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Evaluation of Different Soil Carbon Determination Methods

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Table of Contents

Abstract:

Determining soil carbon (C) with high precision is an essential requisite for the success of the terrestrial C sequestration program. The informed choice of management practices for different terrestrial ecosystems rests upon accurately measuring the potential for C sequestration. Numerous methods are available for assessing soil C. Chemical analysis of field-collected samples using a dry combustion method is regarded as the standard method. However, conventional sampling of soil (may be cores or bulk samples delete "cores") and their subsequent chemical analysis is expensive and time consuming. Furthermore, these methods are insufficiently sensitive to identify small changes over time in response to alterations in management practices, changes in land use or physical disturbances??. Presently, several different *in situ* analytic methods are being developed purportedly offering increased accuracy, precision and cost-effectiveness over traditional *ex situ* methods. We consider that, at this stage, a comparative discussion of different soil C determination methods will improve the understanding needed to develop a standard protocol.

Key words: soil carbon, wet oxidation, dry combustion, inelastic neutron scattering, remote sensing, laser induced breakdown spectroscopy

I. Introduction

Rapid, accurate and inexpensive measurement of the soil's carbon pool is essential to detect and quantify change in the ecosystem dynamics of C. A comparative assessment of present determination methods is needed urgently to identify promising techniques that reduce uncertainty in measuring the soil's C pool and flux at the farm and watershed scale. Evaluation of sustainable land-use and soil management practices to stabilize or increase the soil's C pool also demands a sensitive analytical protocol that will pave the way to establish soil C as a tradable commodity in the global market. Estimates suggest the global C-credit market in 2007 was (we are now in 2008 so do you really know the value?} be about $30 billion, with the potential to grow to $1 trillion by 2020 (Lal, 2007). Accordingly, there is an undeniable need to identify method(s) to determine the rate of change in the soil's C pool over a specific period. Quantifying the site-specific ancillary benefits of soil C sequestration also necessitates establishing a standard protocols for evaluating soil's organic C (SOC) pool and flux over multiple scales.

Credible estimates of soil C pool and its fluxes also are required to identify policies and site-specific management practices to increase or at least stabilize the SOC pool. Depending on such practices and land uses, the C pool can play dominant role as either a net source or net sinks of atmospheric carbon dioxide (CO_2). To a depth of 1-m (the biologically active soil layer) (in many soils the active layer is much deeper , would be better to drop this or go at least to 2 meters) the soil's C pool is 2500 Pg (1 Pg = 10^{15}g or Gt), comprising 1550 Pg of soil organic C (SOC) and 950 Pg of soil inorganic C (SIC) (Lal, 2001). The former is mainly composed of (1) soluble organic compounds (sugars and proteins), (2) amorphous organic compounds (humic acid, fat, waxes, lignins and polyuronides), and, (3) organomineral complexes (Schnitzer, 1991). (You do not saying that "Charcoal" is present in many soils and also needs to be measured. Keep in mind there is a major effort to add biochar to soils and this needs to be considered) The latter comprises primary and secondary carbonates (Eswaran et al., 1995). Simple inexpensive methods are needed to measure the pools and fluxes in both, SOC and SIC. However, most studies have focused on measuring the SOC pool and relating it to land use and soil management as this is the pool that can be easily effected by changes in agriculture practices and land use management. The SOC content can be determined directly or indirectly from the difference between the total soil C (TSC) and the SIC concentration, measured separately. The indirect method is unsuitable for carbonate-rich soils or those with low SOC due to inherent uncertainties when subtracting large values (there is not a problem in subtracting large values problem may be in measuring the pool size need to rewrite). The SIC can be determined quantitatively by treating a soil sample with HCl and measuring the CO_2 released from carbonates either by gas chromatography or by pressure calcimetry (Sherrod et al., 2002). For soils lacking SIC, TSC value represents the SOC value. (you do not address charcoal in his all at) However, when the parent material is enriched in carbonaceous mineral, such as limestone and dolomite, the SIC must be measured to determine the sample's SOC. The common principle underlying SOC evaluation is the *ex situ* chemical- or high temperature-destruction of the soil organic matter (SOM) from field samples in a laboratory. However, several non-destructive, *in situ* methods currently being developed promise to increase the accuracy and reduce the time and cost of conventional field soil sampling and laboratory analyses. The objective of our review is to consider fully the information on *ex situ* and *in situ* methods of determining the SOC pool, and offer a critical comparative analysis of sensitivity, predictability, and time and cost-efficiency of these novel approaches.

II. *Ex Situ* Methods

Ex situ methods involve collecting representative soil samples and measuring the C concentration via dry or wet combustion techniques. The latter process involves the oxidation of organic matter by an acid mixture and measuring the evolved CO_2 by gravimetric, titrimetric or manometric methods. In the 19th century, Rogers and Rogers (1848) reported that dichromate-sulfuric acid solution could oxidize organic substances. After unsuccessful attempts by Warrington and Peake (1880), and Cameron and Breazeale (1904), Ames and Gaither (1914) accomplished the complete oxidation of organic substances by the dichromate-sulfuric mixture (is it really complete, most work shows that it is not). Schollenberger (1927) introduced the titrimetric determination of unused chromic acid in the oxidation reaction with ferrous ammonium sulfate using several indicators (diphenylamine, o-phenanthroline, or N-phenylanthranillic acid (Tabatabai, 1996). Walkley and Black (1934) and Tyurin (1935) developed a complete quantification method of SOC by wet oxidation without necessitating external heating. However, Tinsley (1950) and Meibus (1960) proposed applying external heat for an extended period of time to increase the recovery of SOC. If you need heat to recover then you are not getting complete quantification the above statements contradict each other.

Rather (1917) introduced the technique of estimating SOM from the weight loss of soils on ignition. He also suggested first destroying the hydrosilicates by treating the samples with hydrochloric and hydrofluoric acids to eliminate the loss of hydroxyl groups during heating, but invariably some SOM is prone to decompose during this treatment. Mitchell (1932) described a low temperature ignition method to remove the soil water by heating the sample at 110°C and exposing the dried soil at 350-400°C temperature for 8 hours in a furnace. Jackson (1958) recommended using an induction furnace wherein heat is generated from high frequency electromagnetic radiation. (There have been many more recent studies on using lose on ignition need to check them out)

Tabatabai and Bremner (1970) introduced an automated CO_2 analyzer based on thermal conductivity measurements of the effluent gases. Current automated total C analyzers follow the principles described by Tabatabai and Bremner (1991). Table 1 summarizes the key features of wet and dry combustion methods for evaluating SOC.

