times slowly in a circular motion being careful not to excite the soil particles and loose material out of the crucible. Weigh the sample again and record the starting weight for the next incineration.

7. Repeat steps 1 to 6 on the same sample in the crucible until no further weight loss is observed (point of depletion). Calculate the total weight loss by adding the individual recorded weight losses together.
8. Clear the crucible and clean it out with a felt rag to remove all previous residues from the inner surface.
9. Prepare a new sample from the same batch as in point (f) above and follow the steps again.

h) **Recording of RIFT data**. Five analyses per soil form were conducted in order to obtain a more accurate reading and to be able to detect large deviations in results that could have been caused by handling error, residual moisture from incomplete drying or contamination of samples. These different analyses can then be collated and a mean total weight loss on incineration can be determined as illustrated in Table 2 below.



Sample	6		**Flame length**	2 cm		**Total Loss**	0.080
Analysis 1			**Flame dist**	4 cm		**total %**	6.504
	Incineration time	Crucible weight	Crucible + Sample weight	Incineration weight	Sample weight	Loss on Incineration Weight	%
	1 min	1.53	2.760	2.690	1.230	0.070	5.691
	1 min	1.53	2.690	2.680	1.160	0.010	0.862
	1 min	1.53	2.680	2.680	1.150	0.000	0.000
	1 min						
	1 min						
Sample	6		**Flame length**	2 cm		**Total Loss**	0.080
Analysis 2			**Flame distance**	4 cm		**total %**	6.375
	Incineration time	Crucible weight	Crucible + Sample weight	Incineration weight	Sample weight	Loss on Incineration Weight	%
	1 min	1.53	2.785	2.710	1.255	0.075	5.976
	1 min	1.53	2.710	2.705	1.180	0.005	0.424
	1 min	1.53	2.705	2.705	1.175	0.000	0.000
	1 min						
	1 min						
Sample	6		**Flame length**	2 cm		**Total Loss**	0.065
Analysis 3			**Flame dist**	4 cm		**total %**	5.328
	Incineration time	Crucible weight	Crucible + Sample weight	Incineration weight	Sample weight	Loss on Incineration Weight	%
	1 min	1.53	2.750	2.700	1.220	0.050	4.098
	1 min	1.53	2.695	2.685	1.165	0.010	0.858
	1 min	1.53	2.685	2.680	1.155	0.005	0.433
	1 min	1.53	2.680	2.680	1.150	0.000	0.000
	1 min						

Table 2: Example of recording system for data showing three RIFT measurements (Five measurements were conducted for each sample for the final analysis). Note that when no further weight loss occurred (highlighted in yellow) the individual loss on incineration weights were added to calculate the total loss.

3.4. Statistical analysis

The RIFT method entails the measurement of the loss of weight in a sample, as is the case with LOI, and in that sense the generally accepted method of comparison is by reporting a least squares regression relationship with SOC. Then to establish the confidence limits in terms of a correlation equation and to determine the level to which they have been observed to co-vary as is evident from work by Howard & Howard (1989), Miyazawa *et al*. (2000), De Vos *et al*. (2005), Ghimire *et al*. (2007), Konare *et al*. (2010) and Wang & Wang (2011). These methods are confirmed by a statistically significant correlation, r (the Pearson correlation), between SOC and for instance LOI (Szava-Kovats, 2009).

The best calibration model is chosen on the basis of highest regression prediction and the lowest values of the root mean square error (RMSE). Wang & Wang (2011) state that regression equations for relating results of the LOI method with other organic reference C determination methods can be obtained by using the least squares regression procedure. Mostly it appears that fitting a linear regression line to the data seems to be the preferred method to obtain a high correlation in order to calibrate an alternative method with an accepted reference method (Ben-Dor & Banin, 1989; Wright *et al*., 2008; Konare et al., 2010). Since the accepted reference method is dry combustion, it was thought prudent to also compare how RIFT performs alongside another currently accepted indirect method like WB, which in some instances, as mentioned earlier, in the past was also used as a reference method.

However, Bland & Altman (1999) caution that some widely used statistical approaches like correlation, regression, and the comparison of means could be misleading; they also warn that the correlation coefficient measures the strength of a relationship between two variables and not the agreement between them. They offer a number of alternatives using simple graphical statistics of which the most relevant to this study is the use of a scatterplot that provides a line of equality (unity line) where y = x as well as plotting the difference between the methods against their mean in order to measure agreement (Bland & Altman, 1986). These potential pitfalls are recognised, as well as the fact that their work was mostly aimed at medical statistics and therefore aims to utilise aspects of relevant comparison statistics related to the comparison of a direct (total carbon) method to two indirect (derived carbon) methods.

In support of the above it may also be important to assess the statistically significant probability (p-value) as well in order to test the validity of the correlations. The p-value represents the probability of error that is involved in accepting our observed result as valid and a low p-value, generally at least < 0.05 (Hill & Lewicki, 2007), indicates the limits at which one can accept the results.

Similarly the standard error (SE) of the regression can be used to assess the precision of the predictions. Approximately 95% of the observations should fall within plus/minus 2*standard error of the regression from the regression line, which is also an approximation of a 95% prediction interval (Hill & Lewicki, 2007).

The correlation can also be illustrated in conjunction with a further comparison in a 1:1 plot. In this instance there will be no bias indicating a high accuracy, on the average, for the calibration model, meaning that if the mean error is positive then there is a systematic over-prediction, if it is negative then there is systematic under prediction (Konare *et al*., 2010).

Box plots can be utilised to determine the variability between the three methods compared to one another in terms of distribution and range for each soil form or for groups of soil forms based on their key features. Box plots will also contribute to illustrating accuracy and consistency within methods.

Lastly it was deemed important to conduct multiple regressions and the use of analysis of variance (ANOVA) to test for significant differences between the means for the soil forms and the testing methods. This could be helpful in determining the validity of comparisons between the testing methods considering there were at least 55 observations per treatment.

CHAPTER 4 RESULTS

4.1. Analysis and results for different soil forms

A general standard baseline assessment of important general quantitative and qualitative soil features and characteristics for the 11 soil forms was conducted. Apart from possibly finding correlations between variables (like clay content and other mineral characteristics), it was thought that some of these features may retrospectively elucidate specific behaviours of specific soil forms under specific treatments and therefore included assessment for:

a) Soil textural class
b) Percentage clay, silt, sand
c) pH (KCl)
d) Resistance (Ohm)
e) H+(cmol/kg)
f) Available P & K (mg/kg)
g) Exchangeable cations (cmol(+)/kg) – Na^+, K^+, Ca^{++}, Mg^{++}
h) CEC (pH 7) - cmol(+)/kg
i) Base Saturation % of Na, K, Ca, Mg
j) T-Value cmol/kg

While the baseline analysis and the blind samples for analysis by wet chemical oxidation and dry combustion were conducted by Bemlab, the student also tested the 11 soil forms independently for SOM using the RIFT method and recorded the results (see Tables 3 & 4 below). Since the RIFT method oxidises SOM which normally contains 50% C by weight according to Brady & Weil (2008) it was decided to convert the values for RIFT back to SOC in order to facilitate visible comparisons in simple graphs (although it did not make a difference in regression analysis). The equation to derive SOC from RIFT SOM was established as:

$$\text{SOC \% from RIFT} = ((\text{dry weight} - \text{Incinerated weight} / \text{dry weight}) \times 100)/2 \qquad \text{(Equation 1)}$$

Therefore apart from the baseline assessment there were also separate tests for soil C using wet chemical oxidation (Walkley-Black) and dry combustion (with a Leco device) in the

