Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use

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Abstract. *A simple method of estimating changes in biologically active soil carbon (C) could help evaluate soil quality impacts of alternative management practices. Most reports of permanganate for active C determination use highly concentrated solutions (0.333* M*) that are difficult to work with and tend to react with a large fraction of soil C that is not well distinguished from total organic C. We report on a highly simplified method in which dilute, slightly alkaline $KMnO_4$ reacts with the most readily oxidizable (active) forms of soil C, converting Mn(VII) to Mn(II), and proportionally lowering absorbance of 550 nm light. The amount of soil C that reacted increased with concentration of $KMnO_4$ used (0.01 to 0.1* M*), degree of soil drying (moist fresh soil to air-dried for 24 hour) and time of shaking (1–15 minutes). Shaking of air-dry soil in a 0.02* M *$KMnO_4$ solution for 2 minutes produced consistent and management-sensitive results, both in the laboratory and with a field kit that used a hand-held colorimeter. Addition of 0.1* M *$CaCl_2$ to the permanganate reagent enhanced settling of the soil after shaking, eliminating the need for centrifugation in the field kit. Results from the laboratory and field-kit protocols were nearly identical (R^2 = 0.98), as were those from an inter-laboratory sample exchange (R^2 = 0.91). The active soil C measured by the new procedure was more sensitive to management effects than total organic C, and more closely related to biologically mediated soil properties, such as respiration, microbial biomass and aggregation, than several other measures of soil organic C.*

Key words: active soil carbon, analytical methods, field kit, microbial biomass, permanganate oxidizable carbon, soil organic matter, soil aggregate stability, soil quality assessment, soil quality indicators

Introduction and Background

Soil organic matter (SOM) and related soil properties are probably the most widely acknowledged indicators of soil quality (Gregorich et al., 1994; Wander and Drinkwater, 2000). Since SOM has no definite chemical composition, soil organic carbon (SOC), the dominant elemental constituent of SOM, is more commonly measured and reported in scientific literature. Soil organic C is naturally variable across landscapes, soil types and climatic zones. It is generally characterized by high levels of C in recalcitrant or humified forms. Small changes in SOC resulting from changes in soil management are often difficult to measure, but can have pronounced effects on soil behavior and microbial processes. It may take many years for contrasting soil management practices to cause measurable differences in SOC (Sikora et al., 1996).

Changes in small but relatively labile fractions of SOC may provide an early indication of soil degradation or improvement in response to management practices. The labile fractions of soil C are important to study in their own right as these fractions fuel the soil food web and therefore greatly influence nutrient cycles and many biologically related soil properties. The labile fractions of soil C are often termed the active C pool, to distinguish it from the bulk of the soil C, which belongs to a highly recalcitrant or passive C pool that is only very slowly altered by microbial activities. Fractions of SOC that are thought to represent the active C pool, and serve as sensitive indicators of changes in management-induced soil quality, include microbial biomass C (Islam and Weil, 2000; Kennedy and Papendick, 1995), particulate organic matter (Janzen et al., 1992; Wander and Bidart, 2000) and soil carbohydrates measured as anthrone-reactive C (Deluca and Keeney, 1993; Saviozzi et al., 1999).

Scientists, extensionists and farmers are increasingly interested in making simple assessments of soil quality in the field, to help guide management decisions (Liebig and Doran, 1999; Wander and Drinkwater, 2000). The USDA Natural Resources Conservation Service (NRCS) has

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therefore developed several tools for field assessment of the impact of management practices on soil quality, including a qualitative Soil Health Assessment Card (USDA–NRCS, 1999) and a more quantitative Soil Quality Test Kit (USDA–NRSC, 1998). The current version of the NRCS Soil Quality Test Kit contains tests for nine soil parameters (USDA–NRSC, 1998). However, it does not include any test for either the active fraction or total SOM or SOC. Determinations of such labile C fractions as particulate organic matter, extractable carbohydrates or rapidly mineralizable C are time consuming and require complex laboratory manipulations that limit their use. Total SOC content can be readily determined in the laboratory by wet acid dichromate oxidation (Islam and Weil, 1998b; Walkley and Black, 1947), CO_2 released by dry combustion (e.g., LECO Corp. CHN Analyzer) and loss of mass on ignition (Magdoff, 1996). However, a practical field test for total or active organic C is not yet available.

In earlier work, Islam and Weil (1997) showed that anthrone-reactive C, extractable after a short-term microwave treatment, was a good predictor of a soil quality index that integrated 11 physical, biological and chemical soil properties. However, there are several limitations of the anthrone-reactive C as a measure of soil quality:

- the procedure requires expensive laboratory equipment (microwave oven, water bath, shaker, centrifuge, spectrophotometer, etc.);
- the anthrone reagent is both unstable and a toxic irritant containing concentrated H_2SO_4, which is too hazardous for routine use in the field;
- the results show poor repeatability and high sensitivity to operator technique; and
- the anthrone reaction is subject to interference by such common soil constituents as Cl^-, NO_3^- and Fe^{2+} (Doutre et al., 1978; Johnson and Sieburth, 1977).