A. Wet Combustion

The analysis of SOC content by wet combustion long has been regarded as standard procedure since Schollenberger (1927) introduced it; it produces results in agreement with those of the dry combustion technique (Nelson and Sommers, 1996). (looking at large data bases show that the comparison varies greatly with soil depth and soil type I did this with the NRCS data base not published but results were all over the board for 10,000 plus samples between wet and dry table two shows some ideas of the differences but more may be needed) Wet combustion involves oxidizing SOM to CO_2 with a solution containing potassium dichromate ($K_2Cr_2O_7$), sulfuric acid (H_2SO_4) and phosphoric acid (H_3PO_4), following the reaction

$$2Cr_2O_7^{-2} + 3C^0 + 16\,H^+ = 4\,Cr^{3-} + 3CO_2 + 8\,H_2O \qquad \text{Eq.1}$$

This reaction generates a temperature of 210°C and is sufficient to oxidize carbonaceous matter. The excess $Cr_2O_7^{-2}$ (not used in oxidation) is titrated with Fe $(NH_4)_2(SO_4)_2.6H_2O$, and reduced $Cr_2O_7^{-2}$ is assumed to be equivalent to the sample's SOC content. Calculations for SOC content are based on the fact that C present in soil has an average valence of zero.

The wet combustion method has undergone a number of modifications related to the type and concentration of the acids used and whether external heat is applied or not. Schollenberger (1927) suggested heating the soil- H_2SO_4-$K_2Cr_2O_7$ mixture to complete SOM oxidation and thereby increase the recovery. Others soon realized that the temperature and its duration were critical and must be standardized to ensure the oxidation of a constant proportion of SOM; for that, a consistent amount of dichromate must be thermally decomposed during digestion. Tyurin (1931) incorporated a definite heating time and temperature for soil-chromic acid mixtures in a test tube. However, Walkley and Black (1934) reported satisfactory results with no heating, and suggested using a factor of 1.32 (assuming 76% recovery) to account for the incomplete digestion This percent is all over depending on soil type and soil depth and mineralogy). Table 2 lists the correction factors for various soils. Meibus (1960) proposed boiling the soil-dichromate-sulfuric acid mixture for 30 min in an Erlenmeyer flask connected to a reflux condenser. Subsequently, many researchers tried to modify earlier procedures to enhance the recovery, such as proposed by Meibus (Nelson and Sommers, 1982) and Heans (1984). For dry combustion, Soon and Abboud (1991) reported that the Walkley-Black (WB), modified Tinsley, and modified Meibus methods respectively recovered 71, 95 and 98% of soil C. Thus, we conclude that external heat can improve the SOC recovery, although the WB method is far more popular than the modified methods with external heat. Assuming a recovery of 76% often leads to overestimating or underestimating SOC concentration, depending on the soil's type. The recovery percentage varies from 59% to 88%, and the corresponding correction factor from 1.69 to 1.14 (Table 2). Díaz-Zorita (1999) attributed the low recovery of SOC by the WB method in soil from a graminean pasture system to presence of a high percentage of recalcitrant SOM (e.g., phenolic and lignin compounds). The modified WB method sometimes overestimates the SOC content, while the WB method underestimates it (Brye and Slaton, 2003). Variable recovery percentage of the WB method depends on the soil's type rather than on landuses. Mikhalilova et al. (2003) compared four management regimes (native grassland, grazed, continuous cropping and continuously plowed fallow) and derived a single correction factor of 1.63 independent of from the management regime. De Vos et al. (2007) reported a strong correlation between recovery percentage (using the WB method) and the soil's textural class and pedogenetic horizons. Recovery was higher by 3 to 8% from sandy soils than from loam and silt-loam soils. Similarly, recovery from samples from eluvial horizons was significantly higher than those from A horizons, presumably due to higher SOM content in the upper than lower soil horizons.

Interferences by chloride (Cl^-), ferrous iron (Fe^{2+}), higher oxides of manganese (Mn^{3+} and Mn^{4+}) and coal particles also entail incorrect estimations of SOC content (Nelson and Sommers, 1996). Particularly, these ions participate in chromic acid-oxidation-reduction reaction, wherein Fe^{2+} and Cl^- lead to a positive error, and MnO_2 to a negative error. Large concentrations of Fe^{2+} occur in highly reduced soil and are oxidized to Fe^{3+} by $Cr_2O_7^{-2}$, giving high values for SOC content (Eq. 2). This error is more prevalent when the soil sample is not dried before analysis.

$$Cr_2O_7^{-2} + 6\,Fe^{2+} + 14H^+ = 2Cr^{3+} + 6Fe^{3+} + 7H_2O \qquad \text{Eq. 2}$$

In case of salt affected soils, Cl^- ion reacts with dichromate producing chromyl chloride that consumes of $Cr_2O_7^{-2}$ (Eq. 3).

$$K_2Cr_2O_7 + 4KCl + 6H_2O = CrO_2Cl_2 + 6KHSO_4 + 3H_2O \qquad \text{Eq. 3}$$

The interference of Cl^- ions can be eliminated by washing the soil with Cl^- free water, precipitating Cl^- by adding Ag_2SO_4 or by stoichiometric correction (Eq. 4). Heans (1984) concluded that adding Ag_2SO_4 either before or after $K_2Cr_2O_7$ failed to control Cl^- interference, and suggested separate assay and stoichiometric correction as the only permissible alternative for assessing SOC by the WB method.

$$\text{Corrected-SOC (\%)} = (\text{Estimated SOC\%}) - (\%\ Cl^-/12) \qquad \text{Eq. 4}$$

The higher oxides of Mn (mainly MnO_2) often produce a negative error by competing with oxidizable substances in soil-chromic acid mixtures (Eq. 5). This interference produces an error of small magnitude in calculations because even in highly manganiferous soils, a minute quantity of MnO_2 competes with $Cr_2O_7^{-2}$ for the oxidation of SOC (Nelson and Sommers, 1996) (Eq. 5).

$$2MnO_2 + C^0 + 8H^+ = CO_2 + Mn^{2+} + 4H_2O \qquad \text{Eq. 5}$$

The presence of carbonized materials (e.g., charcoal, coal, coke and soot) also is responsible for poor recovery in the wet digestion process. Without applying any external heat, the percentage recovery of SOC present in carbonized materials is low; and with external heat, the recovery is variable depending on the properties of the carbonized materials (Heans, 1984; Skjemstad and Taylor, 1999; and De Vos et al., 2007). Thus, Walkley (1947) reported that the WB method recovered only 2-11% of SOC present in carbonized materials. Microscopic inspection of the digested material revealed charred materials in the remaining organic fragments. Wet digestion methodologies cannot be employed to recover carbonized materials or to separate the SOC fractions from carbonized materials because their oxidation depends on the time and temperature of heating the chromic acid mixture and the carbonized material's properties, such as bonding with organomineral complexes, and the groups present etc. (Also there is now an environmental problem with using and disposing of the compounds containg chromium, cannot use in many labs any more).