Plot	Soil	pH	Resist.	H^+	P Bray II	K	Exchangeable cations (cmol(+)/kg)				CEC (pH 7)	Na^+	K^+	Ca^+	Mg^+	T-Value	Clay	Silt	Sand
	Texture	(KCl)	(Ohm)	(cmol/kg)	mg/kg		Na^+	K^+	Ca^+	Mg^+	Unit	%	%	%	%	cmol/kg	%	%	%
1	Sand	5.2	4220	0.30	7	20	0.11	0.05	0.83	0.39	2.89	6.72	3.09	49.37	23.08	1.69	5	2	93
2	Sand	6.5	4430		31	8	0.05	0.02	1.47	0.32	2.58	2.48	1.06	79.24	17.22	1.85	7	4	89
3	Loam	5.1		1.71	7	202	7.00	0.52	7.38	6.20	14.03	30.68	2.27	32.39	27.17	22.80	30	32	38
4	Loam	5.6	140	0.35	14	122	4.90	0.31	11.19	9.50	16.78	18.67	1.19	42.62	36.19	26.26	29	20	51
5	Loam	5.1	1450	1.26	16	249	0.65	0.64	6.97	3.91	12.92	4.83	4.74	51.95	29.10	13.42	22	32	46
6	Loam	4.6	2580	1.01	1	29	0.27	0.08	1.82	1.16	4.54	6.18	1.74	42.04	26.75	4.34	25	26	49
7	Loam	4.9	2110	0.80	4	62	0.32	0.16	3.60	1.58	7.74	4.91	2.43	55.75	24.52	6.46	21	14	65
8	Loam	4.7	1710	0.86	3	42	0.31	0.11	2.65	1.52	6.58	5.66	1.96	48.63	27.96	5.45	19	28	53
9	Loam	3.7	1260	1.06	4	114	0.21	0.29	0.73	0.30	4.25	8.18	11.27	28.08	11.48	2.59	16	35	49
10	Loam	3.9	4430	3.47	3	39	0.18	0.10	0.47	0.45	7.83	3.77	2.16	10.05	9.56	4.66	21	26	53
11	Clay	4.0	3220	1.56	1	53	0.27	0.14	1.44	1.32	5.13	5.63	2.87	30.49	27.99	4.72	25	32	43

Table 3: The soil analysis report for the standard baseline assessment conducted by the independent analytical laboratory

	Leco 1	Leco 2	Leco 3	Leco 4	Leco 5	Leco Mean	**Leco SDev**	WB 1	WB 2	WB 3	WB 4	WB 5	WB Mean	**WB SDev**	RIFT 1	RIFT 2	RIFT 3	RIFT 4	RIFT 5	RIFT Mean	**RIFT SDev**
S2-Constantia	0.14	0.17	0.17	0.20	0.23	0.18	**0.03**	0.52	2.10	2.17	2.17	2.50	1.89	**0.78**	0.00	0.21	0.41	0.41	0.62	0.33	**0.24**
S1-Lamotte	0.21	0.24	0.24	0.27	0.29	0.25	**0.03**	0.37	2.02	2.25	2.94	3.24	2.16	**1.12**	0.99	1.00	1.16	1.32	1.32	1.16	**0.16**
S11-Klapmuts	1.40	1.41	1.47	1.48	1.48	1.45	**0.04**	1.54	2.00	2.02	2.19	2.48	2.05	**0.34**	1.84	1.84	2.05	2.07	2.67	2.09	**0.34**
S6-Oakleaf	0.93	1.31	1.48	1.84	2.12	1.54	**0.46**	2.58	2.71	2.86	3.15	3.45	2.95	**0.35**	2.66	3.04	3.19	3.25	3.88	3.20	**0.44**
S8-Westleigh	1.16	1.47	1.63	1.67	1.90	1.57	**0.27**	1.57	2.22	2.31	2.52	2.52	2.23	**0.39**	2.22	2.42	2.43	2.44	2.65	2.43	**0.15**
S7-Estcourt	1.57	2.17	2.32	2.64	2.56	2.25	**0.42**	1.55	2.01	2.18	2.18	3.74	2.33	**0.83**	1.86	2.04	2.07	2.25	2.66	2.18	**0.31**
S10-Magwa	1.57	2.61	2.62	2.67	2.76	2.45	**0.49**	1.48	2.15	2.20	3.35	5.13	2.86	**1.44**	2.26	2.49	2.70	2.71	2.88	2.61	**0.24**
S9-Kranskop	3.05	3.97	4.16	4.47	6.19	4.37	**1.15**	1.94	2.43	2.86	3.86	5.81	3.38	**1.53**	3.56	4.20	4.37	4.42	4.74	4.26	**0.44**
S5-Nomanci	4.31	6.38	6.46	7.40	7.63	6.44	**1.31**	3.84	4.63	5.81	6.22	7.01	5.50	**1.27**	6.11	6.20	6.50	7.23	7.45	6.70	**0.61**
S3-Champagne	5.19	6.72	6.79	7.34	7.74	6.76	**0.97**	2.87	3.05	4.27	5.30	6.06	4.31	**1.39**	4.71	4.94	5.17	5.31	5.71	5.17	**0.38**
S4-Tukulu	5.82	6.56	7.68	7.90	7.92	7.18	**0.94**	2.87	3.46	4.09	5.39	6.92	4.55	**1.62**	5.14	5.77	6.14	6.28	6.61	5.99	**0.56**

Table 4: Organic carbon levels for five observations using dry combustion with a Leco device and wet chemical oxidation (WB) conducted by an independent analytical laboratory. The RIFT methodology follows reflecting the 5 observations per soil form and the mean and Standard deviation (SDev) for each of the three methods are included.

laboratory as well as the independent RIFT assessment conducted by the student. There were thus 5 tests for each of the 11 soil forms for each of the three C quantification methods. This equated to n = 11 x 3 x 5 (165 final samples, thus 55 samples per method).

4.2. Datasets for dry combustion, Walkley-Black and RIFT

All available results were compiled into datasets in order to facilitate statistical assessments of the three methods within each of the selected soil forms (*n* = 11). The dry combustion results were considered the standard in all cases based on the fact that it is widely considered as the reference method for C determinations (Howard & Howard, 1989; Konen *et al.*, 2002; McCarty *et al.*, 2002; Blaisdell *et al.*, 2003; Rowell & Coetzee, 2003; Mikhailova *et al.*, 2003; Gehl & Rice, 2006; Chatterjee *et al.*, 2009; Konare *et al.*, 2010; McCarty *et al.*, 2010).

Datasets were separately prepared for dry combustion with a Leco device, for Walkley-Black and for RIFT. The mean SOC% for the five individual tests per soil form were then calculated and for the RIFT treatment the SOM was converted to SOC by the general application of a SOM:SOC factor of 0.5 (Brady & Weil, 2008). This general conversion was initially done because a valid regression for the full dataset had not yet been run and it was the intention of the student to confirm that 0.5 x SOM is an applicable conversion factor for the particular soils.

	Leco	WB	RIFT SOC	Clay %
S2-Constantia	0.182	1.89	0.331	7.00
S1-Lamotte	0.25	2.16	1.159	5.00
S11-Klapmuts	1.448	2.05	2.093	25.00
S6-Oakleaf	1.536	2.95	3.203	25.00
S8-Westleigh	1.566	2.23	2.432	19.00
S7-Estcourt	2.252	2.33	2.177	21.00
S10-Magwa	2.446	2.86	2.608	21.00
S9-Kranskop	4.368	3.38	4.257	16.00
S5-Nomanci	6.436	5.50	6.697	22.00
S3-Champagne	6.756	4.31	5.167	30.00
S4-Tukulu	7.176	4.55	5.989	29.00

Table 5: The combined dataset of means to be used for regressions with RIFT OC and Clay%

Because of the known influence of clay on the SOC levels in soil and the potential for over or under estimation based on structural water loss (Ball, 1964; Howard & Howard, 1989; Schumacher, 2002; Santisteban, 2004; De Vos *et al.,* 2005; Sun *et al*., 2009) it was thought prudent also to include Clay % in the dataset (Table 5).

This may prove useful in understanding particular differences in methods and the different soil forms. At first glance the histogram illustrated in Figure 5 below demonstrated significant comparability between the methods based on multiple iterations of the same test. However, the only aspect that can be derived from this graph was that RIFT was generally closer to Dry Combustion than WB in terms of estimated SOC.

