Soil Analysis Based on Samples Withdrawn from Different Volumes: Correlation versus Calibration

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Soil, particularly in forests, is replete with spatial variation with respect to soil C. The present standard chemical method for soil analysis by dry combustion (DC) is destructive, and comprehensive sampling is labor intensive and time consuming. These, among other factors, are contributing to the development of new methods for soil analysis. These include a near- and mid-infrared (NIR and MIR) spectroscopy, laser-induced breakdown spectroscopy (LIBS), and inelastic neutron scattering (INS). These technologies overcome many of the state-of-the-art DC method's shortcomings and offer advances that it cannot. While NIR and MIR measure C bonds, the other two new methods, like DC, are more specific in measuring C and other elements based on chemical, atomic, and nuclear reactions. In addition to their fundamentally different physical principles, these approaches vastly differ in the volumes they typically sample: LIBS, 10^{-9} m^3; DC, 10^{-7} m^3; NIR and MIR, 10^{-6} m^3; and INS, about 0.3 m^3. Thus, extra care is needed when comparing the findings from any two of these methods. Also, the high heterogeneity of the soil matrix, the nonuniformity of C distribution, and the presence of coarse fragments, particularly in forested ecosystems, further compound the difficulties in making direct comparisons. We investigated the implications of these differences when correlating any two of these methods and reviewed the processes of comparing a volumetric measurement against a point measurement. We also conducted a detailed comparison of the INS method with the standard DC test. We found that the total (soil organic matter and roots) measured by the INS correlated better than its components with the DC analyses($r^2 = 0.97$, $P = 10^{-7}$). The samples for DC analysis were taken from excavations of 40- by 40- by 40-cm plots, in 5- and 10-cm layers.

Abbreviations: DC, dry combustion; INS, inelastic neutron scattering; LIBS, laser-induced breakdown spectroscopy.

The sustainability of agriculture and forestry, as well as soundness of the environment, depends on the integrity of the soil's resources, its health, and productivity. In turn, those attributes are reflected by the level and state of organic C, N, and other nutrients. These components of soil organic matter also are sensitive indicators of the ongoing (induced) changes in soil degradation and exhaustion; accordingly, they were recommended as the minimum information needed for assessing soil quality (Lal et al., 2004, p. 3–21). One limitation to adopting conservation measures is that practitioners cannot always document the impact of their efforts. This is also the case when monitoring the efficacy of changing soil management practices or of reclamation protocols for reforestation and for producing biofuel crops on abandoned mine lands. Measuring soil C with the minimal achievable uncertainty is critically important to forest and agricultural managers, ecologists, and climate modelers (Kulmatiski et al., 2003; Galbraith et al., 2003).

With large landscapes, including topographical differences, the extent of the uncertainties often can be more important to the geochemical interpretation of

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the measurements than the value of the C concentration itself. Several independent factors affect the latter, such as field sampling, which reflects the natural variability, sample preparation, and analysis that contribute error to the same measurement (Ramsey, 1997; Wilding et al., 2001; Johnsen et al., 2004). Soil C variation is particularly abundant in forest soils and abandoned mine lands that exhibit high variability in C content, root distribution, and the size distribution of coarse fragments. These drawbacks entail the requirement for intensive sampling at a scale small enough to integrate across the spatial heterogeneity of the soil matrix, thus making reliable belowground assessments of organic C in these environments very challenging. Furthermore, tundra, both with or without permafrost, that is under consideration as a region for a planned global change experiment (Walker et al., 2006) will pose novel difficulties in sampling.

The current state-of-the-art for soil chemical analysis by DC of soil has many limitations, however, stemming from its destructive nature, the need to comprehensively sample the land unit of interest, and thus the effort required in collecting samples and preparing them for analysis (Gehl and Rice, 2007). The variability in the soil and in the sampling protocols that depend on the terrain's conditions and topography add to the methodology's complexity, and propensity for sampling error. An important concern is that a measurement by this method represents a point in time and space that must be extrapolated to cover large regions, and, in lieu of adequate destructive sampling, cannot be used in true sequential measurements.

There is no single analytical method able to address all the concerns about C analysis in soil. Instead, a method optimal for a particular task should be chosen, while recognizing its inherent limitations and underlying assumptions. During the last decade or so, three new methodologies for C analysis in soil have emerged as possible alternatives or complementary to the well established traditional DC method. They are NIR and MIR spectroscopy (Reeves et al., 1999), LIBS (Cremers et al., 2001), and INS (Wielopolski et al., 2008). Although, the LIBS and NIR and MIR methods require minimal sample preparation and overcome some shortcomings of the DC method, they are destructive, requiring soil cores from which volumes of about 10^{-9} and 10^{-6} m^3 are subsampled, respectively. Soil samples for the DC method are extensively processed and small aliquots of about 10^{-7} m^3, which represent a mean value for the homogenized volume, are analyzed. In contrast, the INS method is nondestructive, measures a volume of about 0.3 m^3 in a stationary mode, or can be used in a continuous scanning mode wherein the integrated counts represent a very large soil volume analyzed.