Other colorimetric methods for measuring sugars in soil, using such reagents as *p*–hydroxybenzoic acid hydrazide (PHBAH) and bisodium bicinchoninic, are described in the literature (Joergensen et al., 1996; Lever, 1972), but each has its own limitations concerning complexity, toxicity, equipment requirements and/or lack of reproducibility, and lack of sensitivity to soil management practices affecting soil quality.

In contrast, potassium permanganate ($KMnO_4$) has many characteristics that are propitious for a routine field method. The intense purple color of the $KMnO_4$ solution enables it to serve as its own indicator. If properly prepared and stored, permanganate solutions can be stable over several months (Swift, 1939). It is so safe to handle that solutions ranging from 0.006 to 0.3 *M* are recommended in human and veterinary medicine as an antiseptic treatment for skin infections and wounds (Brander et al., 1982).

In a neutral to slightly alkaline solution, potassium permanganate ($KMnO_4$) is a powerful oxidizing agent because of the large negative value (–1.45 V) of the potential between the Mn^{2+} and MnO_4^- ions (Cotton and Wilkinson, 1965). At pH 7.2, portions of SOC react with $KMnO_4$ to partially bleach the deep purple permanganate color to light pink or clear (Loginow et al., 1987). Specifically, slightly alkaline $KMnO_4$ is known to hydrolyze and oxidize simple carbohydrates, amino acids, amine/amide sugars, and C-compounds containing hydroxyl, ketone, carboxyl, double-bond linkages and aliphatic compounds, to give a light pink color (Loginow et al., 1987; Skoog and West, 1969; Stanford, 1978). Lefroy et al. (1993) used several concentrations of $KMnO_4$ in an attempt to measure soil C fractions that were related to such soil quality properties as aggregation and infiltration. From these results, Blair et al. (1995) concluded that only one $KMnO_4$ concentration (0.333 *M*) was needed to distinguish labile soil C (oxidized by $KMnO_4$) from recalcitrant soil C (not oxidized by $KMnO_4$). They compared the relative size of these two C fractions in cropped soils to those in nearby uncultivated 'reference sites', to derive a C management index for agricultural systems.

To date, most research on $KMnO_4$-reactive soil C has used the 0.333 *M* $KMnO_4$ method of Blair et al. (1995) to oxidize a fraction of soil C considered active or labile. Blair et al. (2001) report that this reagent appears to react with a relatively labile pool of soil C, and that changes in soil management often influence 0.333 *M* $KMnO_4$-reactive soil C more markedly than they do total SOC. Significant correlations have been reported between 0.333 *M* $KMnO_4$-reactive C and several soil chemical and physical properties (Bell et al., 1998; Blair and Crocker, 2000; Blair et al., 1995; Moody et al., 1997; Whitbread et al., 2000). However, at this high concentration, $KMnO_4$ reacts with a rather large fraction of the total SOC [14–27% of the total organic carbon (TOC) in the 13 soils described by Blair et al. (1995)], rather than with just the most labile fractions. In three of the four cases presented by Lefroy et al. (1993), the C reactive with the tenfold more dilute 0.033 *M* $KMnO_4$ showed a greater relative decline with long-term cultivation than did the fraction reactive with the 0.333 *M* solution.

The 0.333 *M* solution may therefore be better suited as a simple estimate of total organic C than as an estimate of the labile C fractions associated most closely with soil quality. For example, using a range of highly weathered Australian soils, Bell et al. (1998) reported on the relationships between fractions of soil organic C oxidized by 0.033 *M*, 0.167 *M*, and 0.333 *M* $KMnO_4$ solutions and certain critical soil physical and chemical properties. The soil organic C fraction most closely correlated with the properties deemed critical to the quality of these soils (aggregate stability, infiltration rates and effective cation exchange capacity) was that oxidized by 0.033 *M* $KMnO_4$. These researchers suggested that sustainable cropping on these soils would require management practices that maintain adequate concentrations of 0.033 *M* $KMnO_4$-oxidizable soil C.

In addition to the relatively low sensitivity to changes in C cycling just discussed, the Blair et al. (1995) method using 0.333 *M* $KMnO_4$ involves several important limita-

tions that we attempted to overcome in developing a simplified, improved method for determination of active soil C in both laboratory and field settings. First, their procedure requires relatively extensive equipment, many time-consuming steps and a laboratory setting for soil grinding, volumetric measurements, shaking, centrifugation, filtering and visible light spectroscopy. Second, 0.333 *M* is close to the limit of $KMnO_4$ solubility (0.40 *M*). This highly concentrated solution is both difficult to prepare and maintain and somewhat hazardous to use.