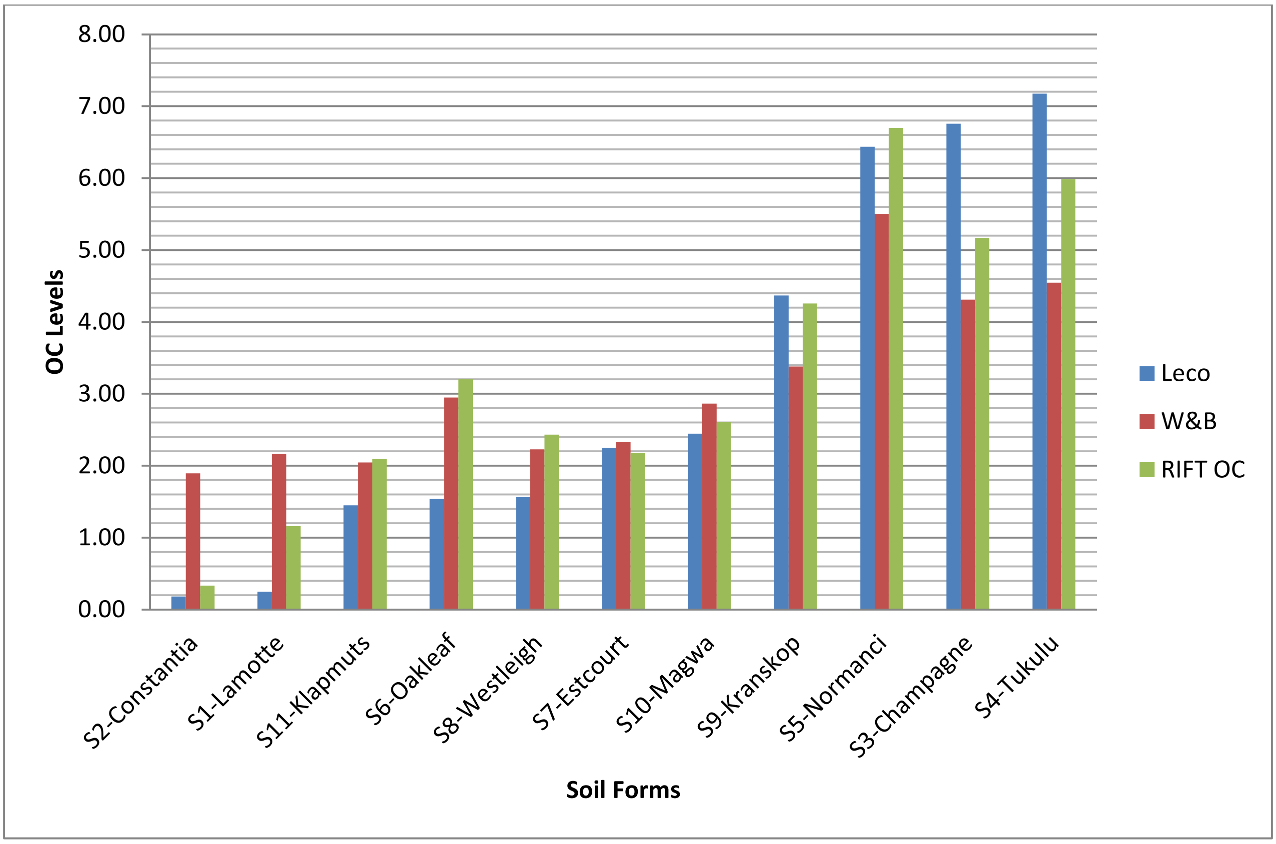


Figure 5: Histogram of the three methods where each bar represents the mean value for 5 observations of the specific treatment from the same sample.

Box plots were employed (using STASTISTICA 12) to determine the range and variability of data within each soil form for each of the three treatments for OC % determination. The box plots in Figure 6 below illustrate the relationship between the three testing methods for each soil form. Logically speaking one would have more confidence in measures on the same sample that are distributed closer together towards the correct value. It is therefore important to analyse the box-plot results with the assumption that dry combustion with a Leco device was accurate with varying degrees of spread (variability).

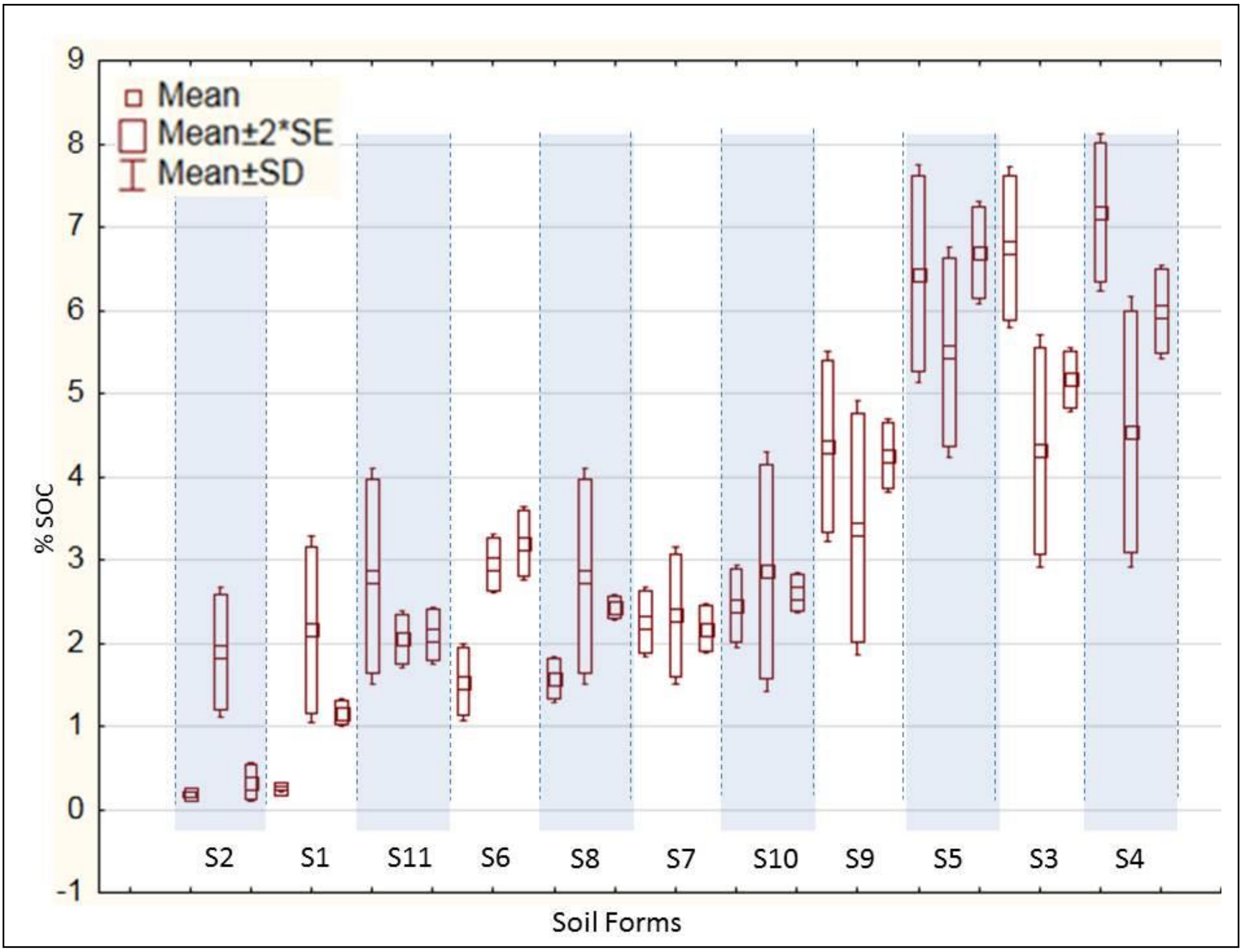


Figure 6: Individual box plots for each soil form (n=5) with the three treatments (per coloured bands) in the following order: Leco, Walkley Black and RIFT.

This provides for two aspects of accuracy: firstly how close to correct and secondly how tightly distributed the observations are. This can be illustrated for the 11 soil forms and the three testing methods by using a table and providing values for accuracy and least spread (see table 6). Most accurate will be Leco because it represents the reference value for SOC determinations and therefore will receive one point in each instance whilst the second most accurate method will receive two points and the least accurate will receive three points.

This accuracy is thus based on the closeness of the means of WB and RIFT to dry combustion with a Leco device. However, all three methods compete for least spread (represented by 1 point) to most spread out or highest variability (represented by 3 points).