Thus, the main impetus for this work was to alert the reader of the large span in soil volumes, ranging from 10^{-9} to 0.3 m^3, analyzed by the various modalities, preventing, in a strict sense, calibration of one method against the other. This problem is particularly acute in the presence of large soil and C heterogeneities.

Calibration vs. Correlation

Very often the terms *calibration* and *correlation* are freely interchanged and misused. To clarify these two concepts and provide consistency in their use, these are briefly reviewed here.

Calibration is the validation of specific measurement techniques and equipment. At the simplest level, calibration is a comparison between measurements, one of known magnitude or correctness made or set with one device and another measurement taken in as similar a way as possible with a second device. The device with the known or assigned correctness is called the *primary standard* or simply a *standard*; very often, such devices assist in producing certified standard materials. Thus, standards and certified reference materials have an assigned value based on fundamental parameters or direct comparison with a reference base. A primary standard usually is under the jurisdiction of a national standards body, such as the National Institute of Standards and Technology (NIST). Secondary, tertiary, check standards, and standard materials may be used as references. A key requirement then would be traceability and an unbroken paper trail of calibrations back to the primary standard. The second device is the *unit under test* (UUT), in a process that establishes its calibration. Therefore, at its simplest, two basic requirements constitute a calibration: (i) using a device with known or assigned correctness in an absolute sense, or a primary or secondary certified or traceable to the NIST's standard materials; and (ii) taking the UUT measurement in as similar a way as possible to that taken with the primary device.

Certainly, soil analysis by the DC method does not qualify as a primary calibration device or as one with a calibrated reading. The standard materials used for the initial calibration of a DC analyzer differ substantially from the various soil matrices under analysis. In addition, as we discussed above, the very large differences in the sampled volumes by each of the methods, combined with the high heterogeneity in the field, represent different domains that require different underlying assumptions; this negates the possibility of calibrating any of the two previously discussed methods.

Instead, we compare two independent variables using *correlation*, indicating the strength and direction of a linear relationship between these two random variables. In other words, correlation tests the hypothesis whether two independent variables are co-related and measures the strength (departure) of two random variables from independence. In this broad sense, there are several correlation coefficients (Pyzdek, 2003), measuring the degree of correlation, adapted to the nature of the data; these are not discussed here.

In principle, each of the soil analytical methods could be calibrated in a strict sense against a known amount of C, albeit with a great deal of difficulty in preparing a proper standard material with a matrix identical to that of the soil. The slopes of the calibration lines (sensitivities of the methods) would differ because of the diverse interactions involved and differing number of C atoms in the dissimilar volumes sampled by each method; however, since all of the methods attempt to assess the same entity, C, they would retain, for a fixed volume, some proportionality. It is, therefore, logical that without any abrupt changes in the C profile and within the instruments' linear domains, there would be linear correlations between any two of the methods. In fact, under the ideal condition of no sampling errors, the slope of the correlation line would be the ratio of the sensitivities of each method.

In reality, however, the quality of the correlation line depends on the inherent inhomogeneities in the soil, the volumes analyzed, and error propagation. This problem is explicitly recognized in the different averaging schemes used to generate the best estimate of C on the local level as, for example, with a LIBS system wherein multiple laser shots are averaged, or multiple cores are taken for the DC method. Similarly, on a field level, researchers use mathematical averaging with or without geostatistical considerations, and mechanical averaging by homogenizing or compositing samples. These procedures are inherent in current standard practices where the findings from limited discrete samples are extrapolated to field or regional levels. Thus, these provisos must be recognized in comparing any two other methods.

MATERIALS AND METHODS

Site Description and Sampling

We selected a complex site with woody and rocky components in the soil matrix. We obtained measurements in a grassland, a pine forest, and a hardwood forest, on adjacent sites in the Blackwood Division of Duke Forest near Durham, NC (35°58'41.430" N, 79°5'39.087" W). The grassland is dominated by the C_3 grass *Festuca arundinacea* Schreb., with minor forbs (herbaceous non-woody plants), and C_3 and C_4 grass species; it is mowed at least once annually for hay. The pine site with loblolly pine (*Pinus taeda* L.) in the overstory was planted in 1983 and has been unmanaged since. The hardwood site is an unevenly aged, 80- to 100-yr-old oak (*Quercus* sp.)–hickory (*Carya* sp.) forest, and also was never managed. Stoy et al. (2006) gave a detailed description of these sites.

Immediately after the INS measurement, we took three soil cores to 40 cm deep for determining the moisture content. Subsequently, we excavated 40- by 40- by 40-cm pits in layers, collected samples from layers of 0 to 5, 5 to 10, 10 to 20, 20 to 30, and 30 to 40 cm, and processed them for analysis. Soil from each layer was sieved to remove live and dead roots >2 mm as well as coarse organic fragments and coarse stone fragments. Roots, coarse woody fragments, and the soil were ground, dried (60°C), and weighed. We analyzed the organic coarse fragments and live and dead roots for C content. Additionally, the total weight of the soil fraction in each layer was determined. Subsequently, we drew from each layer three 5-g samples that were pulverized, and two 0.2-g aliquots from each sample were analyzed for C and N by the DC method (Sollins et al., 1999) using a Flash EA 1112 series Thermo Finnigan NC soil analyzer (Milan, Italy) for a total of 264 aliquots (the 0–5-cm layer was combined with the 5–10-cm layer). Results from the grassland (G), pine (P) forest, and hardwood (H) forest sites were compared with those obtained with the INS system from the same sites.

