Walkley–Black analysis of forest soil organic carbon: recovery, limitations and uncertainty

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Abstract

Organic carbon levels of 542 soil samples from temperate lowland forest were determined by the original and modified Walkley–Black (WB) dichromate methods and total organic carbon (TOC) analysis. The performance and the lower and upper quantification limits of the WB method were assessed. Variable recovery rates were related to laboratory and field conditions and to the sample composition. The percentage carbon recovered by the original WB method was found to be systematically lower than commonly accepted, and the correction factor was estimated at 1.58 instead of 1.30–1.35. However, a good linear relationship with TOC enabled acceptable prediction of soil organic carbon which was most precise when using the original WB method. Texture class and pedogenetic horizon showed significant differences in recovery. Depending on the modifications of the WB method, recoveries varied significantly between laboratories, explaining up to 29% of the total variation of the topsoil carbon assessment of a site. Low recovery from samples was partly attributed to charcoal and resistant elementary carbon particles. No interference was found to be caused by iron or manganese compounds. In order to use WB carbon data of forest soils, laboratory- and method-specific determination of the recovery rate using a total analyser is strongly recommended. The original WB method was unable to predict reliably forest soil carbon contents higher than 8% TOC.

Keywords: Organic carbon, soil carbon, forest soils, Walkley–Black method, recovery, quantification limit, inter-laboratory variation

Introduction

The choice of which carbon assessment method to use is a critical decision which affects the estimation of soil organic carbon (SOC) stocks and their change over time (Brye & Slaton, 2003). Many historical soil databases report SOC values analysed by the Walkley–Black (WB) dichromate method or one of its numerous variants. In some cases these data sets have been recently reconsidered and the locations resampled to assess temporal changes in SOC contents and stocks in the context of greenhouse gas balance assessment (Ronse *et al.*, 1988 and Lettens *et al.*, 2005 in Belgium, Bellamy *et al.*, 2005 in England, Wang *et al.*, 2003 in China). However, as instruments and analysis procedures change, methodological differences are inevitable. The difficulty is

that modifications in analytical methodology, operational range limits and laboratory performance are rarely recognized and quantified in carbon accounting efforts. Skjemstad *et al.* (2000) determined specific WB conversion factors for nine Australian laboratories to allow comparison of data generated over a long period. Taking dry combustion total organic carbon (TOC) as the standard, they found absolute recovery differences of up to 25% between laboratories, but also within laboratories as the WB method was modified with time. They concluded that carbon stock assessment is inaccurate and highly tentative, unless these differences are recognized.

Nelson & Sommers (1982) stated that procedures such as WB should be considered to give approximate or semi-quantitative estimates of SOC because of the lack of an appropriate correction factor for each soil analysed. Indeed, several studies have demonstrated the necessity to determine soil-specific correction factors for WB data sets when these data

are compared with other standardized SOC assessments (Hussain & Olson, 2000; Mikhailova *et al.*, 2003). The majority of WB recovery studies focus on soils under agricultural management, reporting recovery differences resulting from management system (Hussain & Olson, 2000), vegetation (Diaz-Zorita, 1999), soil type (Allison, 1960) and depth (Drover & Manner, 1975). No references were found of recovery studies exclusively focusing on forest soils.

The WB method is based upon several assumptions. The most important is that carbon is the chief reducing agent present in soils and that carbon, oxidizable hydrogen and reducible nitrogen are present in the soil organic matter (OM) in about the same proportions (Walkley & Black, 1934).

Concern has been raised about possible interferences by other soil constituents with dichromate. $CaCO_3$ was found to be without influence in dichromate titrimetric methods, but chloride, ferrous iron and higher oxides of manganese have been shown to undergo redox reactions in chromic acid mixtures leading to incorrect values for OC (Walkley, 1947). Furthermore, elemental carbon, mostly present in forest soils as charcoal as a result of forest fires or human charcoal production, influences the recovery considerably depending on the WB method variant (Bremner & Jenkinson, 1960) and the particle size, origin and nature of the charred material (Skjemstad & Taylor, 1999).

All these factors could influence a WB determination and an attempt should be made to test their relevance for forest soil carbon assessment.

The aim of this work was to (i) determine the recovery and conversion factors that are appropriate for the soils under temperate lowland forest in Flanders, Belgium, (ii) assess the lower and upper quantification limits of the original WB method for these soils, (iii) determine the within- and between-laboratory measurement uncertainty, (iv) examine the factors determining recovery, both in the field and laboratory, and finally (v) estimate the number of samples needed to determine a laboratory- and method-specific recovery factor reliably.