From the accuracy and spread matrix in Table 6, it is evident that RIFT_OC was more accurate than WB and had by far the least variability of all the methods between the 5 values used to construe the means. The RIFT method seemed to be slightly less effective with the Oakleaf, Klapmuts and Estcourt soil forms on the grounds of accuracy but with the Klapmuts and Estcourt soil forms RIFT_OC actually had the least variability as measured through the differences of the residual variances from the means.

	Accuracy between WB & RIFT_OC			Variability between Leco, WB & RIFT_OC		
Soil Form	**Leco**	**WB**	**RIFT_OC**	**Leco**	**WB**	**RIFT_OC**
S2-Constantia	1	3	2	1	3	2
S1-Lamotte	1	3	2	1	3	2
S11-Klapmuts	1	2	3	3	2	1
S6-Oakleaf	1	2	3	2	1	3
S8-Westleigh	1	3	2	1	3	2
S7-Estcourt	1	2	3	2	3	1
S10-Magwa	1	3	2	2	3	1
S9-Kranskop	1	3	2	2	3	1
S5-Nomanci	1	3	2	2	3	1
S3-Champagne	1	3	2	2	3	1
S4-Tukulu	1	3	2	2	3	1
Incidences of most accuracy and least spread		3	**8**	3	1	**7**

Table 6: Matrix illustrating accuracy between RIFT and WB and least spread between the three methods for each soil form. The final score reflects the number of incidences that a method illustrated more accuracy and with the least variability per soil form.

It was therefore only with the Oakleaf soil form that the RIFT method was less accurate and had more variability than Walkley-Black albeit only marginally. In most cases where RIFT was more accurate or less variable than Walkley-Black it was with significantly higher margins.

4.3. Analysis of relationships and interpretation of results

The data for regression analysis were collected into two datasets, firstly per soil form for which the mean of the five observations per soil form is calculated and displayed as 11 data points per testing method and secondly as unique or individual linked observations per sample regardless of soil form displayed as 55 data points per testing method. The relationships between the different testing methods needed to be assessed as well as the potential need for incorporating more predictor variables or factors for consideration of correction of the RIFT observations towards improved accuracy.

Regression analysis commenced on STATISTICA 12 with scatterplots of Leco vs RIFT and Leco vs WB and the lines of equality (1:1 Line) were given on these graphs. This is loosely based on earlier work by Bland & Altman (1986) who suggested its use in assisting to gauge the degree of agreement between measurements within clinical measurement comparisons of a new measurement technique with an established one. In this instance it showed that the correlation of RIFT with dry combustion was significantly higher than WB.

Results from a regression with dry combustion (Leco) as reference variable and RIFT and WB as predictor variables illustrated that RIFT indeed correlates strongly and linearly with dry combustion (with RIFT significantly better than WB). As can be seen in Table 7 above the marked correlations (Highlighted in red or *) are significant at least at p < .01 providing enough grounds for the consideration of a significant relationship.

	Correlations (RIFT Base Data 1) N=11 (Casewise deletion of missing data)		
Variable	Leco	WB	RIFT_OC
Leco	1.000000		
WB	0.930433*	1.000000	
RIFT_OC	0.951999*	0.966076*	1.000000

Table 7: Pearson correlation statistics for the relationship between Leco, WB and RIFT. Marked correlation coefficients (*) indicate p < 0.01.

Furthermore as illustrated by the scatterplots in figures 7 and 8 (11 Soil Forms) and figures 9 and 10 (55 unique samples) against the line of equality, it is visibly obvious that there is less variability between Leco vs RIFT than between Leco vs WB.

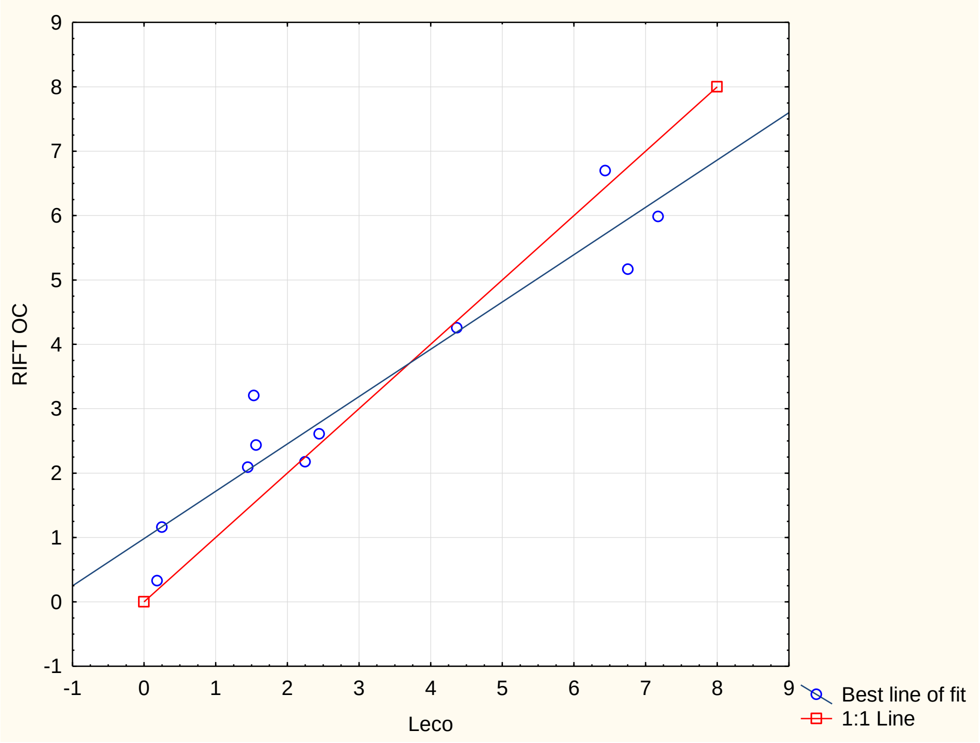


Figure 7: Scatter plot of RIFT vs Leco indicating the line of equality (Unity Line). Regression equation y = 0.9838 + 0.7349*x; Correlation coefficient r = 0.9520, $p < 0.00001$; $R^2 = 0.9063$

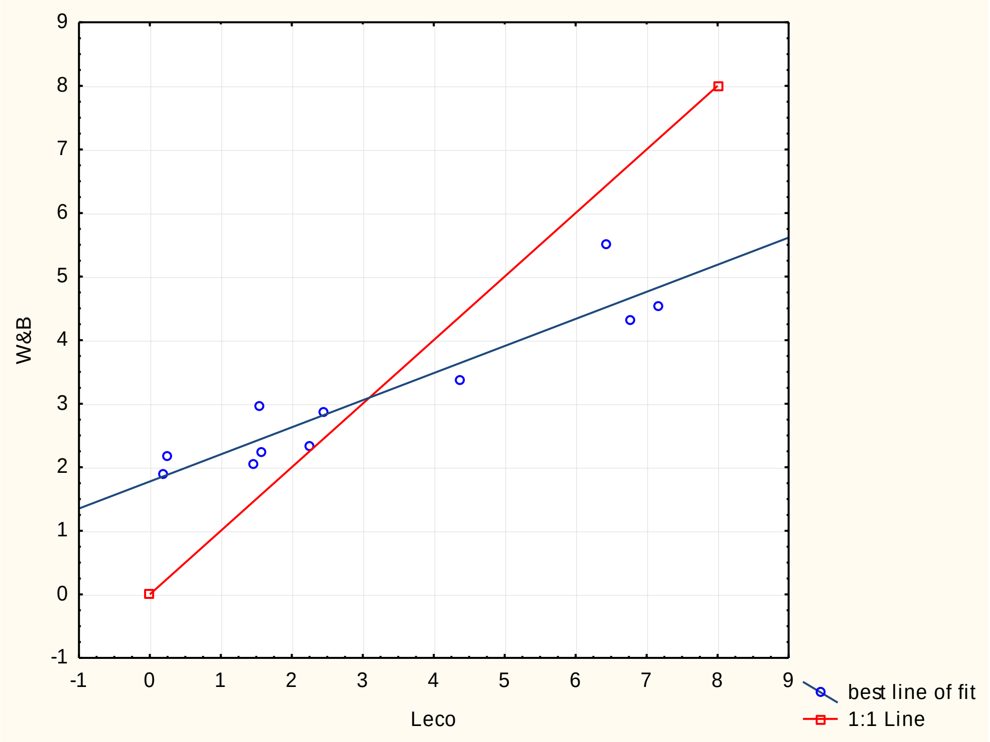


Figure 8: Scatter plots of WB vs Leco indicating the line of equality (Unity Line). Regression equation y = 1.7765 + 0.4263*x; Correlation coefficient r = 0.9304, $p < 0.00003$; $R^2 = 0.8657$