Inelastic Neutron Scattering Instrument

The basis of the nondestructive INS system for C analysis in soil is the spectroscopy of γ rays induced by fast neutrons interacting with the elements present in the soil. An electrical neutron generator produces fast neutrons, and an array of NaI detectors registers the induced γ rays. The nuclear reactions are insensitive to C chemistry and, because the reactions are very fast, we can, uniquely, operate the INS system in static and scanning modes. Its multielement capability simultaneously provides quantitative information on key soil elements, such as, but not limited to, C, Si, O, N, H, and K. Wielopolski et al. (2004, 2008) described the system in detail. Figure 1 shows the INS system with its main components, mounted on a cart. The system is mounted about 30 cm above the ground, and data typically are acquired for 30 to 60 min. The INS's footprint and depth of analysis of about 1.0 m^2 and 30 cm, respectively, uniquely analyzes volumes of about 0.3 m^3 (>300 kg of soil). With previously established conversion from INS counts to grams C per square centimeter, the results, following the data acquisition, are reported immediately in units of grams C per square centimeter. Since, on average, the INS system queries a constant soil volume, its response is proportional to the C concentration. We note that the INS's response is governed by the exponential attenuation functions, Beer's law, of the neutrons penetrating into the soil and the γ rays emanating from it; accordingly, we define the effective volume as that from which 90% of the

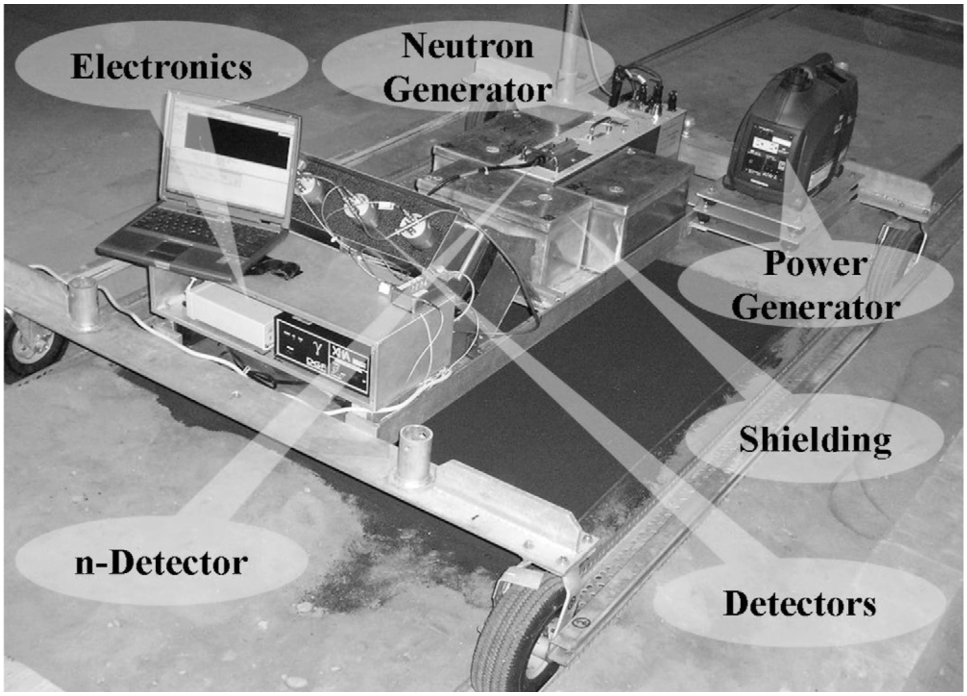

Fig. 1. Picture of the inelastic neutron scattering α prototype, identifying the major components (Wielopolski et al., 2008).

signal is derived. This volume corresponds to a depth of about 30 cm, whereas 99% of the signal derives from an approximate depth of 46 cm; these depths were obtained by Monte Carlo calculation simulating the system. These facts are embedded implicitly in the conversion factor from INS counts to grams C per square centimeter.