Methods

Samples

A data set was assembled of 542 mineral soil samples collected from 135 forest sites across Flanders region. Latitude–longitude co-ordinates of the sampled locations range from 50°42¢ to 51°22¢N and 2°40¢ to 5°41¢E. Altitude above sea level varied from 3 to 118 m. The sampled soils belong to the World Reference Base reference groups: Podzols, Arenosols, Luvisols, Albeluvisols, Cambisols, Gleysols, Fluvisols, Anthrosols and Regosols (FAO, 1998). The sampled sites encompassed all major forest types of the region, ranging from pine stands on poor sandy soils with mor and moder

humus types to beech and oak stands on loamy sands, loams and silt loams, and poplar and ash stands on nutrient-rich alluvial silty clay and silty clay loams with mull humus type. For this study, the soil samples were stratified according to the soil factors: USDA texture class, pedogenetic horizon and sampling depth. Not all samples could be stratified due to missing soil information for specific samples. For the 542 samples, no or incomplete texture data were available for 19 samples, for eight samples no soil horizon was attributed and for 16 samples sampling depth was missing. However for the latter, soil horizon was stored in the database instead.

The number of samples per site ranged from 1 to 40. At two experimental sites, one near Brussels in the Sonian Forest (Silt loam soil – Albeluvisol) and one near Brasschaat (Sand – Podzol), samples were taken from the 0–10 cm soil layer at 20 points located on two parallel transects at 10 m intervals.

All other samples were taken according to pedogenetic horizon. The samples were stored at 2 °C prior to analysis.

Soil samples were oven-dried at 40 °C to avoid chemical decomposition at higher temperatures (UN/ECE, 2006), crushed and passed through a 2-mm mesh sieve. Following this preparation, the 20 samples of 0–10 cm from both experimental sites were split using a rotary cone sample divider (Laborette 27; Laval lab, Quebec, Canada) and sent to three laboratories for independent analysis. Texture was analysed using a laser diffraction method as described in De Vos *et al.* (2005).

Aqua regia extractable Fe and Mn were analysed by ICP-AES following microwave digestion. Exchangeable Fe and Mn were determined by the compulsive exchange method in a 0.1 mol L[1] $BaCl_2$ extract according to ISO 11260 (ISO, 1994).

Total organic carbon and loss-on-ignition analysis

Total organic carbon was measured with a TOC analyser equipped with a solid sample module operated at 900 °C (Shimadzu 5050A with SSM-5000A; Shimadzu, Kyoto, Japan). Analysis was performed according to ISO 10694 (ISO, 1995). Sample quantity was between 0.5 and 1 g and measurable range from 0.1 to 30 mg OC. Each sample was analysed in duplicate and the average reported. Methodology, detection limits and correction for clay content in the estimation of SOM by loss-on-ignition is described in De Vos *et al.* (2005).

Walkley–Black procedures

The WB method was developed in 1934 (Walkley & Black, 1934) based on the Schollenberger method, and further refined by Walkley (1947). Essentially, concentrated H_2SO_4 is added to a mixture of soil and aqueous $K_2Cr_2O_7$. The heat of dilution raises the temperature sufficiently to induce a substantial, but not complete, oxidation by the acidified

dichromate. Residual dichromate is back titrated using ferrous sulphate. The difference in added $FeSO_4$ compared with a blank titration determines the amount of easy oxidizable organic carbon.

The percentage WB carbon is given by the formula:

$$\text{WBC} = M \times \frac{(V_1 - V_2)}{W} \times 0.30 \times \text{CF} \quad (1)$$

where M is the molarity of the $FeSO_4$ solution (from blank titration), V_1 is the volume (mL) of $FeSO_4$ required in blank titration, V_2 is the volume (mL) of $FeSO_4$ required in actual titration, W is the weight (g) of the oven-dried soil sample and CF is the correction factor

The CF is a compensation for the incomplete oxidation and is the inverse of the recovery. This CF was set by Walkley & Black (1934) to 1.32 (recovery of 76%).

To assess differences in laboratory performance, the topsoil samples of the experimental sites were analysed by three different laboratories. The laboratories were highly experienced in WB analysis but used different variants of this method.

Laboratory A applied the WB procedure exactly as described in Walkley (1947), now defined as the original WB method. A recovery of 76% was used.

Laboratory B applied the WB method at the heat of dilution but care was taken to isolate the vessels using an expanded polystyrene sheet. Reaction time was also extended by 45 min compared with the original method. In addition, 10 mL of a 10% $BaCl_2$ solution was added to improve colour change detection. In general, laboratory B assumes a recovery of 77% but they mention an observed range between 70% and 84%.

Laboratory C applied a modified WB method using an external heat source based on the French standard X31-109 (Baize, 2000). However, the temperature of the $K_2Cr_2O_7$–H_2SO_4 mixture was raised to boiling point before removing the heat source instead of boiling for 5 min according to the X31-109 norm. This laboratory determined experimentally a recovery factor of 77%.