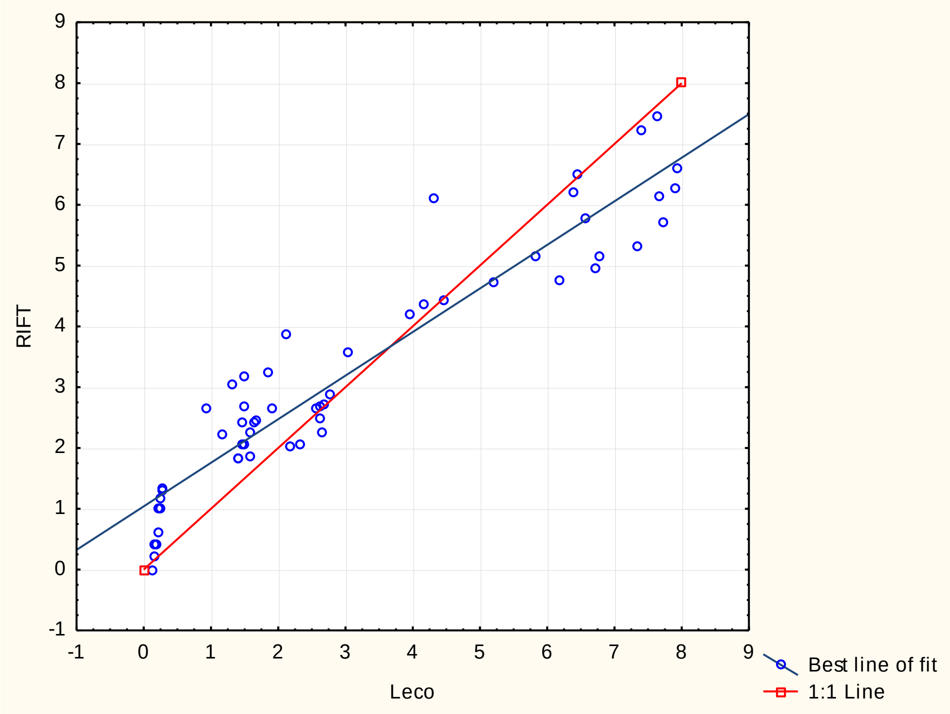


Figure 9: Scatter plot of RIFT vs Leco indicating the line of equality (Unity Line) for the 55 unique observations. Regression equation y = 1.0415 + 0.7164*x; Correlation coefficient r = 0.9434, $p < 0.01$; $R^2 = 0.8899$

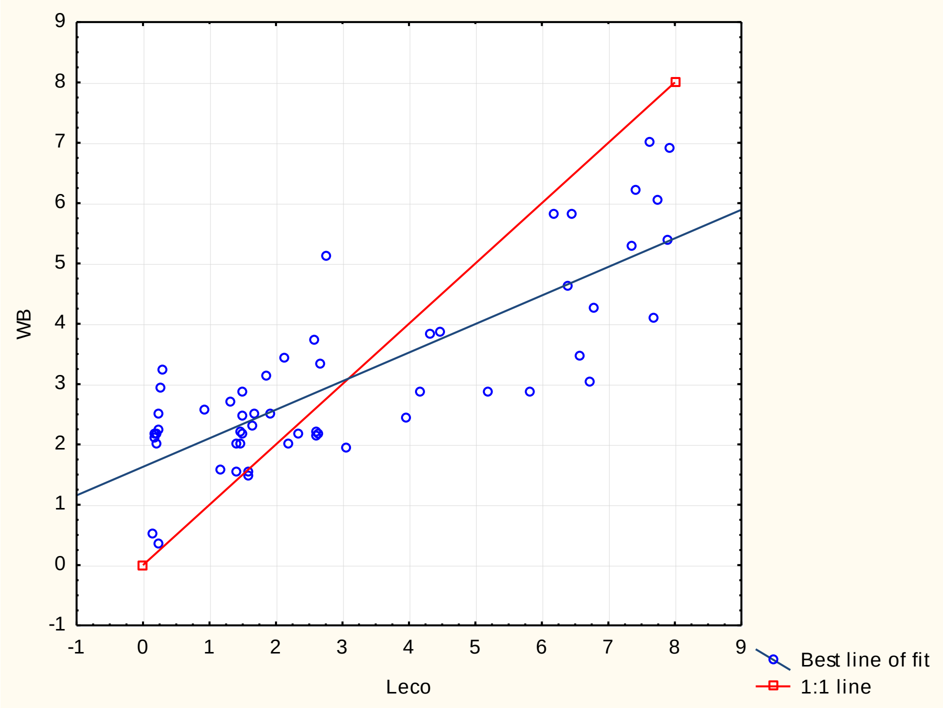


Figure 10: Scatter plot of WB vs Leco indicating the line of equality (Unity Line) for the 55 unique observations. Regression equation y = 1.629 + 0.4734*x; Correlation coefficient r = 0.8047, $p < 0.01$; $R^2 = 0.6476$

In terms of the 11 soil forms, the scatter plot of RIFT vs Leco illustrates a deviation from the 1:1 line that is significantly less than the deviation observed on the scatterplot of WB vs Leco . The scatterplot also represents the correlations as follows:

RIFT vs Leco:

- Regression equation: $y = 0.9838 + 0.7349*x$;
- Correlation coefficient $r = 0.9520$, $p < 0.00001$; $R^2 = 0.9063$

WB vs Leco:

- Regression equation $y = 1.7765 + 0.4263*x$;
- Correlation coefficient $r = 0.9304$, $p < 0.00003$; $R^2 = 0.8657$

Similarly in terms of the 55 unique and linked samples the scatterplot of RIFT vs Leco the deviation from the 1:1 line is significantly less than the deviation observed on the scatterplot of WB vs Leco. The scatterplot also represents the correlations as follows:

RIFT vs Leco:

- regression equation $y = 1.0415 + 0.7164*x$;
- Correlation coefficient $r = 0.9434$, $p < 0.01$; $R^2 = 0.8899$

WB vs Leco

- Regression equation $y = 1.629 + 0.4734*x$;
- Correlation coefficient $r = 0.8047$, $p < 0.01$; $R^2 = 0.6476$

It is therefore evident that RIFT offers a significantly better correlation with dry combustion than WB. The differences in deviation are also described in Table 8 which illustrates the extent to which RIFT SOC is more closely related to Leco for reported SOC% than WB.

It is also evident that SOC determined with RIFT (and the 0.5 x SOM conversion) is generally more accurate than determining SOC by means of the Walkley-Black method for the selected soils. Table 8 represents the three methods and the difference of the means squared from dry combustion for WB and RIFT_OC respectively. The differences of the means were squared in order to render the equations positive so that they could be accumulated in a value for comparison (Where $\sum\Delta^2$ = the sum of the differences from the means squared). It is evident that over the range of the means for 11 soil forms that basic RIFT and the conversion from SOM to RIFT SOC through converting with a factor of 0.5 is more accurate than standard WB.

The lowest residual $\sum\Delta^2$ values (See Table 8) illustrate clearly to what extent in general RIFT_OC is more accurate than WB. Column A shows the sum of residual differences for RIFT SOC to be 8.841, almost three times more accurate compared to WB.