Using colorimetric analyses rather than titrations can increase the precision of the wet combustion method (Soon and Abboud, 1991). There are two approaches to colorimetric determination: (i) determining the unreacted dichromate solution that changes color from orange to green, and (ii) measuring the absorbance of the color complex (violet) produced from the reaction of Cr^{3+} with s-diphenylcarbazide at 450 nm (Tabatabai, 1996). The Cr^{3+} ion has two broad maxima in the visible range, one near 450 nm and the other near 600 nm. The dichromate ion also has an absorption maximum near 450 nm, but not near 600 nm, and hence, it is advisable to determine the absorbance at 600 nm. Soon and Abboud (1991) measured the absorbance of clear supernatant 10-ml aliquot of soil-chromic acid mixture at 600 nm against a set of standard sucrose solutions and achieved 100% recovery by comparison with dry combustion as the reference method. Using automatic titration or digital burettes, coupled with the wet digestion process, also may improve accuracy. Nevertheless, even though the wet digestion method has limitations due to variable recovery percentage, still it is used worldwide throughout the world to measure SOC concentration because of its low cost and minimum requirements. (But a manjor environmental problem, needs to be discontintued)

B. Dry Combustion:

Incinerating SOM and thermal decomposing carbonate minerals generate CO_2 that is measured by (1) dry combustion followed by measuring the changes or mass- loss-on-ignition (LOI), and, (2) dry oxidation of SOC, then collecting and determining the evolved CO_2 with automated instruments (Table 1). Both methods involve oxidizing the SOC at a high temperature. The LOI method entails heating the sample in a muffle furnace between 200-500°C, whereas dry oxidation via automated analyzer is accomplished between 950-1150°C.

1. Loss on Ignition (LOI):

In this method, the SOM is assessed by measuring the weight loss from a dry soil sample (oven-dried at 105°C) after high temperature ignition of the carbonaceous compounds in a muffle-furnace. Three assumptions underlie this method: (a) LOI is due only to the combustion of SOM, and, (ii) the C content of SOM is constant (Christensen and Malmros, 1982). The concentration of SOC can be computed from the LOI-SOC relationship, where SOC is determined by an autoanalyzer or by the multiplication factor of 0.58, assuming that SOM comprises 58% of the SOC. However, this conversion factor (0.58) varies with soil's type, the sampling depth, and types of organic compounds in the SOC. The LOI does not generally represent SOM because LOI can decompose inorganic constituents without igniting the entire SOM pool. Temperature and the duration of ignition are critical to prevent the loss of CO_2 from carbonates and the structural water from clay minerals and amorphous materials (volcanic soils), the oxidation of Fe^{2+}, and the decomposition of hydrated salts (Schulte and Hopkins, 1996 and Ben-Dor and Banin, 1989).

While some hygroscopic water is removed from the soil during ignition at 105°C, sometimes the dehydration of the sample is incomplete; thus the SOM value may be overestimated. Also, different salts present in soil release molecular water at different temperatures above 105°C. For example, gypsum ($CaSO_4.2H_2O$) contains up to 21% water and loses 1.5 H_2O molecules at 128°C, and the remaining H_2O at 163°C. Epsom salts ($MgSO_4$. $7H_2O$) loses six H_2O molecules at 150°C and the remaining one at 200°C. Four H_2O molecules are lost from $CaCl_2.6H_2O$ at 30°C, and the remaining two molecules at 200°C (Schulte and Hopkins, 1996 and Lide, 1993). Dehydroxylation of silicates starts between 350 to 370°C, whereas Na-montmorillonite, vermiculite, gibbsite, goethite, and brucite lose crystal-lattice water between 150 to 250°C (Barshad, 1965). Schulte and Hopkins (1996) reported that gypsum is dehydrated fully at 150°C; they recommended using this temperature for soils dominated by hydrated clays. Volcanic soils have large amounts of water in them that can effect the results)

It is difficult to predict an optimum temperature and duration of ignition to ensure the maximum SOM recovery and avoid loss by the dehydration of clays or decomposition of other soil constituents. Abella and Zimmer (2007) reported that at 300°C, 85-89% of LOI occurred during the first 30 min and 98-99% during the first 90 min. In contrast, Ben-Dor and Banin (1989) suggested that 400°C for 8 hr is suitable, basing their suggestion on using fast and prolonged heating working with natural and synthetic soils of Israel. Donkin (1991) observed that optimum temperature for ignition is 450°C for 6 hr, and concluded that higher temperature does not provide any advantage in terms of recovery percentage. Schulte et al. (1991) recommended that after reaching 360°C, exposure for two hours is suitable for routine soil sample analyses.

Despite these limitations, the LOI and SOC content of soil are correlated strongly, as calculated from organic C data (Table 4). However, the slopes (m) and intercepts (c) are highly variable depending on the ignition temperature and duration, soil types, and the compounds that comprise the SOC. The data in Table 4 show that values of the slope of the regression equation <1 indicate a loss of soil constituents other than SOM during ignition, whereas those >1 represent the incomplete recovery of SOM. Konen et al. (2002) concluded that predictive equations developed by LOI-SOC relationship were significantly different for different Major Land Resource Areas (MLRAs) in the central USA. For all their soil samples, ignition was accomplished at 360°C (Table 4). Different recovery of SOM during ignition was controlled by the heating time and duration and by soil texture (particularly clay %) and type. De Vos et al. (2005) observed that the intercept in LOI-SOC regression equation is determined significantly by the samples' clay content. Spain et al. (1982) reported that a 9% improvement in the predictive capability of the equation using a bivariate function of LOI and clay.

Sample size is another source of variation in LOI measurements. Schulte et al. (1991) reported that LOI value significantly decreased with increase in sample weight. Diffusion of oxygen within the sample inhibits oxidation in large samples, a feature that is critical for organic soils, e.g., peat and muck soil.