In order to compare the results of each laboratory we recalculated the reported WBC levels to their uncorrected value, denoted as WBC_{NC} (NC denotes not corrected).

Walkley–Black recovery and correction factor

The percentage recovery (RC) obtained by the WB method compared with dry combustion TOC is given by the following equation:

$$\text{RC} = \frac{100\text{WBC}_{NC}}{\text{TOC}} \quad (2)$$

The correction factor (CF) is calculated as 100/RC (%). When a CF was applied it is indicated as a subscript ($WBC_{1.32}$).

Limits of detection (LOD) and quantification (LOQ)

Detection and quantification capabilities are considered as fundamental performance characteristics of any chemical measurement process (Currie, 1999).

The LOD is the smallest measure, x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation:

$$x_L = x_{bi} + ks_{bi} \quad (3)$$

where x_{bi} is the mean of the n blank measures, s_{bi} is the standard deviation of the n blank measures, and k is a numerical factor chosen according to the confidence level desired (IUPAC, 1997). This k factor is commonly set at 3 for LOD. The LOD is the concentration at which we can decide whether an element is present or not. The LOQ is generally agreed to begin at a concentration equal to 10 standard deviations of the blank ($k = 10$). Therefore, LOQ = 3.3 LOD. Quantitatively, the relative standard deviation (RSD) of repeated measures is 10% at the LOQ and 33% at the LOD (Thomsen et al., 2003). More information on this matter can be found in Currie (1999).

The LOD was determined based according to equation (3) on 10 blank measurements ($n = 10$), repeated on three separate days in order to simulate intra-laboratory reproducibility.

For the empirical assessment of LOD and LOQ, a reference forest soil sample (silt loam, Sonian forest) of an A horizon with a WBC content of 4.35% (determined as the average of control chart measurements; $n > 120$) was measured 80 times. Subsamples of 10, 15, 20, 30, 40, 50, 75, 100, 500 and 1000 mg were analysed eight times each and their RSDs were calculated. LOD was determined at RSD = 33% using a least-squares curve fit of an equation of the form:

$$y = ax^{-b} \quad (4)$$

The same reference sample was used to estimate measurement error of TOC and WB analysis for the construction of a functional relationship.

Assessment of uncertainty

The uncertainties of the WB measurements are estimated according to the NIST 1297 Guideline (Taylor & Kuyatt, 1994). The standard uncertainty equals the positive square root of the estimated variance.

Each sample of the two experimental sites was split and analysed by the three laboratories. An ANOVA model was applied according to a nested classification scheme with 'between samples' and 'between lab' as components. Within laboratory A only, all samples were analysed in triplicate to assess the within-laboratory variability and the measurement uncertainty. It was assumed that this within-laboratory variability is the same for laboratories B and C.

Statistics were calculated using S-plus 6.2 professional (Insightful Corp, Seattle, WA, USA). Differences among ANOVA factor means were tested according to the Tukey method at $P ¼ 0.05$. The root mean squared prediction error (RMSPE) was calculated according to the method described in De Vos *et al.* (2005).

Results and discussion

Relationship between WB carbon, TOC and OM

The total organic carbon determined by dry combustion varied between 0.09% and 15.12%, between 0.08% and 9.77% for the uncorrected WBC, and between 0.10% and 12.85% for WBC corrected with a CF ¼ 1.32. For less than 1% of the total data set ($n ¼ 542$ samples), WBC_{NC} values were higher than TOC, while in 19% of all instances the corrected $WBC_{1.32}$ exceeded TOC.

A linear relationship between WBC_{NC} and TOC was found for the complete data set (Figure 1). The intercept of the linear regression model was negative and not significantly different from zero ($P ¼ 0.149$) and was therefore omitted, resulting in:

$$TOC ¼ 1.58WBC_{NC} \quad R^2 ¼ 0.96; P < 0.01: \qquad ð5Þ$$

$$TOC ¼ 1.20WBC_{1.32} \quad R^2 ¼ 0.96; P < 0.01: \qquad ð6Þ$$

Because there is a measurement error on the *X*-axis, a functional relationship was calculated according to the instructions provided by Webster (1997). Based on 30 replicate measurements, the error of WBC and TOC analysis was estimated as $s^2_{WBC} ¼ 0.033$ and $s^2_{TOC} ¼ 0.093$. However, this functional relationship was not significantly
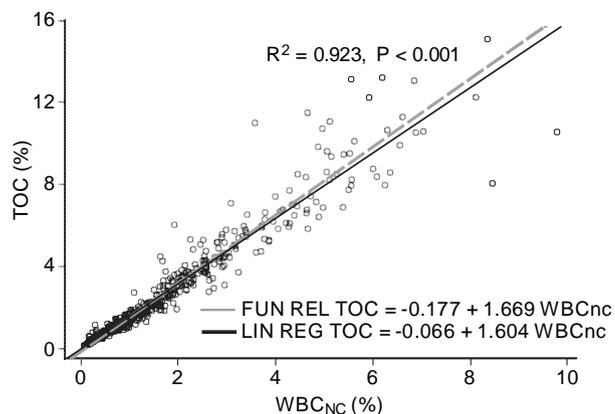
different ($P ¼ 0.88$) from the linear regression model. The observed mean absolute difference between the predicted values of both regression models was 0.075% carbon.