Objectives

The objectives of our study were:

1. To develop a rapid, reproducible and safe method for measuring a soil C parameter that would be a sensitive indicator of management-induced soil quality changes.
2. To simplify this method for use in a user-friendly kit that farmers and conservationists could use in the field. This objective required a method that would not require elaborate equipment and would use a minimum number of reagents, none of them highly toxic, hazardous or unstable.
3. To evaluate the suitability of the method for use on soils with a wide range of properties, and assess the relationships between the measured C fraction and soil microbial properties.

Our hypotheses were that:

1. A $KMnO_4$ solution considerably more dilute than 0.333 *M* would consistently react with a smaller and more labile C fraction.
2. The C measured by the dilute $KMnO_4$ solution would better reflect soil management effects and microbial activity.
3. The dilute solution would be easier to prepare and maintain and would require fewer dilution steps to bring it into a range of absorbance readable on an inexpensive hand-held colorimeter.
4. The fine grinding, weighing, mechanical shaking, centrifuging and filtration steps could all be eliminated in a simplified method without seriously degrading the precision or accuracy of the results.

Materials and Methods

Soils used

A total of 209 surface soil samples were used to evaluate various aspects of the proposed method. These soils either represented farm fields or experimental plots in Maryland (38 farm fields and 12 experimental plots), New Jersey (4 farm fields), North Dakota (9 experimental plots), Pennsylvania (23 farm fields and experimental plots), central Honduras (105 on-farm plots) and southern Brazil (16 farm fields). All sites were in agricultural use, but tillage regime, crop rotations and organic amendments varied. The samples therefore represented a range of management systems, including conventionally plowed continuous corn (*Zea mays* L.), heavily compost-amended organic fruit orchard, and lightly grazed native prairie. The soils were classified in the soil taxonomy suborders Udults, Udepts, Udox, Ustolls, Ustepts, Udalfs and Ustalfs. They ranged in pH from 4.5 to 7.4, in clay concentration from 150 to 500 $g\,kg^{-1}$, in clay mineralogy from kaolinitic to smectitic, and in total organic C concentration from 4 to 69 $g\,kg^{-1}$. All samples were composite samples obtained from the upper 7.5 cm of soil, except the nine North Dakota Mollisol samples, which represented the upper 15 cm of soil.

Modifications to the permanganate oxidizable C method of Blair et al. (1995)

We substantially modified the Blair et al. (1995) 0.333 *M* $KMnO_4$ method to develop a $KMnO_4$ oxidation method that would be more sensitive to the effects of soil management, more rapid, reliable and user-friendly to carry out, and suitable for routine use in a field kit. The procedural factors modified and evaluated were as follows.

Molarity of $KMnO_4$ solution used to react with the active soil C. We aimed to find a solution concentration that would be easier to make and handle than the 0.333 *M* $KMnO_4$ solution prescribed by Blair et al. (1995) and that would react consistently with a management-sensitive labile C fraction in soils. To do so, we tested a series of $KMnO_4$ solutions, ranging from 0.005 to 0.1 *M* (adjusted to pH 7.2), using a set of soil samples from a minimum tillage treatment and a conventional plow tillage treatment from a long-term replicated experiment. Continuous corn grain had been grown for on these plots for 15 years. The soil was a Hagerstown silt loam (Ultic Halplustalf). We reacted 1.0 g oven dry equivalent (ODE) of air-dried soil with 20 ml of neutral $KMnO_4$ in water at concentrations of 0.005, 0.01, 0.0125, 0.025, 0.05 and 0.1 *M*. The soil–$KMnO_4$ suspensions were shaken at 200 rpm for 15 min at room temperature in screw-cap polycarbonate centrifuge tubes. After shaking, the tubes were centrifuged at 3000 rpm for 5 min to separate the soil particles from the solution. We then transferred 0.20 ml of the clear centrifugate to a glass cuvette tube, diluted with 10.0 ml of distilled water, using a strong stream to assure complete mixing. We measured the absorbance of 565 nm light using a Bosch and Lomb 2500 spectrophotometer and compared the absorbance readings to a standard curve constructed using 0.20 ml of each unreacted $KMnO_4$ solution plus 10.0 ml distilled water.

Absorption wavelength and standard curve. To determine the most effective wavelength for measuring changes in absorbance by $KMnO_4$ solutions as a result of reacting with soil C, we constructed standard curves using 1 ml aliquots of 0.005, 0.01 and 0.02 *M* $KMnO_4$ (adjusted to pH 7.2) diluted to 50 ml with distilled water. We read the absorbance of these solutions on a Bosch and Lomb 2500

Spectrophotometer using 550, 560, 565, 570 and 580 nm light settings and used linear regression to determine the standard curve parameters for each wavelength.