Analysis

Carbon analysis by the DC method is a three-step process: soil sampling, sample preparation, and analysis of a sample aliquot; each step contributes to error propagation. The total amount of C in the sample, C_T, is

$$C_{\mathrm{T}} = \int_V C_{\mathrm{v}} \mathrm{d}v \qquad [1]$$

where C_v is the true C concentration in soil volume element dv = dxdydz located at x,y,z, and V is the volume of the sampled soil. The sample is dried, sieved, and homogenized, thus creating an artificial analogous mixture with uniform C distribution weighing M kilograms. From this homogeneous mixture, subsamples weighing m kilograms are withdrawn, pulverized, and, typically, 0.001- to 0.005-kg aliquots undergo chemical analysis by DC. Using a previously established conversion factor k from a DC system signal, S_{DC}, to the C mass, the total C is estimated as C_{DC}, and, for a single spot is given as

$$C_{\mathrm{DC}} = \frac{M}{mkS_{\mathrm{DC}}} \qquad [2]$$

We note that C_{DC} is an estimate of the soil's C content to a given sampling depth; this result typically is multiplied by the soil's bulk density or divided by the surface area of the coring device to express the C content in grams C per cubic centimeter, or the more utilitarian unit of grams C per square centimeter.

As discussed above, a fundamental problem in comparing systems is that the new modalities analyze substantially different soil volumes; the IR and LIBS analyze much smaller volumes than DC, <<0.1%, whereas the INS volume is about five orders of magnitude larger. Nevertheless, by analogy to Eq. [1], we can consider that the total C, C_T', sampled by the INS is an integral across a larger volume V':

$$C_{\mathrm{T}}' = \int_{V'} C_{\mathrm{v}} \mathrm{d}v \qquad [3]$$

Since there are no intermediary steps, the INS signal is measured directly from the soil C; however, each C atom carries a weight, w_v, depending on its position in the soil relative to the detection system. Thus, the INS signal, C_{INS}, is a weighted integral, proportional to the total number of C atoms in the interrogated volume V'. The position-dependent weight, w_v, is associated with the attenuation of neutron penetration into the soil, and γ-ray emission toward the detector. The k' factor is a proportionality constant, i.e., the sensitivity of the system in converting counts to grams of C, and differs from k in Eq. [2]. Thus, again, under steady-state conditions, the number of counts registered is proportional to the total number of C atoms in the interrogated volume:

$$C_{\mathrm{INS}} = k'S_{\mathrm{INS}} = \int_{V'} w_{\mathrm{v}} C_{\mathrm{v}} \mathrm{d}v \qquad [4]$$

We earlier demonstrated a linear relationship between the INS's response and soil C content (Wielopolski et al., 2008) by chemical analysis by the DC method. We also showed that the interrogated volumes by either method are mostly reproducible. Thus, on average, for steady spatial conditions, C_T and C_T' are related by a constant. Therefore, we would expect the INS response in Eq. [4] to correlate with the response of DC in Eq. [2], even though the methods sample different volumes. We will confirm that this correlation improves with increasing overlap in the sampled volumes.

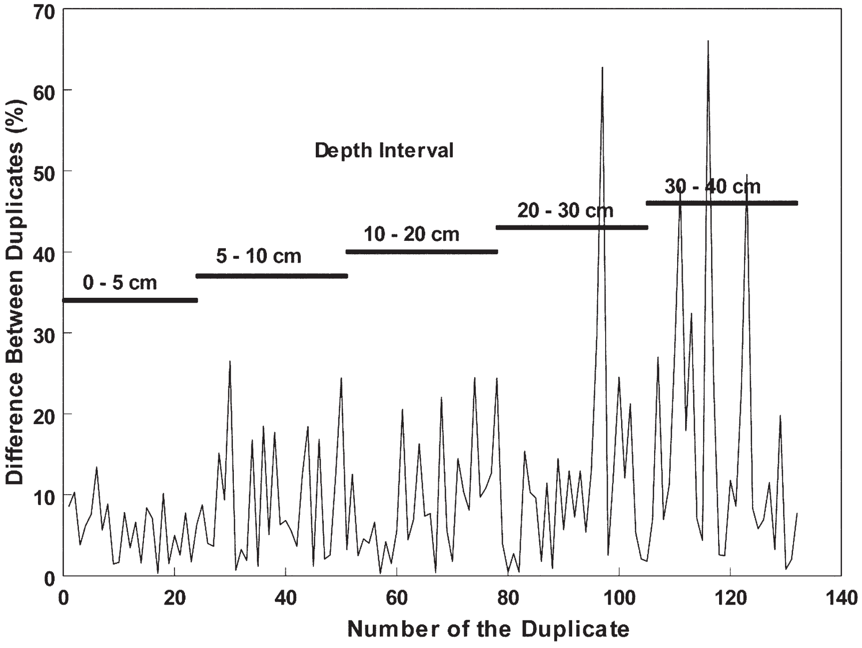

Fig. 2. Distribution of the differences between the duplicates organized with increasing depth of the sampling interval.