The spread around the regression line increases with increasing carbon content. For TOC values above 7.7% the slope of the linear regression is no longer significant ($P ¼ 0.076$), indicating that the original WB method is unable to predict carbon contents higher than 8% reliably. This confirms the findings of De Leenheer & Van Hove (1958). They reported a relatively stable recovery of 75% up to 7.5% C. Above 10% TOC these authors considered the original WB method was unsuitable. Applying the commonly accepted CF of 1.32 (Skjemstad *et al.*, 2000) to the results of the forest soil samples analysed by laboratory A results in a systematic underestimation of the total carbon content by 10–20%.

Based on loss-on-ignition analysis of forest soils in the Flemish region, OM was found to contain 58% of TOC (De Vos *et al.*, 2005). This factor is commonly accepted and used worldwide.

Substituting TOC ¼ 0.58 OM in equation 6 results in:

$$OM ¼ 2.07WBC_{1:32} \quad \text{or} \quad OM \lozenge 2WBC_{1:32}: \qquad ð7Þ$$

In order to estimate the OM content of forest soils, the corrected $WBC_{1.32}$ content (original method) should be multiplied by a factor 2, and TOC should be multiplied by 1.72. Although factor 2 is confirmed by many authors (Nelson & Sommers, 1982; Bellamy *et al.*, 2005) and mentioned in many procedures and handbooks (e.g. ISRIC – Van Reeuwijk, 2002), confusion remains and these conversion factors are often applied without reference to the specific recovery of the applied WB method. Hence, modified WB methods that equal TOC performance due to more complete oxidation conditions should use the 1.72 conversion factor instead of 2.

Determination range

While the upper limit of reliable WB analysis was found to be 8% TOC, the lower limit can be assessed by LOQ. The standard deviations of three sets of 10 blanks analysed on separate days were 0.041, 0.043 and 0.031 mL $FeSO_4$, respectively, for exactly 10 mL of 1 N $K_2Cr_2O_7$.

The average LOD for a 1-g soil sample is therefore theoretically estimated at $0.044 \pm 0.018\%$ and the LOQ at $0.14 \pm 0.06\%$ carbon. This is valid for a sample free of matrix effects, as no soil is present in the blanks.

Figure 2 shows the empirically observed relationship between carbon content and precision. Using the equation (4) of the least-squares curve fit through these data points, an LOD of 1.27 mg carbon could be interpolated for an RSD of 33%. Consequently, in a 1-g soil sample, 0.127% of carbon can be detected, but not reliably quantified.

Using an estimated LOD of 1.27 mg C, LOQ is 4.20 mg OC. This is in close agreement with the inflection

Figure 1 Linear regression and functional relationship between WBC_{NC} (Walkley–Black uncorrected values) and total organic carbon.

In figure:
$R^2 = 0.923, P < 0.001$
FUN REL TOC = -0.177 + 1.669 WBCnc
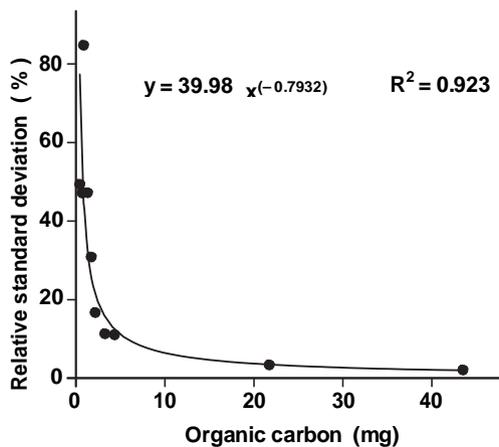LIN REG TOC = -0.066 + 1.604 WBCnc
TOC (%) vs WBC_{NC} (%)

Figure 2 Empirical determination of the precision versus organic carbon content of a test sample of a forest soil.

point of the curve which could be set at 4.35 mg OC or about 100 mg of the soil.

The test also shows that a minimum sample of 100 mg is needed for a reliable OC determination. Furthermore, it indicates that the empirical LOQ of actual soil material is about 0.42% C on a 1-g sample, which is about three times higher than the theoretically determined LOQ. Indeed, it is often observed that instrument detection and quantification limits are about two to five times lower than matrix detection and quantification limits.