				A	**B**
Soil Form	**Leco**	**WB**	**RIFT_OC**	**Rift_OC Δ^2**	**WB Δ^2**
S2-Constantia	0.182	1.892	0.331*	0.022	2.924
S1-Lamotte	0.250	2.164	1.159*	0.827	3.663
S11-Klapmuts	1.448	2.046	2.093	0.416	0.358
S6-Oakleaf	1.536	2.950	3.203	2.780	1.999
S8-Westleigh	1.566	2.228	2.432	0.749	0.438
S7-Estcourt	2.252	2.332	2.177*	0.006	0.006
S10-Magwa	2.446	2.862	2.608*	0.026	0.173
S9-Kranskop	4.368	3.380	4.257*	0.012	0.976
S5-Nomanci	6.436	5.502	6.697*	0.068	0.872
S3-Champagne	6.756	4.310	5.167*	2.524	5.983
S4-Tukulu	7.176	4.546	5.989*	1.410	6.917
			$\sum\Delta^2$	**8.841**	**24.311**

Table 8: Extent to which RIFT SOC is more closely related to Leco for reported SOC% than WB. (* Instances where RIFT_OC values were more accurate than WB values)

In order to ascertain whether clay % as a variable may increase the accuracy of RIFT SOC predictions, a regression summary was obtained through STATISTICA 12 by plotting the residual values (Δ^2) of RIFT and WB against clay %. The variable clay % illustrated a much more significant correlation with RIFT than with WB (Figures 11 and 12).

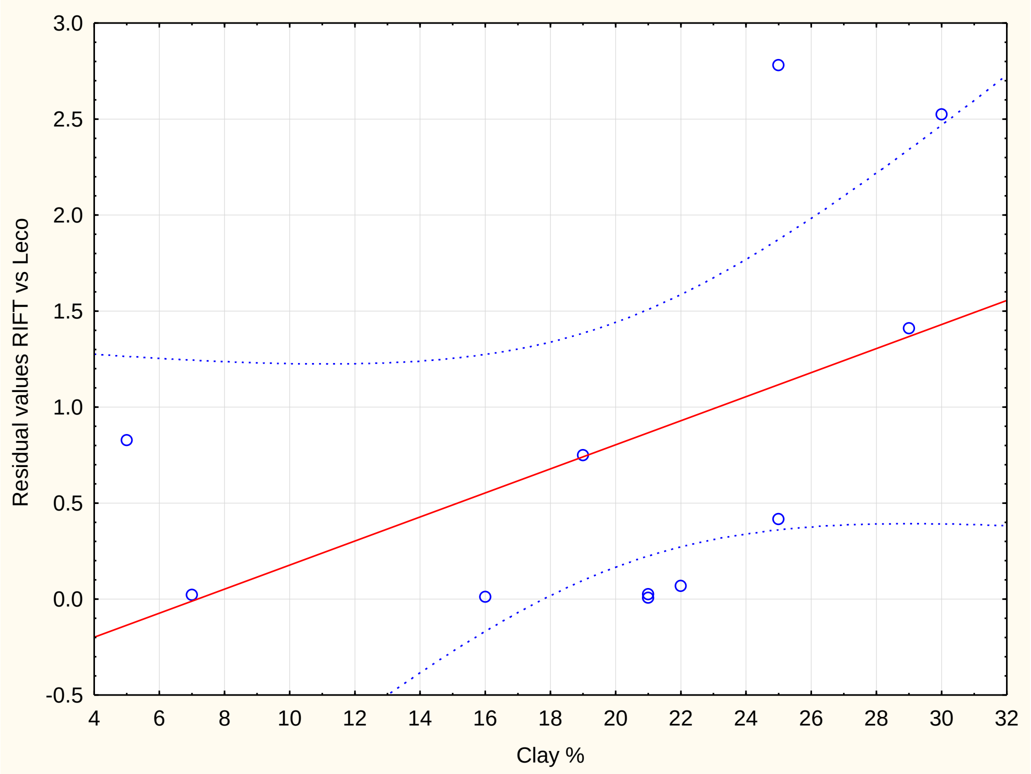


Figure 11: Scatter plot of RIFT residuals (Table 8: column A) vs Clay % with regression equation: $y = -0.4491 + 0.0626*x$; Correlation coefficient $r = 0.4943$, $p = 0.1222$; $R^2 = 0.2443$

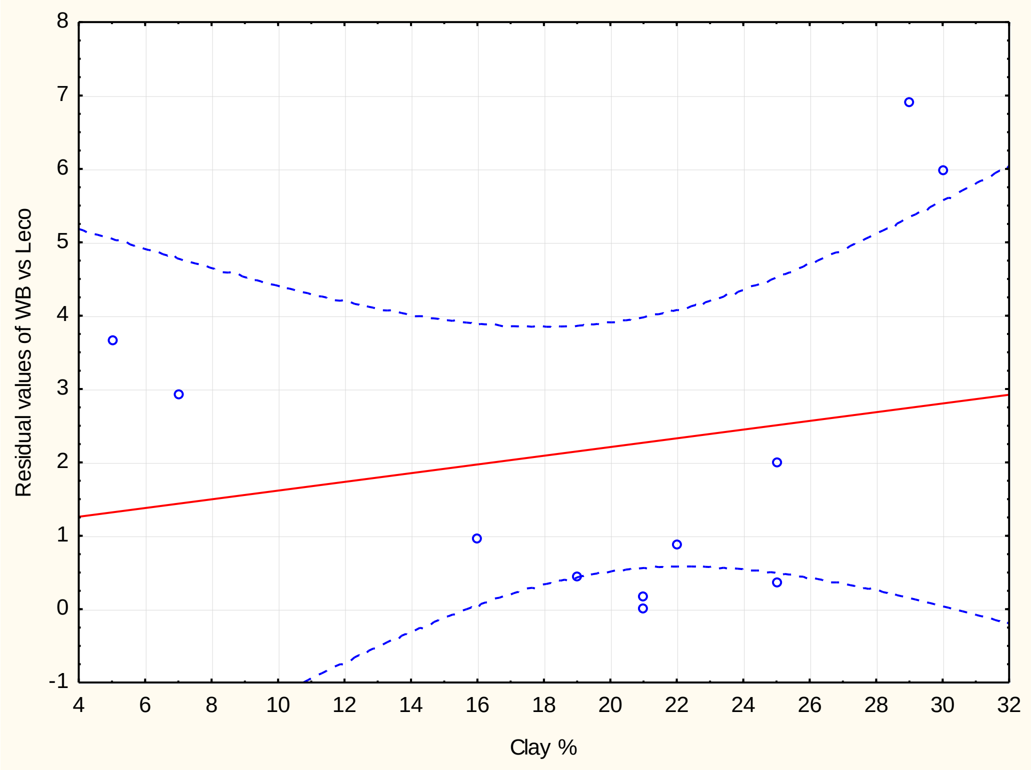


Figure 12: Scatter plot of WB residuals (Table 8: column B) vs Clay % with regression equation: $y = 1.0219 + 0.0594*x$; Correlation coefficient $r = 0.1986$, $p = 0.5582$; $R^2 = 0.0395$

However the correlation of RIFT_OC Δ^2 with clay % was not very significant as illustrated by the correlation coefficient of r = 0.4943 (Figure 11). Clay % as a variable was therefore not used in this study to obtain further refinement of RIFT predictions. It was also observed that for RIFT the correlation with clay % was more significant at very low clay % values and at very high clay % values. This reflects on the condition where the soils with very little to no clay contents were generally more sandy and low in SOC and the soils with high clay contents generally contained more SOC. Soils with intermediate clay contents contained variable amounts of SOC making it unsuitable for a linear regression analysis.

Previous researchers have also speculated about this feature of clay content (Konen *et al*., 2002) and attributed it to variability of SOM, clay content and variation in clay mineralogy (Konare *et al*., 2010) across land types. It was also noticed in this study that this variability exists across soil forms and one cannot base assumptions of higher or lower SOC content merely on the soil form, rather its particular clay mineralogy and many other features need to be considered. Clay content could potentially be of significant value in improving the accuracy of RIFT in predicting SOC but potentially through an alternative predictive tool not constrained by the linear nature of regression statistics.

CHAPTER 5 DISCUSSION AND RECOMMENDATIONS

5.1. General observations

South African soils are generally low in SOM (Du Preez *et al*., 2011); about 58% of top soils contain less than 0.5% organic carbon and only 4% of top soils contain more than 2% organic carbon. We will also find that variations (sometimes large) exist within and between soil forms and that each soil sample may have unique RIFT/SOC relationships depending on its particular climatic conditions, vegetative cover, topographical position and soil texture.