While the LOI is a simple, rapid, and inexpensive technique of determining SOC content, the LOI-SOC regression equation must be determined for particular soil type and depth. Inclusion of the clay percentage in the bivariate regression equation can increase the correlation between LOI and SOC content. Finally, consistency should be assured for ignition temperatures, exposure times, and the samples' size and information on these three parameters included at the time of publishing the research data (Konen et al., 2002; Heiri et al., 2001).

b. Automated Carbon Analyzer:

Use of the automated analyzer for determining of total C has evolved to become the standard method. Following are the major steps in detecting C in an automated CN analyzer: (i) Automatic introduction of the sample into a high-temperature oxidation zone wherein soil C is converted to CO_2; (ii) carriage of CO_2 by a carrier gas (generally helium) and separated from other gasses (N_2, NO_X, H_2O vapor, SO_2) either by a gas chromatographic system, or a series of selective traps for the individual gases; and, (iii) detection of the concentration of CO_2 mainly by thermal conductivity, mass spectrometry or infrared gas analyzing methods (Smith and Tabatabai, 2004). Method of CO_2 detection varies with instruments' manufacturer and model. Table 5 is a short list of automated analyzers, the detection principles of detection, manufacturers and contact information. Automate analyzer is calibrated with glutamic acid and generally samples are replicated to ensure the quality of the run. The main advantages of automated analyzer are (i) rapid and precise, (ii) no loss of soil C during combustion; (iii) potential for simultaneously measuring nitrogen and sulfur (depending on model); and, (iv) can be connected to mass spectrometer for stable isotope analysis.

Special care must be taken in homogenizing the soils and ensuring its fineness. In most cases, 100-200 mg of soil sample is used for auto analyzer analyses. Pérez et al. (2001) suggested that simple crushing was not sufficient to guarantee homogeneity of small soil samples, and precision generally is better for finely ground samples (<177 μm). They also concluded that a 100 mg of soil sample is adequate to obtain the best results from an auto analyzer. In contrast, Jimnez and Ladha (1993) recommended soil samples of 60 mg with fineness of 150 µm ; this can be achieved by roller grinding or ball milling the sample after passing it through a 2-mm sieve.

The sample size must be large enough to create detectable signals and generate representative data within the limits of its combustibility. However, sample size can not indefinitely be increased because of incomplete oxidation under a low O_2 supply and the physical limitations of the sample's container. Most automated analyzers (like Elementar Vario Macro) offer options to increase O_2 dosing and combustion time. In particular, samples of organic soil in particular must be analyzed under a sufficient O_2 supply or with a sample of lesser weight, although the latter may contribute to uncertainty in the sample's representativeness. Extremely small soil samples with low SOC content also generated very low detector signal to noise ratio, and hence, poor accuracy and precision (Jimnez and Ladha, 1993).

Complete combustion of the sample also depends on the temperature within the combustion furnace, generally held between 950 and 1200°C. For some models, soil samples are encapsulated in a tin foil that raises the combustion temperature to about 1800°C. Wright and Bailey (2001) compared two combustion temperature profiles, 1040 and 1300°C, concluding that 1300°C is essential for accurately measuring total soil C. They observed that under lower combustion conditions (1040°C), carbonate decomposition from samples of pure $CaCO_3$ is minimized to 5%, whereas it is maximized to 98% at higher temperatures (1300°C).

Dry combustion with auto analyzers have higher precision than wet combustion or LOI, but also costs more due to the expense of buying the analyzer (US $40,000 to over $50,000) and the associated such as an ultra-microbalance, computer, and printer. The operating costs of auto analyzers also are slightly higher due to the required consumables and high purity gasses (He and O_2). The instrument consumes a significant amount of electricity in heating the furnace. Jimnez and Ladha (1993) estimated that the cost per sample for analyzing TSC using the Perkin-Elmer 2400 CHN analyzer ranges between $3.8 and $6.50 for running 100 samples and 10 samples, respectively, in a single operation. Analyzing few samples increases the cost of analysis because of the extended time required for their stabilization and calibration and the increase in the quantity of standard runs for each operation. Running a large batch of samples can reduce cost of analysis by economies of scale. Many systems can be set to run with automatically with out some one sitting at the machine this will also reduse the cost of the human input)

Comparing different *ex situ* methods to determine soil C, reveals that high precision and low analysis cost cannot be achieved using the same method. Thus, automated dry combustion analysis provides high precision, whereas the LOI method involves low cost. The expense of assessing soil C can be lowered provided the relationship between LOI and automated dry combustion is established for a particular soil type. However, it is rare to find out a strong linear relationship between the two (Abella and Zimmer, 2007). Further, wet digestion, the Walkley and Black method, carries a wide variation in recovery percentages, and also does not demonstrate a strong correlation with the automated dry combustion technique (De Vos et al. 2007) and chemical disposal is an environmental problem. In general, we conclude that automated dry combustion is the only reliable, comprehensive method to determine soil C concentration with the added benefit of also measuring N and S at the same time. With a limited budget, LOI method might be used rather than the automated technique, but the correlation factor in between them should be reported with the results.

Although soil sampling in the field and automated dry combustion is considered as the standard method, the whole process is expensive, time consuming and labor intensive. The automated analysis of prepared soil samples alone costs around $12 per sample. (Ture but the samples are also used for measurements of many other elements that are needed for agronominc practices so bulk sampling is still needed for these, do not be one dimensional (P, K and amny micro nutrients) Moreover, without intensive soil sampling, it is hard to detect changes in soil C over large landscapes due to spatial heterogeneity (Freibauer et al., 2004). All laboratory analyses use a small quantity of homogenized samples, generally between 0.1 to 1 g. The percentage of soil C present assessed is converted to a mass per area basis using the soil's bulk density value, which is measured separately. Determining soil bulk density is a challenge, particularly in soils with a high percentage of coarse fragments (Lal, 2006). Averaging the soil C concentration based on bulk density can lead to uncertainties about the value of the soil's C pool and may mask the impact of recommended management practices (RMPs) on C dynamics. (this is not clear what are the problems you aer worried about? You really do not discuss bulk density limitations? These major limitations with *ex situ* methods instigated the development of alternative methods, particularly *in situ* ones, to achieve higher precision, faster analyses, and lower costs and than the present *ex situ* determination methods.

III. In Situ Methods:

New *in situ* soil C methods promise high precision without as much sample processing time and their subsequent analysis. *In situ* methods mainly are based on remote sensing and spectroscopic measurements in the field (Table 6). Spectroscopic methods include infra-red reflectance near-infra-red (NIR) and mid-infra-red, laser-induced breakdown spectroscopy

(LIBS) and inelastic neutron scattering (INS). Currently these methods are being calibrated with standard soil core sampling and *ex situ* determination methods. (You compare to methods you say do not work well so what do the results mean?)