RESULTS

Correlation of the Inelastic Neutron Scattering Signal vs. Dry Combustion

We combined into one data set the DC results from the grassland, pine forest, and hardwood forest, and evaluated the differences between every two paired aliquots, 132 pairs, that were withdrawn from the same sample. In Fig. 2, we plotted the variability between paired aliquots, calculated as 100[ABS(Aliquot 1 – Aliquot 2)/MIN(Aliquot 1,Aliquot 2)], where ABS is the absolute value of the difference and MIN represents the minimum value of these two. The differences between the paired aliquots increased with depth where the C values were low. The large differences between the aliquots are surprising, since we took special care in homogenizing and pulverizing the samples. From Fig. 3, the histogram density function of the differences, the most probable difference is about 6%, with a higher mean of 10.2%, and standard deviation of 11.0%. There was a nonsubstantial change in the distribution of the

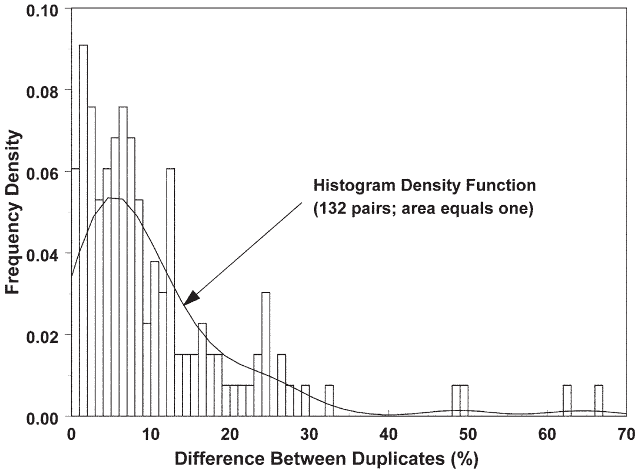

Fig. 3. Histogram and the histogram density function of the differences between aliquots drawn from the same sample.

differences when calculated relative to the maximum or the mean values of the two aliquots. After averaging the paired aliquots, however, the maximum difference between the samples from the same layer relative to their mean declined, although the mean differences remained about the same, 10.6%, as shown in Fig. 4. We also calculate the standard error of the mean (SEM) for n = 6 aliquots per layer:

$$\mathrm{SEM}=\frac{\mathrm{SD}}{\sqrt{n}} \qquad [5]$$

where SD is the standard deviation of the six aliquots. This averaging, smoothing process yields a mean SEM per layer of only 3.3%. Thus, when assessing the total surface C density per pit as the sum of the C surface density in each layer in a pit, the error is propagated according to

$$\mathrm{SEM}=\sqrt{\sum \mathrm{SEM}_j^2} \qquad [6]$$

where SEM_j is the standard error for each layer, and the summation is across the number of layers, j = 1 to 5 per pit. Table 1 summarizes these results, revealing that after the averaging processes for the multiple samples from different layers, the final standard error for the soil C surface density is reduced to about 1.6%.

We derived a regression line between the values for soil C (minus plant roots) given in Table 1 determined by the number of counts in the C peak by the INS method and chemical analysis; these results are summarized in Table 2 and plotted in Fig. 5 as the mean C line together with the 95% confidence levels. In principle, we might select the maximum or the minimum from each layer, particularly if we were to reduce the number of samples or aliquots used, and consequently affect the regression results as depicted in Fig. 5. We note that the lines for the mean, maximum, and minimum are almost parallel. Furthermore, the outcome might include the maximum values from the high end of the regression, and the minimum values from the low end, and vice versa; these two cases, denoted as Mix1and Mix2, respectively, are also plotted in Fig. 5 and summarized in Table 2. The changes in the slopes for the various cases are apparent, as are the predicted C concentrations due to the hypothetical readings of the low, 5000 counts and the high, 25,000 counts in the C peak. The r^2 values for all these regressions are similar, signifying the same quality of fit for all these cases that cannot be known in advance. Thus, reducing the number of points measured by chemical analysis will lower the quality of the correlation. This problem is further aggravated when using local correlations used to predict large fields that are highly inhomogeneous. Interestingly, all the regression lines are bound by the 95% confidence limit.

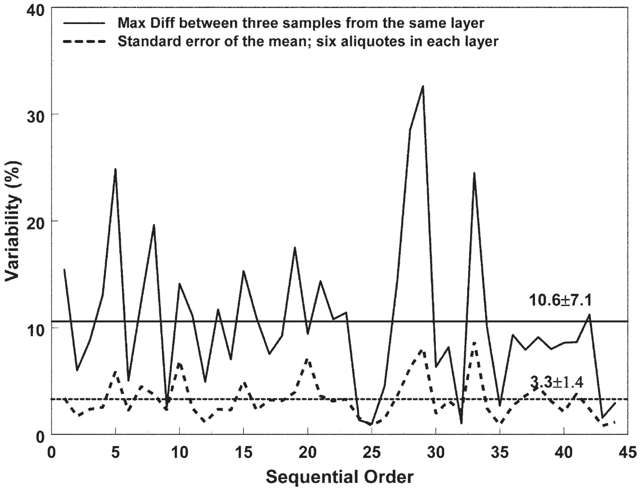

Fig. 4. Maximum difference between three samples from the same layer divided by the mean of the three, and the standard error of the mean of the six aliquots per layer. The horizontal lines represent the mean values and their standard deviations.