This variability is probably the reason why a universal equation predicting SOC from for instance, LOI does not exist (Konen *et al*., 2002). It is therefore assumed that soil texture characteristics like loam, clay, sand and silt will behave differently with the application of intense heat on account of unique balances of different soil fractions. It is known that LOI overestimates the organic matter content (Gehl & Rice, 2006) and may require unique correction equations to compensate for structural water from clays, oxide colloids (sequioxides), carbonates, and elemental C like charcoal (De Vos *et al*., 2005). With the exception of areas close to the coastline, soils of the Garden Route region do not normally include soils with high carbonates that may oxidise at high temperatures and lead to an overestimation of the organic fractions. The absence of free Ca in the soils from the study area is mainly related to the relatively high rainfall conditions. However the most significant factor to consider for correction remains clay content. It is therefore assumed that RIFT may also overestimate organic matter content where high levels of structural water occur. Although the relationship was not statistically significant between RIFT residuals and clay %, it is expected that higher clay contents may lead to bigger differences between Leco and RIFT.

A further soil characteristic that may influence the prediction of SOC is bulk density. It relates to the relationship between soil organic carbon, bulk density, and soil depth, where in most soils, the highest soil carbon values were linked with low bulk density values and *vice versa* (Rantoa, 2009). Heuscher *et al*., (2005) states that SOC shows a negative relationship with bulk density in that bulk density increases as SOC decreases. Many studies have shown the effect of SOC on the bulk density in various soils (Bernoux *et al*., 1998; Heuscher *et al*., 2005) where measurements of SOC content was used to predict bulk soil density. However, as mentioned before, such data are considered too labour intensive and time-consuming for this methodology.

Because of the ubiquitous nature of clay in most soils it is also assumed that the repetitive intense heat of the RIFT method will mostly destroy all bound water. Structural water may start declining at temperatures as low as 280–400 °C (Heiri *et al*., 2001) and different salts present in soil release molecular water at different temperatures above 105°C, whereas some clays like Na-montmorillonite, vermiculite, gibbsite, goethite, and brucite lose crystal-lattice water between 150 to 250°C (Chatterjee *et al*., 2009).

Soil organic matter can only be indirectly measured in soils because of its complex nature and mixing with inorganic materials and it is therefore preferred to use SOC content as a measure of organic matter and not *vice versa* (Konen *et al*., 2002; De Vos *et al*., 2005). However it is assumed that generally SOM equates to 2 times SOC (Brady & Weil, 2008) and this assumption was to be applied to the selected soil forms in general and can also be tested through experimentation.

Konare *et al*. (2010) and Konen *et al*. (2002) propose that calibration will also be needed for soil populations at least at regional level. De Vos *et al*. (2005) recommend that calibration coefficients be considered at the ecosystem level too.

LOI determination of organic carbon is found to be more accurate in soils with higher organic matter content than sandy soils low in organic matter e.g. soils with lower than 5% organic matter (De Vos *et al*., 2005). It will therefore be important to assess the usefulness of the RIFT method for soils below 5% SOC content.

Lastly it may be necessary to develop unique RIFT/SOC equations for each different soil type in the study if it is found that the SOM ratio to SOC does not generally equate to a stable ratio for most soils, e.g. 2:1 as suggested by Brady and Weil (2008).

5.2. Variability in WB, Leco and RIFT

It was noted that great variability exists within the reference method as well as the alternative methods. It was also noted that there was much less variability observed within the RIFT data than within the data of the conventional laboratory tests for dry combustion and WB. This can be attributed to a few possibilities, i.e.:

a) That the individual soil samples for the same soil form differed markedly
b) That lower thresholds for operator consistency could exist within the conventional laboratory standards for testing with both dry combustion and WB
c) That lack of equipment calibration may have allowed for more variability
d) That there was simply more attention paid to detail by the student in the RIFT method to ensure repeatability.
e) That the structural or chemical characteristics of the particular soils caused more variability in the readings than would normally be the case.

The student took the utmost care to ensure homogenisation of the soil samples by sieving in a < 2mm sieve drum and removing larger stones and organic materials repeatedly. The student also noticed that while making up smaller samples, when soil is manipulated, the finer fractions may settle out and the larger grainier fractions may end up in the upper layers of a sample. This was countered by mixing the soil samples uniformly in the mortar with a spatula each time before scooping it into the sample sachets and sealing it before sending it to the professional laboratory and for use in the RIFT method. However, it could have occurred that the laboratory staff did not mix their samples before adding them to their devices, but this is mere speculation. This is an important aspect of soil measurement though because if a researcher pours a sample from a sample bag then only the upper part of the sample may end up in the receptacle. This could mean that the test is done on a part of the sample with a very different physical nature than another part of the sample.

To put it in perspective one may need to look at the data again and recognise that dry combustion had the least variability for the two sands (Lamotte & Constantia). This could be on account of the fact that there wasn’t much carbon or other volatiles that could be detected by the analyser. Similarly the RIFT method also had little variability but the WB method illustrated great variability for the measurements of the two sands. The general reasons for variability may also become evident if one investigates these issues related to each individual soil type further.

It is recommended that where small samples of soil are tested that the utmost care be taken in ensuring total homogenisation of the sample in order to test the full complement of textural variation within a sample and not possibly just one or two of those fractions.

5.3. Accuracy of RIFT compared to standard testing methods

The RIFT method illustrated significant potential as an accurate test for SOC. In some instances this led the student to question the accuracy of the current acceptable methods, the indirect methods (like LOI and WB) as well as even the reference method. This study highlighted the notion that the nature and characteristics of the soil may best indicate which method of testing is ideally suited for determining its SOC content accurately.

In terms of WB and RIFT, both techniques correlated well with SOC determined by dry combustion and both techniques were good predictions of SOC. However RIFT with a conversion factor of 0.5 was more effective in reporting SOC for at least 72% of the particular soil forms than WB. Furthermore the variances in accuracy for the 55 unique linked samples showed that RIFT was more effective in 63.6% of observations and has 2.7 times less residual distance than WB from the reference method.

The deviations from the 1:1 lines as illustrated by Figures 7 to 10, are a function of the nature of the indirect methods, i.e. the consumption of organic matter through incineration and wet chemical oxidation. The fact that the deviations are much less pronounced in RIFT than WB could be ascribed to the more complete consumption (point of depletion) that occurs during the RIFT method. Both, however, overestimate SOC significantly at very low levels (under 3% SOC) and both underestimate SOC at higher levels (above 6.5 % SOC) although to a lesser extent.

A possible explanation for this phenomenon as far as RIFT is concerned is that with very low SOC soils the structural water fractions are less dense or pervasive leading to more complete consumption. It is suggested that in higher SOC soils (which also contain more than 25% clay) the consumable products are more dense or compact offering resistance to full consumption. This explanation is supported by the fact that in the instances where both SOC and clay % were high, it took many incinerations (from 4 to 6) before the consumables were deemed depleted. Inversely where SOC and clay % was very low, it took only from 2 to 3 incinerations before the consumables were deemed depleted. This could be remedied by more intense incineration or an extended incineration regime in future. This disparity could also be remedied by employing a gravimetric device with more sensitivity. In this instance an Adam Portable Precision Balance with an increment ability of 0.005g was used. There is therefore a gap between 0.001g and 0.005g that remained unaccounted for with the gravimetric device that was used. It is suggested that a gravimetric device be used that can discern a load of a little as 0.001g.

It is also recommended that a predictive tool be developed based on the findings of the research and compiled into a matrix for correction of clay for RIFT SOC. Such a tool could be based on simple rules associated with the observed behaviours of the soil samples under specific testing methods and could report a corrected RIFT SOC value based on the residual percentages of significant over or under estimation of SOC. This could establish a factor mean for each soil condition based on residual factor percentages of over or under estimation within the context of clay as major influence on the outer values (very low and very high clay %) whilst standard RIFT observations remain consistent in the mid region SOC values.

The tool should take cognisance of the fact that at relatively high or very low levels of clay, the correction factor can be attributed to clay content. In between very low clay and very high clay e.g. the mid regions between 10% and 25% clay, it was found that there is very little consistency in terms of the clay content as a linear predictor of SOC content. This could also be on account of the type of clay in the mid regions, which can differ significantly based on their clay characteristics e.g. the amount of clay-bound organic matter can differ significantly between kaolinite and smectite (Konare *et al.*, 2010).