A. Infrared reflectance spectroscopy:

Infra-red reflectance spectroscopy is a rapid technique for measuring soil C based on the diffusely reflected radiation of illuminated soil (McCarty et al., 2002). Within diffuse reflectance spectroscopy, both the near infrared region (NIR, 400-2500 nm), and the mid infrared (MIR, 2500-25000 nm) region have been evaluated for quantifying soil C (Morón and Cozzolino, 2002, McCarty et al., 2002 and Russell, 2003). NIR uses a quantitative determination of components of complex organic compounds, whereas MIR spectroscopy involves the spectral interpretation of chemical structures. McCarty et al. (2002) reported that organic and inorganic C pools can be measured simultaneously by spectral analysis; they observed that useful calibrations for soil C can be developed using MIR, and to lesser extent, NIR analysis. NIR is based on the absorption of the C-H, N-H, and O-H groups found in organic compounds. These absorptions are overtones and combination bands of the much stronger absorption band seen in MIR spectra (Murray 1993, Batten 1998, Deaville and Flinn 2000, Reeves 2000). Multiple regression statistics (Partial Least Square and Principal Component Analysis) relate the NIR data at selected wavelengths to reference values for calibration (Deaville and Flinn, 2000, Cozzolino and Morón, 2006). The major limitation of NIR is the continual need for calibration and quality control. Due to differences in particle size and soil mineral absorption intensities, NIR absorption by soil is not linearly related to the individual soil matrix components (Russell 2003). The NIR has excellent performance (R^2= 0.961 to 0.975) when applied to a calibration set of samples of a similar particle size distribution. However, predictability is low in samples with heterogeneous particle size and high variability in moisture content (Madari et al., 2005). However, NIR simultaneously measures quantitatively and qualitatively certain soil parameters (like forms of C), in addition to

C content. Commercial field portable NIR instruments are available, and widen the use of NIR for *in-situ* measurement of soil C. COST?

B. Laser Induced Breakdown Spectroscopy (LIBS):

Laser induced breakdown spectroscopy (LIBS) is based on atomic emission; the soil's C content is determined by analyzing the unique spectral signature of C (at 247.8 or 193 nm, or both). A laser beam at a specific wavelength, e.g., 1064 nm, is focused on each sample with a lens of 50 mm focal length to form microplasma that emits light that is characteristic of the sample's elemental composition (Ebinger et al., 2006) (Fig. 1). The emitted light is spectrally resolved using a grated-intensified photodiode array detector. Intact soil cores or discrete, pressed samples are used for analysis; spectra are collected along a soil core or from each discrete sample. The performance of LIBS is comparable to that of the dry combustion method. (Reference) The spatial variability of C in soil profiles is accounted for by the ability to analyze and average multiple spots. Cremers et al. (2001) compared the data from LIBS measurements with those from dry combustion and observed a high correlation of 0.96 for soils of similar morphology. They also reported that LIBS quickly determines C (in less than a minute) with excellent instrumental detection limit of ~300 mg kg^{-1} and a precision of 4-5%. The greatest advantage of LIBS is its capability for remote surface chemical analysis of samples although the utility of this feature for soil C analysis remains to be demonstrated. The rapid determination of soil C and the portability of LIBS systems afford the potential to collect and analyze thousands of measurements to characterize soil C content, its distribution and heterogeneity over a large area; nevertheless, these undoubted advantages need to be balanced against the very small volumes analyzed. How do > 2mm particles affect the determination and how do you deal with coarse fragments in the soil and how this affect the total volume of SOC? How many cores are needed in the field? Cost?

Soil properties (e.g., texture, carbonate and moisture content) influence LIBS analyses; thus, numerous calibration curves based on soil texture were required. However, this practice is unacceptable for a field deployable instrument. There is urgency in developing a "universal calibration curve," an essential tool for soil C measurements. The new approach of using multivariate analysis for quantifying soil C builds upon and extends the preliminary observations of Cremers et al., 2001 and Martin et al., 2002. Multivariate statistical analysis (MVA) helps control the variability in C concentrations due to the influence of the soil's matrix, which accounts for the textural dependence of calibrations. Acid washing of soil samples to remove calcium carbonate reduces the standard deviation by almost 8% after normalization the C signal to the silicon (Si) signal. The reproducibility of LIBS analyses can be improved by (i) increasing the number of shots and averaging the spectra over more shots, (ii) applying the method of intensity ratios of C with either Si or Al and, (iii) using the MVA techniques (Martin et al., 2003). Commercialization of a portable LIBS system has reduced its unit cost and might increase its employment for high resolution soil C analyses. Future research is needed to reduce the variability in the LIBS signal caused by the presence of rock fragments, roots, and other materials. A collaborative soil C detection and quantification effort using LIBS was initiated between Oak Ridge National Laoratory and Los Almos National Laboratory. (again to report volume you still need bulk density. And if you need to remove carbonates you still need to do field sampling and this has the same problems as you discuses for Ex-situ methods. You seems to miss the need for field samples and processing in this part. Also if used in the field how deep do you take cores and how many cores to get around variability? You would need to take many cores. Do not discuss over all cost of time in the field.

C. Inelastic Neutron Scattering (INS):

The new inelastic neutron scattering (INS) system for soil C analysis is based on spectroscopy of gamma rays resulting from fast neutrons interacting with the nuclei of the elements in soil. A 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 NaI detectors. The peak areas in the measured spectra are proportional to the soil's elemental content. The key elements measured presently are C, Si, O, N, H, Al, and K. Since the INS is based on nuclear processes that are very fast, it is insensitive to the chemical configuration of the element and can be used in a scanning mode. Figure 2 shows the main components of the self-contained INS alpha prototype field unit mounted on a cart. Having placed the INS system in position, it hovers about 30 cm above the ground; data acquisition typically is set for an interval between 30 to 60 min. The INS subsequently analyzes the acquired spectra for spectral peak intensities (counts), and, using an established calibration line, reports the results instantly in units of kg C m^{-2} (Wielopolski et al., 2008).