Volume Impact on the Correlation

The possibility of calibrating the INS against the DC method is negated by the very large difference in the volumes analyzed by each of the methods, in addition to other reasons. The responses of both systems, however, are directly proportional to the true C content (g C cm^{-2}) in the volumes that each analyzes. Furthermore, the volume analyzed by the DC method occupies a subspace of that used by the INS method. Therefore, assuming an average spatial consistency in the distribution of soil C, these two methods are proportional to each other, with the regression

Table 1. Mean soil C surface density and standard error of the mean (standard deviation divided by √6, the number of aliquots per layer); G1 to G3, H1 to H3, and P1 to P3 denote three sites in the grassland, hardwood forest, and pine forest, respectively.

Pit	Mean soil C	SEM
	g C cm^{-2}	%
G1	0.56	1.45
G2	0.77	1.72
G3	0.44	1.32
H1	0.57	1.73
H2	0.51	2.04
H3	0.72	1.70
P1	0.43	1.79
P2	0.57	1.31
P3	0.58	1.09

Table 2. Parameters of the inelastic neutron scattering (INS) regression lines fitted to soil C results by dry combustion; their means, maxima, and minima of two synthetic mixtures of C were selected for regression analysis.

Regression to	Slope (SE)	Intercept (SE)	r^2	P	Predicted C on 5000†	Predicted C on 25,000‡
					g C m^{-2}	
Mean	34,459 (6,986)	−3,148 (3511)	0.777	0.0017	0.236	0.817
Max	31,485 (6,700)	−3,176 (3620)	0.759	0.0021	0.260	0.895
Min	38,696 (8,148)	−3,541 (3727)	0.763	0.0021	0.221	0.738
Mix-1	24,051 (5,692)	1,714 (2971)	0.718	0.0039	0.137	0.968
Mix-2	49,149 (12,459)	−10,426 (6204)	0.690	0.0056	0.314	0.721

† Predicted surface C content based on hypothetical INS readings of 5000 counts.

‡ Predicted surface C content based on hypothetical INS readings of 25,000 counts.

line slope as the proportionality constant. This constant depends on the volumes used for comparison and implicitly presumes linearity between the signal and the soil's C content for each method. Such signal linearity with soil C was verified in 1500-kg homogeneous synthetic soils (sand mixed with known amounts of C), with an r^2 value of 0.995 (Wielopolski, 2006). This calibration also was derived analytically by solving the neutron-γ transport equation using Monte Carlo simulations normalized at one point for source intensity.

Figure 6 depicts these considerations on volume where we show the INS regression lines against C determined by DC in root residues and the soil. We also show the total C content in layers from 0 to 5 cm and from 0 to 40 cm in Fig. 6a and 6b, respectively. The quality of the regression is improved by (i) combining the C in the roots and soil, and (ii) increasing the soil's volume. Table 3 summarizes the systematic improvement in the r^2 and P values as the volume analyzed by the DC method approached that sampled by the INS. This table sums up the gradual improvements in the quality of the regressions for various depths of analysis; we plotted them for total C in Fig. 7. Initially, as expected, the slope of the regression line changed; however, beyond some depth, the INS's effective range of ~30 cm, adding more C by the DC method simply shifted the regression line to the right without changing its slope. This feature is reflected in the increase of the intercept of the regression line. Figure 8 shows the main regression line of the INS signal vs. total C to the depth of 30 cm with all the data points; thus, this is the line for converting INS counts into grams C per square centimeter.

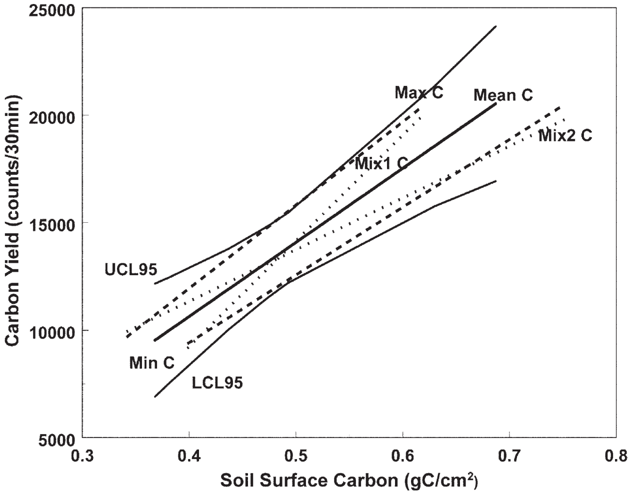

Fig. 5. Regression lines of inelastic neutron scattering vs. C content in the pits; solid lines represent the mean C values and upper (UCL) and lower (LCL) 95% confidence limits; dashed lines represent the maximum and minimum C values; and, the dotted lines denote the extreme mixture of maximum and minimum C values.

DISCUSSION

None of the existing and the newly emerging methods for C analysis in soil can be calibrated strictly and absolutely. The main obstacle to absolute calibration is the lack of standards duplicating the high heterogeneity (Conant et al., 2003; Poussart et al., 2004) in natural soil matrices. These difficulties are exacerbated in comparing one methodology to another because of the very large differences in the volumes each analyzes, basically negating any possibility of intergroup calibration.