In order to determine reliably less than 0.42% WBC in a forest soil sample, more than 1 g of sample is needed or about 4 g soil to enable determination at 0.1% OC level. Walkley (1947) allowed up to 10 g of soil weighed in, but many laboratories commonly use 1 g or less (Van Reeuwijk, 2002).

Variation in recovery and field factors

Reported recovery ranged between 59% and 94% (Allison, 1960; Nelson & Sommers, 1982). Walkley & Black (1934) found that on average 76% of the OC was recovered by the heat of dilution procedure, but the actual recoveries of the soils tested varied from 60% to 86%. The average RC of our data set was 68%, but individual recovery values varied considerably: from 27% to 105% with a 10–90 percentile range between 54% and 82%. Recoveries less than 54% were typically related to greater TOC values (median 4.29%) while RC >82% were related to low TOC values (median 1.02%). In general, recovery decreased with increasing TOC content according to a weak (R^2 ¼ 0.09) but significant linear relationship: RC ¼ 71.29 – 1.41 TOC ($P < 0.001$). So, each percentage of TOC lowers the recovery by 1.41%.

Sandy textures showed the greatest recoveries, which differed significantly from loam and silt loam soils showing 3–8% lower recoveries (Table 1). Differences were also found

Table 1 Mean recovery (RC) according to USDA texture class. Texture data were available for n ¼ 523 out of 542 samples

Texture class	Samples (n)	RC[a] (%)
Sand	303	70.0 a
Loamy sand	36	67.1 a
Sandy loam	13	66.7 a
Loam	22	58.3 b
Silt loam	149	63.7 b

[a]Within a column, mean values with different letters are significantly different at P ¼ 0.05 (Tukey method).

Table 2 Mean recovery (RC) according to pedogenetic horizon. Data on soil horizons were available for n ¼ 534 out of 542 samples

Soil horizon	Samples (n)	RC[a] (%)
A	232	65.6 a
E	169	70.3 b
B	102	68.6 ab
C	31	66.1 ab

[a]Within a column, mean values with different letters are significantly different at P ¼ 0.05 (Tukey method).

Table 3 Mean recovery (RC) according to soil depth class. Information on soil depth was available for n ¼ 526 out of 542 samples

Soil depth class (cm)	Samples (n)	RC[a] (%)
0–15	252	66.4 a
15–30	146	69.4 a
30–45	54	67.6 a
45–60	19	67.3 a
60–120	55	66.8 a

[a]Within a column, mean values with a letter are significantly different at P ¼ 0.05 (Tukey method).

between pedogenetic horizons (Table 2). In general, eluvial horizons showed significantly higher RC values than A horizons. This is consistent with our findings that soil layers depleted in clay or low in OM show higher recoveries. The same was observed when stratifying the soils according to fixed depth classes (Table 3). The upper 15 cm topsoil showed a smaller RC than the underlying 15–30 cm layer, although this difference was not significant. Below 30 cm RC decreased again presumably due to clay illuviation (Luvisols) and/or humus enrichment (Podzols).

The same differences in recovery were observed between coniferous stands, mostly located on sandy soils in Flanders region and broad-leaved forest on soil types with a higher clay content. When stratified according to dominant tree species, RC values were found to increase from poplar stands,

Table 4 Mean values of Walkley–Black carbon (%) analysis as reported by three different laboratories for 19 topsoil samples of a silt loam and 20 topsoil samples of a sandy site

Laboratory	Silt loam TOC ¼ 8.93 (2.62)			Sand TOC ¼ 3.04 (1.53)		
	WBC_{NC}^{a}	WBC_{COR}^{b}	RC_{NC}	WBC_{NC}	WBC_{COR}	RC_{NC}
			60.8 a (7.8)	1.90 a (0.87)	2.54 a (1.16)	65.3 a (10.9)
A	5.37 a (1.46)	7.15 a (1.95)	75.5 b (9.1)	2.36 a (1.04)	3.06 a (1.35)	81.5 b (14.0)
B	6.67 b (1.75)	8.66 b (2.28)	75.4 b (11.4)	2.29 a (1.13)	2.98 a (1.46)	76.8 b (14.1)
C	6.69 b (2.05)	8.69 b (2.67)				

Values within parenthesis are SD. WBC_{NC}, uncorrected values; WBC_{cor}, corrected values; RC_{NC}, % recovery.
[a]Within a column, mean values with different letters are significantly different at P ¼ 0.05 (Tukey method).
[b]Standard correction factors applied by each laboratory: A ¼ 1.32, B ¼ 1.30, C ¼ 1.30; WBC_{cor} are the reported values.

through to Corsican and Scots pines on sandy sites. However, the strong confounding effect between tree species and soil texture could not be quantified because of data set limitations.