Therefore the RIFT SOC and clay correction matrix tool could be a partial correction matrix and applies only to the areas where RIFT significantly overestimates or significantly underestimates SOC. The matrix should also be able to take easily observable rudimentary clay content of the particular soil into consideration. Simple rules that should be associated with the RIFT and clay matrix:

- Where clay is very low, the factor mean is considered for overestimation (this is calculated as the mean of all the residual values for samples with lower than 10% clay content).
- Where clay is high, the factor mean is considered for underestimation (this is calculated as the mean of all the residual values for samples with, for instance, higher than 27% clay content).

The rules for the proposed RIFT and clay content correction matrix also support the models for all soil samples grouped according to their USDA texture classes (De Vos *et al*., 2005), in that low clay up to 10% represents the range from sand to loamy sand to sandy loam and that medium clay from about 10 to 15% represents loam soil, from 15 to 27% clay represents silt loams, and higher than 27% represents clay loams.

However, for such a model to be effective and valid many more soil forms over a much larger geographical area need to be tested and incorporated into the model. This way the model will grow more comprehensive and therefore more accurate, as data points are added based on the rules above.

CHAPTER 6 CONCLUSION

6.1. Variability of soils and techniques

The selected soils were representative of the spectrum of soil forming conditions, morphology and variability of classification features of the study area. Based on the clay, silt and sand percentages there was a good spread from sands to loams to clays. It was notable that the sandy soils contained very little clay (5 to 7%) and very little SOC (0.18 to 0.25%). Inversely soils containing relatively high clay (29 to 30%) also contained relatively high SOC (6.76 to 7.18%). In between these extremes there was high variability in SOC content (1.45 to 6.44%) and soil texture features.

Significant differences in the variability and spread of the three techniques were observed. Although the RIFT technique showed least spread of results to obtain the means, dry combustion with a Leco device showed least spread with the two low carbon sands and the Westleigh soil form. WB had the least spread on determining the means for the Oakleaf soil form. This variability could be attributed to operator consistency and error but it is most likely that structural or chemical properties of the particular soils caused more or less variability in the readings due to the particular chemical or thermodynamic effect of the different techniques.

6.2. Correlations between techniques and deviations from the unity line

RIFT correlated better with the reference method than WB in terms of the SOC means of the 11 soil forms. A scatter plot of RIFT vs Leco illustrated a deviation from the 1:1 line that is significantly less than the deviation observed on the scatterplot of WB vs Leco. The correlation coefficients and probabilities for RIFT vs Leco were $r = 0.9520$, $p < 0.00001$; $R^2 = 0.9063$ as opposed to WB vs Leco with $r = 0.9304$, $p < 0.00003$; $R^2 = 0.8657$. In this instance RIFT with a conversion factor of 0.5 was more effective in reporting SOC for at least 72% of the particular soil forms than WB

In terms of comparing the 55 unique soil samples through each technique the correlation coefficients and probabilities for RIFT vs Leco were $r = 0.9434$, $p < 0.01$; $R^2 = 0.8899$ as opposed to WB vs Leco with $r = 0.8047$, $p < 0.01$; $R^2 = 0.6476$. As far as the variances in accuracy are concerned for the 55 unique linked samples, RIFT was more effective in 63.6% of observations and has 2.7 times less residual distance than WB from the reference method

Deviations from the 1:1 lines were observed for both RIFT and WB vs Leco and can be attributed to the nature of the particular techniques, i.e. the consumption of organic matter through incineration and wet chemical oxidation. The deviation is much less pronounced in RIFT than WB and could be ascribed to the more complete consumption that occurs during the RIFT method. Both however overestimate SOC significantly at very low levels (under 3% SOC) and both underestimate SOC at higher levels (above 6.5 % SOC) although to a lesser extent.

A possible explanation for this phenomenon as far as RIFT is concerned is that with very low SOC soils the structural water fractions associated with low clay are less dense or pervasive leading to more complete consumption under the direct and intense incineration regime. It is suggested that in higher SOC soils (notably in excess of 25% Clay) the consumable products associated with clay are more dense or compacted, potentially offering resistance to full consumption by a regulated incineration regime. This explanation is supported by the fact that in the instances where both SOC and clay % were high, it took many incinerations before the consumables were deemed depleted. Inversely where SOC and clay % were very low, it took but a few incinerations before the consumables were deemed depleted.

6.3. Validity of RIFT as an acceptable test for SOC

The validity of RIFT as an acceptable test for SOC needs to be viewed against the current conventions of what are considered as acceptable methods for the determination of SOC. Apart from dry combustion, which is a direct method and the generally accepted reference method for SOC determination, the only other common methods are dichromate oxidation (like WB) and LOI which are both considered indirect methods but which have also been responsible, until recently, for the production of most of the datasets for estimating soil C in analytical laboratories around the world. Of these two methods the WB method is the most widely used and occasionally serves as reference method for predictions of SOC through the use of other methods like LOI, the thermo-gravimetric method and in South Africa even laboratory and field spectroscopy

Apart from a gravimetric device and an incinerator, the only other expenditures are rudimentary and include aluminium thimbles, butane gas and basic lab equipment like spatulas, mortar and pestle, etc. No expensive chemicals are used, no electricity (except for minimal charging of devices) and no sophisticated lab equipment like drying ovens, desiccators, muffle furnaces, combustion chambers or chemical analysers. Furthermore drying ovens and muffle furnaces are usually used for between 8 and 24 hours at temperatures varying from 105˚ C (for oven drying overnight) to an average of 450 ˚ C (for LOI incineration) implying comparatively high basic electrical costs for LOI, WB and dry combustion.

The RIFT device is mobile, light and can be deployed in any workspace (or in a vehicle) in close proximity to the field. Although soil samples are still collected traditionally the RIFT method introduced innovative and rapid methodologies for soil preparation, incineration and measurement with the added benefit of very low maintenance cost and high accuracy.

Because the RIFT method is based on principles found in currently acceptable methods of SOC testing, especially related to dry combustion and LOI, it warrants consideration as a valid test for SOC. RIFT has proven through empirical experimentation that it is more accurate than WB and more consistent for the selected soils of the study and additionally it is safer, cheaper and faster.

The evidence also shows that RIFT is effective in testing soils with lower than 5% organic matter and could potentially be more so once a RIFT/clay correction matrix tool is developed, based on a wider and more extensive sampling regime. Therefore its ability to rapidly, accurately, and inexpensively measure the soil’s carbon pool as desired by Chatterjee *et al*. (2009) and its potential ability to detect and quantify change in the ecosystem dynamics of C, should legitimise the RIFT as an acceptable and routine test for SOC. The hypothesis proposed in section 1.3 is therefore validated by the results of this research.

6.4. Limitations, recommendations and further research

Due to the inherent nature of soil structure and its colloidal fraction, the RIFT method has a profound impact on the soil sample in terms of the loss-of-weight experienced during incineration. The method is non discriminative and at first may appear too crude. However, its effect on the soil is such that it is more “absolute”, and that the incinerations reach a point of depletion, where most of the oxidizable material has been digested. To have an understanding of those oxidizable fractions is critical in order to render RIFT more effective. However, through simple processes at the level of citizen’s science and the potential use of a simple correction matrix, those fractions can be approximated and determinations can be made with a much greater margin of accuracy. Nevertheless, even without such a matrix, the RIFT method proved more accurate in most cases for the selected soils than Walkley-Black.

The RIFT technique only takes a few minutes per sample and it is recommended that more than one reiteration of RIFT per sample takes place (e.g. three to five) and that an average is determined (especially initially) in order to determine spread and consistency of RIFT with differing soil forms. It is also recommended that a finer scale gravimetric device be employed e.g. with an increment ability of 0.001 g.

The use of clay % as a variable for the refinement of SOC results through standard regression analysis proved inconclusive with a correlation coefficient r = 0.4943 between RIFT vs Leco residuals and clay%. However there exists potential to develop an effective RIFT/clay correction matrix. This matrix could be based on reducing the overestimation for very low clay and reducing the underestimation for very high clay contents through calculating the residual means as a correction factor. For this a broader and more extensive sampling regime would also need to be employed.

Further research is necessary to test many more soil forms over a much broader landscape. This is imperative to start obtaining a picture of the full spectrum of RIFT test conditions relating to the influence of clay fractions, dry soil mass-to-volume ratio, and even possibly carbonates (where appropriate) in future.

CHAPTER 7 REFERENCES

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