We demonstrated a good correlation between INS measurements and the current standard method of chemical analysis by DC of combined soil samples obtained from 40- by 40- by 40-cm plot excavations from a pasture, a pine forest, and a hardwood

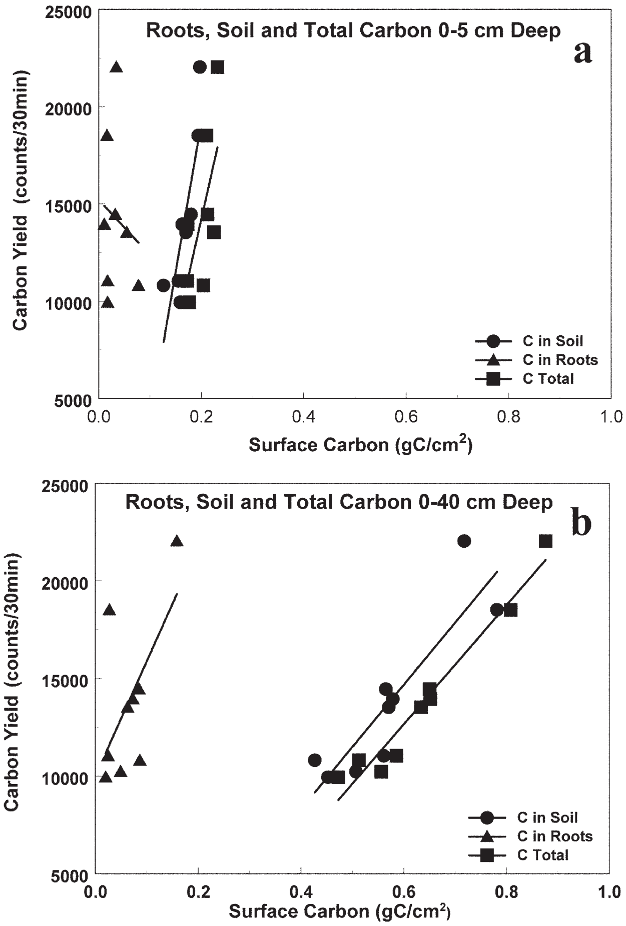

Fig. 6. Inelastic neutron scattering signal regressions vs. surface C in the roots above 2 mm, in the sieved soil, and the total combined C in the roots and soil determined by dry combustion in (a) the top 0- to 5-cm layer, and (b) from 0 to 40 cm.

Table 3. Summary of the regression parameters fitted to the soil, roots, and total belowground C as a function of increasing soil depth.

Soil depth	Location	Slope	Intercept	r^2	P
cm					
	soil	161675.9	−16518.9	0.7456	5.7×10^{-3}
0–5	roots	−43319.6	12088.4	0.05423	5.8×10^{-1}
	total	110928.7	−11586.3	0.3727	1.1×10^{-1}
	soil	105293.6	−17698.5	0.7785	1.6×10^{-3}
0–10	roots	32755.6	8759.2	0.0426	5.9×10^{-1}
	total	77224.0	−13890.4	0.6714	6.9×10^{-3}
	soil	47143.2	−8639.6001	0.8508	4.0×10^{-4}
0–20	roots	56182.8	6855.7960	0.3512	9.3×10^{-2}
	total	40319.5	−8376.3625	0.9797	3.5×10^{-7}
	soil	35860.1	−7361.7251	0.8155	8.5×10^{-4}
0–30	roots	55242.1	6759.0243	0.3282	1.1×10^{-1}
	total	33232.9	−8203.9298	0.9532	6.5×10^{-6}
	soil	33028.9	−8631.6176	0.8226	7.4×10^{-4}
0–40	roots	54573.7	6758.7757	0.322	1.1×10^{-1}
	total	30924.7	−9437.8525	0.9527	6.8×10^{-6}

oak forest, albeit from a total of only nine points. We highlighted the importance of using a large number of samples to represent a plot even when the plots were represented by excavated pits stratified and homogenized by depth. We also demonstrated an improvement in the INS's response vs. total C content when the C in the roots was combined with that in the soil. Thus, by undertaking sequential measurements at sites with and without roots, it might be possible to establish a root growth rate under different soil management practices.

Forests present considerable challenges for estimating soil C due to the lack of tillage, the presence of large perennial root systems, and the potential for plentiful coarse fragments. We worked with a set of plots ranging from pasture to forest with highly heterogeneous conditions. For example, across plots, the percentage of each pit made up of coarse stone fragments ranged from 4 to 29%. Tilled and no-till agricultural soils would probably simplify soil sampling using standard sampling via soil coring. Certainly, for a homogeneous soil with a uniform C distribution and no error in preparing the sample, the size and number of samples used for the analysis are immaterial; however, the errors in measurement and in sample preparation, together with the soil's natural variability in the field, necessitate multiple sampling with volumes as large as practically possible. The C depth profile (Jobbágy and Jackson, 2000) also contributes to uncertainty; for example, a homogenized 40- by 40- by 5-cm sample will yield a different C content than a 10- by 10- by 80-cm sample of the same volume. We cannot overstate the importance of adequate discrete sampling for DC to represent the volume interrogated by the INS. The microvariability of C in the soil, a priori an unknown, may affect the slope of the INS regression line and thus the field prediction from a scan. We detailed the effects of the volume of the sampled soil on the quality of the regression between these two methods and the resulting prediction of the soil's C content (Table 2). Thus, having information on the location and depth of the samples is essential for any meaningful C comparisons among methodologies.