These results suggest that the use of a separate forest soil recovery factor for (i) sandy soils and (ii) loamy and silt loam soils is worthwhile. The suggested recovery factors are 70% and 63% respectively. Diaz-Zorita (1999) proposed for Graminean pasture systems on Typic Hapludolls a recovery factor of 59% and 74% for the same soils under agricultural management.

Differences in WB application methodology

Differences between the laboratories were often related to effects of digestion conditions (Walkley, 1947). Table 4 illustrates the inter-laboratory differences.

For the silt loam, the average corrected WBC values were always less than the TOC values; however, the difference between these two values was only significant for laboratory A (P ¼ 0.0053). For the sandy sample, the WBC reported by laboratory B agreed most closely with the TOC, although the values reported by the other laboratories were not significantly different. The recovery rate for laboratory A was, as expected, always lower than for other laboratories, confirming that the original WB method recovers substantially less carbon than the modified versions. However, the modified methods of laboratories B and C applying external heat typically show more variable results, as illustrated by their greater SD in Table 4; this was reported earlier by De Leenheer & Van Hove (1958). Consequently, when for each laboratory its specific RC_{NC} was used to predict TOC, the RMSPEs were 1.12%, 1.19% and 1.62% for the silt loam, and 0.50%, 0.61% and 0.63% for the sandy sample for laboratories A, B and C respectively. It shows that the prediction of TOC based on WB is most precise for laboratory A, although recovery is lowest.

Based on a nested-ANOVA analysis, the variance components of the results from the three laboratories are given in Table 5.

Table 5 Estimated variance components (s^2) of Walkley–Black analysis results of a silt loam site (19 samples) and a sandy site (20 samples) independently analysed by three laboratories. Percentage of each component in brackets

Site	s^2_{total}	$s^2_{between-sample}$	$s^2_{between-laboratory}$
Silt loam	3.51 (100)	2.49 (71)	1.02 (29)
Sand	1.07 (100)	0.93 (87)	0.134 (13)

Values within parenthesis are in percentage.

Of the total variance, most variation was attributed to between-sample or field variation, which includes field sampling and spatial geochemical variability.

The within-laboratory variance, estimated for laboratory A only, was 0.27 (7.7% of total) and 0.06 (5.6% of total) for the silt loam and sandy site respectively. The within-laboratory variance was 26% and 45% of the between-laboratory variance. This inter-laboratory difference, most likely resulting from differences in WB method, amounted to 29% of the total variation.

It is commonly accepted that not more than 20% of the total variance should be sampling and analytical variance. In this comparison, between-laboratory variance alone is already more than 29%, indicating that more attention should be paid to the standardization and calibration of the methods.

The average standard uncertainty for a laboratory A measurement was 0.5% C and 0.2% C for the silt loam and sand samples respectively. The average relative uncertainty, which is the standard uncertainty relative to the mean C content, was 10.6%.

Factors explaining recovery differences

Effect of temperature. Reaction temperature is an important factor in determining the recovery (De Leenheer & Van Hove, 1958). Most modifications of the WB method change temperature conditions and hence RC. Temperature depends on acid strength, size of the reaction vessel, air temperature and even air flow rate in a fume cupboard.
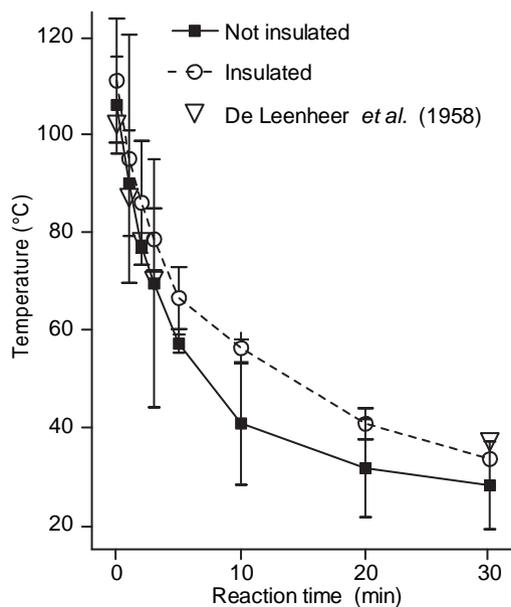
Figure 3 Evolution of reaction temperature for non-insulated and insulated reaction vessels. Error bars are 95% confidence limits of five replicates. No values were provided for 5, 10 and 20 min by De Leenheer & Van Hove (1958).