The INS system, which by-and-large sees a constant volume, is linear from zero up to very high levels of soil C. It has multi-elemental capabilities, for example, the H peak was calibrated against soil moisture (to be published). The following are its unique and invaluable features: (a) Interrogation of large volumes containing over 200 kg of soil; (b) a large footprint of about 2 m2; and, sampling the soil to a depth of about 30 cm (is that deep enough fcor many soils I think not). Since the INS response is governed by the exponential attenuation functions, Beer's law, of the neutrons penetrating into the soil and the gamma rays emanating from it, these values are not strictly defined but rather effective, or on average. Implicitly, the linear regression of the INS calibration line embeds them. Calibrating the INS system with synthetic soils in which sand was mixed with known amount of carbon yielded an r^2 value of 0.99 (Wielopolski et al., 2004). The system also was calibrated in grassland, pine forest, and hardwood forest in the Blackwood Division of the Duke Forest near Durham with a r^2 value of 0.97. The latter calibration was accomplished via chemical analysis by dry combustion of samples taken after homogenizing an excavation pit of $40 \times 40 \times 40\ cm^3$.

How do you deal with volume measurements and account ofr coarse fragments? Can SIC, SOC, charcoal be separted or do all of them came as C? Cost? Enviromental hazards of Nuclear material? What is the cost?

D. Remote Sensing:

Since 1960, remote sensing has been explored as an alternative non-destructive method for SOC determination, at least of surface soils (Merry and Levine, 1995). Reflectance of various spectral bands was correlated with soil properties, including SOM content (Chen et al., 2000, 2007). Spectral sensors were developed and examined to measure SOM (Pitts et al. 1983; Griffis, 1985; Smith et al., 1987; Shonk et al., 1991). Sensors usually operate with wavelengths between 0.3 μm and 1 m and are divided into four groups: (1) Visible (0.4-0.7 μm), (2) reflective infrared (0.7-3 μm), (3) thermal infrared (8-14 μm), and (4) microwave (1 mm -1m). A wavelength between 0.4 μm to 2.5 μm, is suitable for soil with >2% SOM content (Baumgardner et al., 1970). 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 (Baumgardner et al., 1970; Smith et al., 1987; Sudduth and Hummel, 1988; Henderson et al., 1992). Ben-Dor and Banin (1995) successfully correlated statistical data and Landsat TM imaging analysis with the sand, clay and SOM content of different soils in Madison County, Alabama, USA. Chen et al., (2000) proposed a process of mapping SOC with remotely sensed imagery (bare field) that includes image filtering, regression analysis, classification and reclassification. With this method, they obtained a high correlation (0.97-0.98) between predicted and measured values at field scale level (of area 115 ha) in coastal plain region of Georgia, USA. Mapping of SOC with remote sensing has proven to be both accurate and economic; however this method requires separate sampling and mapping for each crop field. Chen et al. (2007) proposed to group field based on image similarity and mapping them together as one group to reduce sampling costs.

Although there is a strong relationship between remotely sensed spectral data and SOC content, prediction at different spatial scales has not been achieved. Moreover, to draw inferences of SOC content from satellite imagery on a large scale necessitates having surrogate indices such as vegetation type and species or soil moisture (Merry and Levine, 1995). Beside these shortcomings, remote sensing with its high resolution monitoring abilities is applicable for predicting SOC distribution, which is not feasible by any other means. Continued development of inventory and integrating remote sensing data can improve the SOC accounting (West et al., 2008). (COST depth of sampling how do you deal with the need for volume measurements needing bulk density? Can this go to 1 meter as you give as the active depth needed?

All methods have pros and cons and so, they should be matched to specific measurement needs and applications before they are selected or rejected. The choice of an instrument or measurement techniques will depend upon the researchers' need and resources, such as the funding allotted for the project.

You do not discuss the IR used on the VERUS system? Has been demonstrated in the field, has problems but does need to be discussed)

IV. SUMMARY AND CONCLUSIONS

Considering both *ex situ* and *in situ* measurements, we suggest that three methods, automated dry combustion, LIBS, and INS have higher precision than others. Table 7 compares these three based on their precision and analytical advantages and disadvantages. The major drawbacks of the automated dry combustion method are labor-, cost- and time-intensive soil sampling, along with its dependence on the soil bulk density value. (you need BD for LIBS and INS if you are going to report C on a voloume basis as we need to do and LIBS and INS many not go deep enough INS is 30 cm and LIBS depents on the core depth but many cores are needed) For the LIBS and INS systems, the main expenditure is limited to the initial cost of instrumentation, and its operating expenses (e.g., technician, consumables, and safety personnel, etc). Until the advanced methods of measurement described in this review are calibrated properly, methods for determining soil C will follow the legacy of standard field soil core sampling and automated dry combustion analysis. You can take samples from multible areas in a field (standard fertility sampling and measure SOC in them and if you take a volume sample even get that at the same time. You do not discuss the cost of LIBS and INS as you did with Ex-suti methods, need to out down costs. Need to discuss depth limitations with INS and LIBS and now you deal with soils high in coarse fragments which will effect the volume measurements.

How do INS and LIBS deal with SOC measurements? You need to add a cost line to table 7. You gloss over the cost for LIBS and INS. Also in table 7 need to discuss the need to deal with Bulk density and need for volume measurements.

Need to realte to measurements taken on many farms for fertility work, that will need to go on and may be a way to get a better handled on SOC. Again now is SIC handled in LIBs and INS? You go into the old methods in depth but seem to give to little detail for the new methods and the limiatiaons they have, How can you get cores in soils with coarse fragments and even in soils with dense layers where even a 1 inch core many only go to 30 or so CM because of the dense material or coarse fragments? I have worked in sandy soils in NE talking cores and if there is a carbonate later and many dry land soils have this a core will not penetrate the soil,

New methods have promise but have many problems not discussed.

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Table 1: Features of *ex situ* soil C determination methods

Method	Principle	CO_2 determination	Advantages/Disadvantages
I. Wet combustion			
Combustion train	Sample is heated with $K_2Cr_2O_7$-H_2SO_4-H_3PO_4 mixture in a CO_2-free air stream to convert OC in CO_2.	Gravimetric/Titirimetric	Gravimetric determination requires careful analytical techniques and titrimetric determination is less precise
Van-Slyke-Neil apparatus	Sample is heated with $K_2Cr_2O_7$-H_2SO_4-H_3PO_4 mixture in a combustion tube attached to the apparatus to convert OC in CO_2.	Manometric	Expensive and easily damaged apparatus
Walkley-Black	Sample is heated with $K_2Cr_2O_7$-H_2SO_4-H_3PO_4 mixture. Excess dichromate is back titrated with ferrous ammonium sulfate.	Titrimetric	Oxidation factor is needed. Variable SOC recovery. Generate hazardous byproducts such as Cr.
II. Dry combustion			
Weight-loss-on-ignition	Sample is heated to 430°C in a muffle furnace during 24 hours.	Gravimetric	Weight losses are due to moisture and volatile organic compounds. Over estimate the Organic matter content
Automated	Sample is mixed with catalysts or accelerator and heated in resistance or induction furnace in O_2 stream to convert all C in CO_2.	Thermal conductivity, gravimetric, IR absorption spectrometry	Rapid, simple, and precise but expensive. Slow release of contaminant CO_2 from alkaline earth carbonates with resistance furnace

Table 2. Correction factors for soil organic C not recovered by the Walkley-Black method.