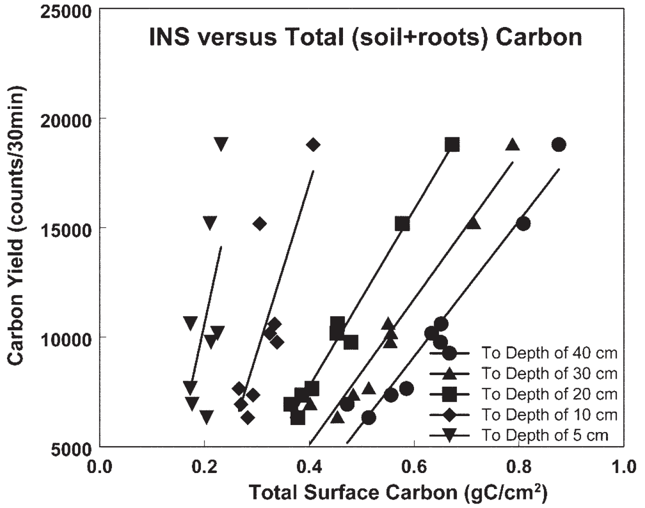

Fig. 7. Inelastic neutron scattering (INS) correlation with total surface C measured by dry combustion from various depths.

Because the soil C distribution varies laterally and with depth, intensive sampling is required so that C changes with time can be estimated. Improvement of such estimates on the landscape or higher levels frequently can be assisted by compositing core subsamples to arrive to better mean values; however, on a site like ours, high amounts of coarse stony fragments make random sampling and subsampling using soil cores difficult to impossible. Frequently, C estimates at a landscape level might be improved by using advanced geostatistical methods, albeit complicated ones (Simbahan et al., 2006). The INS scanning capability affords averaging across the scanned area and thus should provide a better estimate of the C level at a landscape scale, in particular if it is riddled with a high degree of inhomogeneities.

The number of samples required to represent the INS volume clearly depends on the microsite variation in C, which is

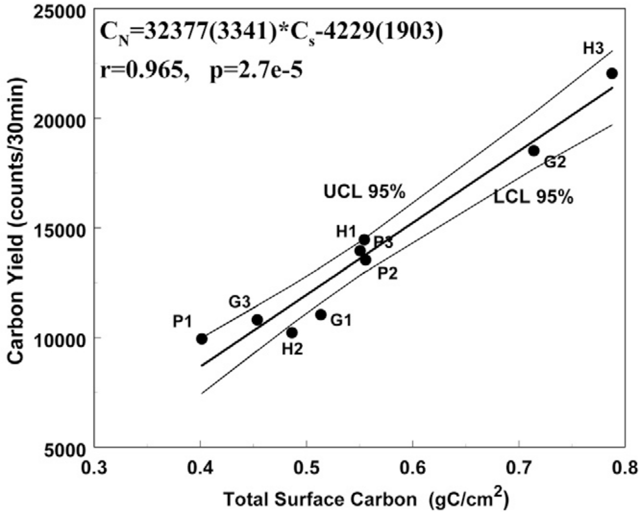

Fig. 8. Inelastic neutron scattering correlation, C net counts (C_N), with chemical analysis by dry combustion, C surface density (C_s), to a depth of 30 cm, including upper (UCL) and lower (LCL) 95% confidence limits.

impacted by past management practices. Using excavations and homogenizing soil from different layers, as reported here, yielded excellent correlations with INS measurements. In several of our other studies using soil cores, the regressions were not as good, with r^2 values in the range of 0.4 to 0.8 (data not shown) with the exception when using synthetic homogenized soil, where r^2 was 0.99 (Wielopolski, 2006). Furthermore, using cores may have resulted in our ignoring coarse fractions >2 mm, thus introducing error in the C determination. We clearly showed that including the C in coarse fractions >2 mm improved the fit.

We propose that with adequate sensitivity and a larger number of detectors, the INS will afford an excellent tool for monitoring belowground C sequestration in forests and other terrains.

SUMMARY

We outlined the concerns when comparing soil C analysis derived by various methods from vastly different soil volumes and their possible impact on correlation between any two methodologies. Specifically, we demonstrated the proportionality between the INS signal and the conventional DC chemical analysis. We also described the correlation between the INS system and the DC method, and elaborated on the implications from comparing point measurements vs. volumetric measurements. The unique characteristics of the INS system combined with its being a field unit afford a new sampling paradigm and C monitoring ability. In addition, using the new INS instrument entails an extensive reduction in time and effort, and consequently sampling costs, not possible previously. Once fully demonstrated, the INS could become an important method for monitoring soil C changes with time.

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