We compared the change of the reaction temperature under the working conditions of laboratory A to that described by De Leenheer & Van Hove (1958) for the same 10:20 (v:v) mixture of $K_2Cr_2O_7$ and H_2SO_4 (Figure 3). Initial temperature of the heat of dilution of the mixture was between 100 and 111 °C. Walkley (1947) mentioned temperatures between 110 and 120 °C and De Leenheer & Van Hove (1958) between 102 and 105 °C.

In the first 3 min, the reduction in temperature was similar regardless of the method, but then cooling was more rapid in laboratory A. Insulation measures and ambient laboratory temperature help explain this difference. When the vessels were insulated by standing on expanded polystyrene, the rate of temperature reduction was significantly reduced (Figure 3), resulting in a relative increase in recovery of 7% (4.3% absolute). In addition, performing the analysis in a fume cupboard can make an absolute difference in final recovery of 2–4%, depending on the air flow. Walkley (1947) mentioned a 2% lower RC in 500 cm^3 vessels than in 250 cm^3 ones and he observed that recovery increased by about 1% when ambient air temperature was raised by 5 °C.

Recovery increases when external heat is applied, and many laboratories modify their WB method empirically in various ways. However, heating above 150 °C leads to the risk of thermal decomposition of dichromate, decreasing the oxidation potential.

All these factors make RC differences between laboratories inevitable and these differences are not recognized in most soil carbon databases (Skjemstad et al., 2000). Therefore, determination of a laboratory-specific WBC recovery factor based on comparison with a total analyser is essential in order to obtain equivalent results.

Charcoal and resistant elementary carbon particles. Forest soil samples may contain considerable amounts of carbon fractions that resist wet oxidation. Dichromate methods that involve no external heating give poor RC of OC present in carbonized materials such as charcoal, graphite, coal and soot (Nelson & Sommers, 1982), whereas methods involving external heat show substantially higher but also very variable recoveries (Bremner & Jenkinson, 1960; Skjemstad & Taylor, 1999).

We experimented with wood charcoal containing $98.4 \pm 0.8\%$ TOC. Determination of WBC_{NC} on a 0.2-g milled subsample of the charcoal resulted in values of only 2.5–3.2% carbon. Supplementary analysis of milled charcoal from beech and ash showed WBC_{NC} values between 4.7% and 6.0%. When 0.5 g of a reference forest soil sample with $3.3 \pm 0.1\%$ WBC_{NC} was spiked with variable amounts (2–50 mg) of charcoal, WBC_{NC} increased only slightly from 3.3% to 3.5% but the RC decreased from 56% to 25% (Figure 4). So original WBC was almost unaffected by a charcoal admixture and greatly underestimated the elemental carbon stocks. If only one-fifth of the SOC consisted of charcoal carbon (only 1.3% of sample mass) RC decreased by 11%. Microscopic inspection of the remaining fraction after digestion of forest soil samples showed a lot of charred particles together with other organic fragments that withstood dichromate oxidation.

Interferences by other forest soil constituents. The rapid dichromate methods are subject to interference by certain
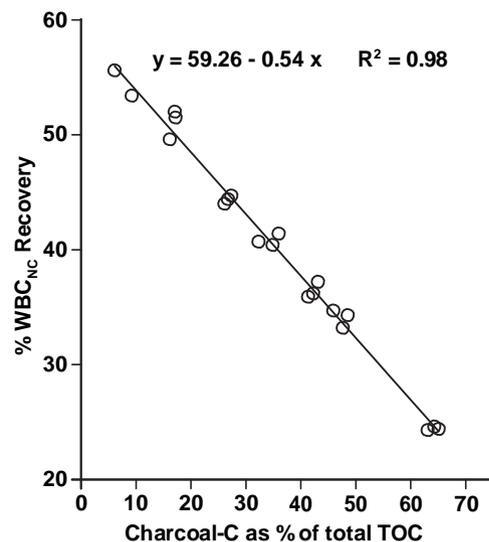