Sampling location	Recovery%	Average Correction factor	Reference
Forest soils, Belgium	63	1.58	De Vos et al. (2007)
Calcareous soil, Italy	77	1.30	Santi et al. (2006)
Sierra Leone	83	1.20	Kamara et al. (2007)
Native Prairie, Arkansas,USA	66	1.51	Bryre and Slaton (2003)
Agricultural soil, Arkansas,USA	63	1.59	
Russian Chernozem	61	1.63	Mikhailova et al. (2003)
Graminean pasture, Argentina	59	1.69	Díaz-Zorita, (1999)
Mineral soils, New Zealand	80	1.25	Grewal et al.(1991)
Canadian Prairie	71	1.40	Soon and Abboud (1991)
Australia	88	1.14	Lowther et al. (1990)

Table 3. Modifications in wet digestion methods for determining SOC (adapted from Nelson and Sommers, 1996).

Method	Reagent concentrations (N)		Ratio of H_2O to acid (v:v)	Digestion Conditions	C.V.%
	$K_2Cr_2O_7$	H_2SO_4			
Schollenberger (1927)	0.058	18		Tube heated by flame at 175°C for 90 s	1.4-1.9
Tyurin (1931)	0.066	9	1.00	Flask with funnel boiled at 140°C for 5 min	8.5
Walkley-Black 1934)	0.055	12	0.50	Flask with no external heat, max temp is 120° C	1.6-4.2
Tinsley (1950)	0.027	7.2	0.67	Flask with condenser refluxed for 2 h at 150° C	0.8-3.1
Mebius (1960)	0.045	10	0.42	Flask with condenser refluxed for 30 min at 159° C	1.2-1.8
Modified Mebius (1982)	0.033	10.8	0.67	Flask with condenser refluxed at 150° C for 30 min	1.0-3.6
Heans (1984)	0.055	12	0.50	Tube heated in block at 135° C for 30 min	4.1

Table 4. Relationship between soil organic C (determined by wet oxidation and dry combustion) and weight loss-on-ignition (LOI).

Soil type	Temperature (°C)	Duration (h)	SOC = m*LOI + c			Reference
			m	c	(r^2)	
Forest soils, USA	300	2	0.4315	0.1603	0.69	Abella and Zimmer (2007)
Sierra Leone	375	2	-6.55	0.64	0.93	Kamara et al. (2007)
	550	3	0.5783	-1.2875	0.96	De Vos et al. (2005)
Nebraska sand hills, USA	360	2	1.414	- 0.6791	0.94	Konen et al. (2002)
Central loess plains, USA	360	2	0.6717	- 4.5359	0.94	Konen et al. (2002)
Southern Wisconsin & Minnesota till prairies, USA	360	2	0.5743	0.1025	0.98	Konen et al. (2002)
Central Iowa and Minnesota till prairies, USA	360	2	0.6824	- 2.8696	0.97	Konen et al. (2002)
Illinois and Iowa deep loess and drift, USA	360	2	0.6094	0.1949	0.98	Konen et al. (2002)
<u>Tasmanian acidic soils</u>						
Non-basalt derived	375	17	0.726	-1.598	0.96	Wang et al. (1996)
Basalt derived	375	17	0.469	-0.941	0.95	Wang et al. (1996)
Canadian Prairie	375	16	-9.36	0.633	097	Soon and Abboud (1991)
	450	6	0.568	0	0.98	Donkin (1991)
	450	16	0.914	0	0.99	Lowther et al. (1990)
Various soils of U.K.	550	3	0.840	-1.68	0.98	Howard & Howard (1990)
	400	8	0.972	-0.37	0.97	Ben-Dor & Banin (1989)
	450	12	1.04	-0.03	0.92	David (1988)

Table 5. Current automated dry combustion CN analyzers, description, and operating principles

Manufacturer	Address/website	Model (s)	Operating principle/detection system
Costech Analytical Technologies	26074 avenue Hall, Suite 14, Valencia, California-91355, USA www.costechanalytical.com	ECS 4010 CHNSO	The sample within tin capsule reacts with oxygen and combust at temperatures of 1700-1800 °C. Combustion of sample generates mixture of N_2, CO_2, H_2O and SO_2. The gases are separated by gas chromatographic (GC) separation column and are detected sequentially by the TCD (thermal conductivity detector). The TCD generates a signal, which is proportional to the amount of element in the sample.
LECO Corp.	3000 Lakeview Avenue, St. Joseph, Michigan 49085-2396, USA www.leco.com	TruSpec series	Sample encapsulated in tin foil is combusted at 950°C and detection by infrared
PerkinElmer Life and Analytical Sciences	710 Bridgeport Avenue, Shelton, Connecticut-06484-4794, USA www.perkinelmer.com	2400 Series II CHNS/O Elemental Analyzer	Based on the Pregl-Dumas method. Samples are combusted with user flexible mode and gases are separated by frontal chromatography and eluted gases are measured using TCD.
Elementar Analysensysteme GmbH	Donaustrasse 7 D-63452 Hanau, Germany www.elementar.de	vario Macro, vario Max, vario EL III	Samples are dropped into the combustion tube at user selected temperature up to 1200°C. The use of tin vessels further elevates the temperature up to 1800°C. Complete combustion is ensured with O2 jet injection. Except for N_2, other gases are retarded into specific adsorption trap. After TCD signal for N2 is received, adsorption traps are thermally desorbed and the corresponding gases detected with TCD sequentially.
Thermo Scientific (part of Thermo Fisher Scientific Corporation)	81 Wyman Street, Waltman, MA 02454, USA www.thermo.com	Flash EA 1112 NC	Detection by TCD.