Figure 4 Decrease in recovery (uncorrected WBC) by spiking a forest soil reference sample with wood charcoal powder.
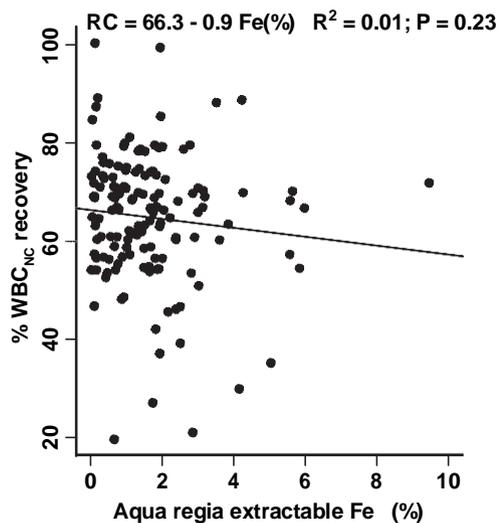
Figure 5 Relationship between WBC recovery and % Fe extracted by aqua regia in forest soil samples.
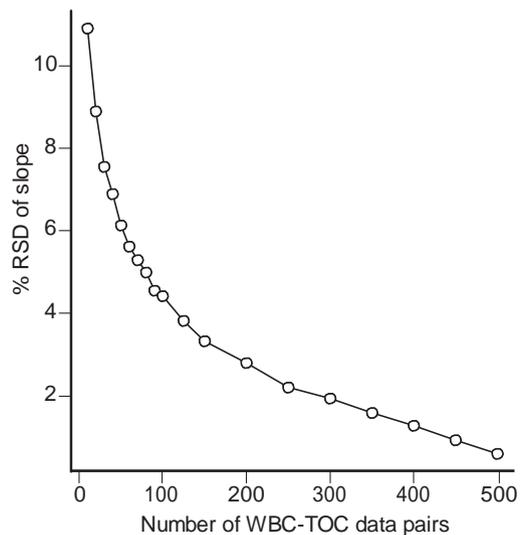

Figure 6 Simulation of number of data-pairs needed to reduce the relative standard deviation of the recovery rate. RSD ¼ relative standard deviation.

soil constituents that lead to spurious results (Walkley, 1947). The presence of appreciable amounts of Fe^{2+} or Cl^- in the soil will lead to a positive error, whereas MnO_2 will result in a negative error and lower values for OC (Nelson & Sommers, 1982).

In the forest soils studied, chloride levels were low. Because all soil samples were oven-dried, no appreciable concentrations of ferrous iron were expected to remain in the samples. However, Nelson & Sommers (1982) reported that metallic iron in soil samples may also lead to positive interference in dichromate methods. This was recognized by Walkley (1947) in recommending that iron or steel mortars be avoided. In practice soil sampling and laboratory equipment do contain iron, and contamination leading to high recoveries is possible.

We therefore tested a subset of our data (n ¼ 143 samples) where the recovery was plotted against the amount of aqua regia-extractable Fe (Figure 5). The slope of the regression was not significantly different from zero. Similarly, no significant relation was found with $BaCl_2$-exchangeable Fe (data not shown).

Higher oxides of Mn compete with $Cr_2O_7^{2-}$ for oxidizable substances when heated in an acid medium leading to a negative error when any reactive MnO_2 is present (Nelson & Sommers, 1982). However, we found no significant influence of aqua regia-extractable manganese on the WB recovery for the data subset. The regression function was RC ¼ 64.5 + 0.023 Mn (P ¼ 0.65, R^2 ¼ 0.001) with manganese expressed as mg kg^{-1} DW. In addition, $BaCl_2$-exchangeable manganese had no effect on the recovery (RC ¼ 64.4 − 4.49 Mn_{ex}; P ¼ 0.71, R^2 ¼ 0.0008). This reinforces the conclusions of Walkley (1947) that the quantity of reducible oxides of Mn in most soils is small because only freshly precipitated MnO_2 takes part in redox reactions.

Number of samples for determining recovery factor

Based on our forest soil data set we estimated the number of WBC–TOC data pairs needed for a reliable calibration of the specific recovery for laboratory A. The RSD of the slope was calculated on 1000 realizations of a regression based on 10–500 data pairs selected at random without replacement. The simulation shows that the inflection point is located between 125 and 150 data pairs (Fig. 6). This number will suffice for a reliable estimation of the laboratory-specific recovery.

Conclusions

Large between-laboratory differences in C recovery with WB analysis of forest soil samples were observed. The original WB method using the heat of dilution showed 10–15% lower recovery than modified versions. However, good linear relationships enabled reliable prediction of TOC when laboratory-specific recovery factors were determined. Prediction was found to be most precise with the original method in spite of a lower recovery. Original WBC analysis can be reliably performed within the range of 0.42–8% TOC based on a 1-g soil sample. A larger sample is needed to lower the quantification limit. Other methods of C determination are recommended for samples containing more than 8% OC.

In the field, differences in absolute recovery fell within the range of 0–7% and were attributed to soil texture and horizon. Sandy sites with conifers showed 6% higher recoveries than broad-leaved species on heavier textured soils.

In the laboratory, recovery may be explained by all measures influencing reaction temperature. Their influence may change recovery from 1% to 7%.

Charcoal and resistant elementary carbon particles in soil samples cause a decrease in recovery, but the presence of iron and manganese compounds showed no significant effect.

For each laboratory and type of soil, it is recommended that specific recovery factors are determined in order to standardize the results. Analysing 125–150 samples randomly taken from all soils of interest will suffice to estimate a reliable recovery factor.

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