Table 6. Features of *in situ* soil C determination techniques

Method	Principle	Penetration in soil (cm)	Sampled volume(cm^3)	Features
Mid and Near-infrared reflectance spectroscopy (MIRS/NIRS)	NIRS (400-2500 nm) and MIR (2500-25000 nm) region utilized to quantify soil C. Based on the absorption of C-H, N-H and O-H groups found in organic constituents	0.2-1	~10	Invasive, MIR region needed KBr dilution because of strong absorptions. Strength of these absorptions may result into spectral distortions and nonlinearities.
Laser induced breakdown spectroscopy (LIBS)	Laser is focused on sample forming microplasma that emits light characteristic of the sample elemental composition	0.1	~10^{-2}	Able to provide data at 1 mm resolution, invasive, roots and rock fragments presence may cause C signal variability.
Inelastic neutron scattering (INS)	Based on inelastic scattering of fast, 14 MeV, neutrons from C nuclei and subsequent detection of gamma rays emitted from first C excited level	30	~10^5	Non-destructive, multi-elemental, scanning modality, analytic response function

Table 7. Comparison of soil C determination methods using automated dry combustion, LIBS and INS techniques

Automated dry combustion	LIBS	INS
Sampling and processing		
Destructive soil sampling and processed to finely homogeneous powder	Destructive soil sampling using cores, no processing needed	Non-destructive
Analysis time		
Sampling to final result needs at least week	Few minutes	One hour
Foot print		
Core diameter (2-3.5 cm)	Laser beam diameter (200µm)	~1.5 m^2
Analysis		
In most cases, thermal conductivity of CO_2 (evolved from combustion of soil) converted to percent C using homogenized sample weight	Spectra normalized to the total detected emission and calibration by standard samples to determine total C	Spectra normalized to a monitored neutron generator output. Trapezoidal peak net areas converted instantaneously to elemental C.
Future developments		
None	Improved sensitivity	Improved sensitivity, measuring depth profile

Figure Captions

Figure 1. Schematic presentation of the LIBS system, collection of microplasma, detection, and the spectral resolution of a sample (adapted from Cremers et al., 2001).

Figure 2. Different major sections of an Inelastic Neutron Scattering (INS) alpha prototype

Figure 1.

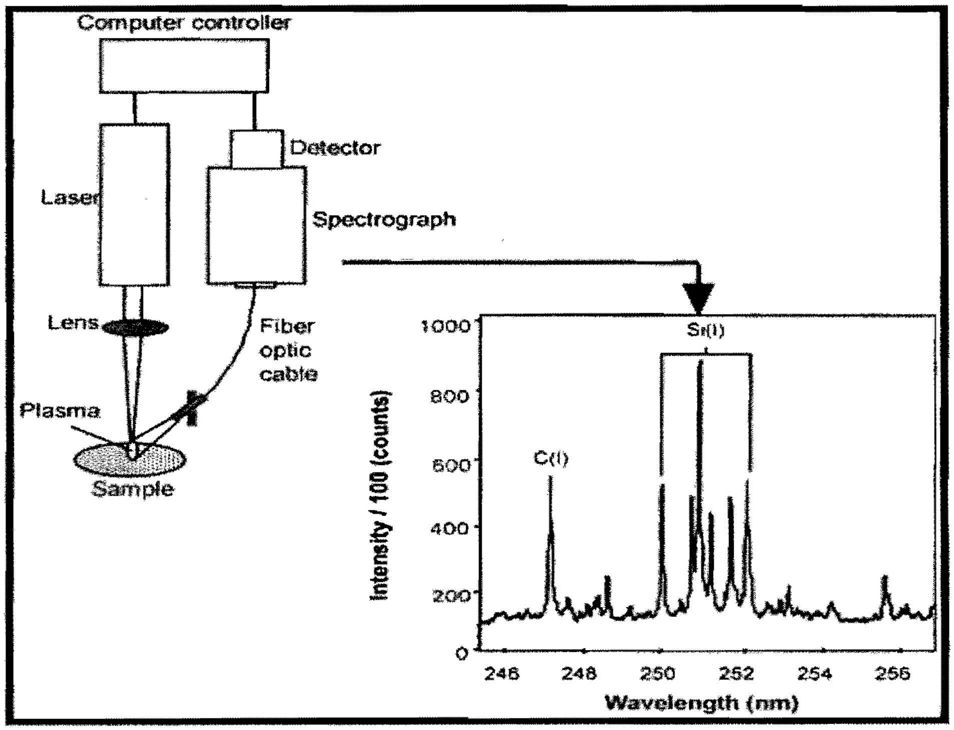
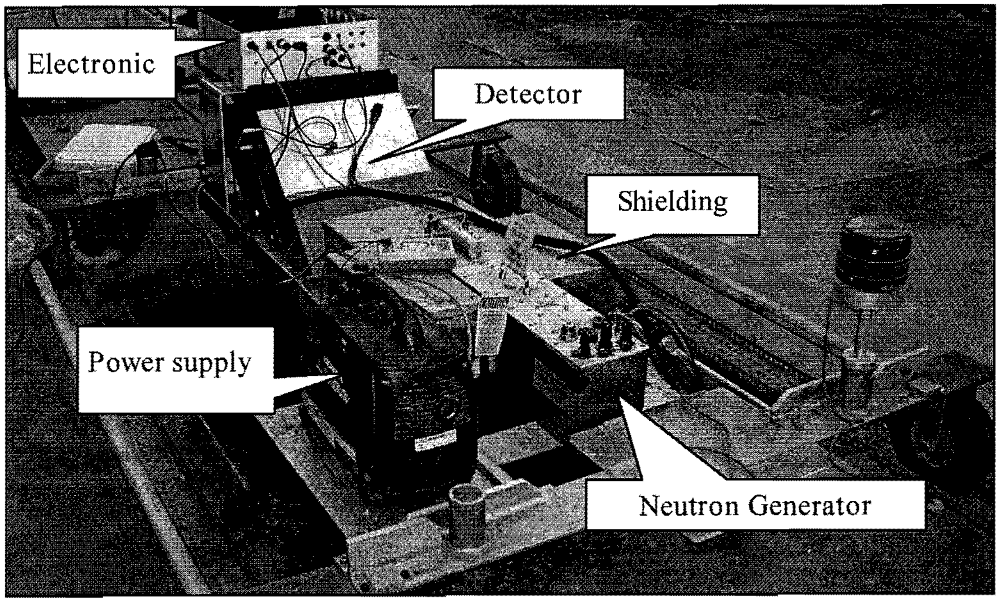

Computer controller
Detector
Laser
Spectrograph
Lens
Fiber optic cable
Plasma
Sample
Si(I)
C(I)
Intensity / 100 (counts)
0
200
400
600
800
1000
246
248
250
252
254
256
Wavelength (nm)

Figure 2.


Electronic
Detector
Shielding
Power supply
Neutron Generator