Shaking time. For field adaptation of the method, various times of wrist shaking were compared using soils from eight cover crop test strips in each of two adjacent Maryland farm fields. The samples of Myersville silt loam soil (pH 6.1) were collected in spring after a winter with either bare soil or rye (*Secale cereale* L.) cover. Both fields were cropped to corn, wheat (*Triticum aestivum* L.) and double-crop soybean [*Glycine max* (L.) Merr.] using no-till management, but the farmer judged one field to have higher soil quality than the other, based on past productivity.

An aliquot of 20.0 ml of 0.02 *M* $KMnO_4$ was added to 50 ml graduated propylene tubes and followed by a 5 ml scoop of air-dried soil (equivalent to 4.9 ± 0.3 g oven-dry soil). The soil–$KMnO_4$ mixture was wrist-shaken vigorously for 1, 2, 4 or 15 minutes, and then was allowed to stand for 10 minutes. Using a 1 ml graduated disposable bulb pipette, 0.50 ml of the aliquot was taken from the upper 1 cm depth and added to approximately 45 ml distilled water in another set of polypropylene tubes. To wash out the residual $KMnO_4$ solution in the pipette, it was filled and emptied three times with the diluted solution, returning the washing solution to the same tube from which it had come. After making to volume up to the 50 ml mark, the tube was capped and shaken to mix, and then about 15 ml of the diluted solution was poured into an optically calibrated glass vial (designed for the colorimeter used) and the light absorption measured by a 550 nm fixed wavelength palm-top Hach® 'generic' colorimeter. The results were subjected to ANOVA and the calculated amounts of oxidizable soil C, standard errors and *F*-values for the difference between fields were compared for the different shake times.

Supernatant clarification. The use of a salt (0.1 *M* $CaCl_2$) to stimulate soil flocculation and rapid settling in the soil–KMnO4 suspension was evaluated as an alternative to the centrifugation (3000 rpm for 5 min) and filtration required by the Blair et al. (1995) method. Three concentrations of $KMnO_4$ (0.005, 0.01 and 0.025 *M*) were shaken, as described above, with four Maryland soils (Hapludults with total organic C contents of 6–33 g kg^{-1} and clay contents of 125–305 g kg^{-1}). One set of subsamples was shaken in $KMnO_4$ solutions made in distilled water and a second set was shaken in the same concentrations of $KMnO_4$ made up in 0.1 *M* $CaCl_2$. The samples shaken in the pure $KMnO_4$ solutions were then centrifuged at 3000 rpm for 5 min and filtered through glass wool (as per Blair et al., 1995) while the samples shaken with $KMnO_4$ made up in 0.1 *M* $CaCl_2$ were not centrifuged or filtered, but were allowed to stand for 10 minutes. Otherwise, the procedures for the two sets of subsamples were as described above.

Dryness of soil samples. Sandy loam and clay loam soils collected from replicated experiments in Virginia and Maryland were analyzed as field-moist, after spreading in a thin layer in the sun to dry for 15, 30 or 60 min, or after air drying indoors for 24 h. The soil samples were then shaken for 2 min with 0.025 *M* $KMnO_4$ made up in 0.1 *M* $CaCl_2$ and analyzed as above.

Fine grinding of soil prior to analysis. Air-dried soil samples previously sieved to pass a 2 mm mesh were crushed with a mortar and pestle to <0.1 mm. Uncrushed <2 mm sieved soils and crushed <0.1 mm subsamples were then shaken with 0.02 *M* $KMnO_4$ for 2 min and analyses made as described above.

Repeatability and comparability of laboratory and field-kit protocols

Comparability of laboratory and field kit. Fifty-nine air-dried, sieved (<2 mm) soil samples from Maryland, New Jersey and North Dakota were analyzed by both the laboratory version of the proposed method (5.0 g soil in 20.0 ml of 0.02 *M* $KMnO_4$ solution dispensed with an automatic pipette, 2 min mechanical orbital shake time, centrifugation, absorbance read on Bosch and Lomb 2500 spectrophotometer set to 550 nm) and the field-kit version of the proposed method (5 g of soil, wrist-shaken for 2 min in 20 ml of 0.02 *M* $KMnO_4$ and 0.1 *M* $CaCl_2$ dispensed with a disposable 1 ml graduated bulb pipette, no centrifugation, and absorbance measured with single wavelength (550 nm) palm-top Hach® colorimeter). Linear regression was used to compare the two versions of the proposed method. In order to evaluate the coefficient of variation (CV) of the two protocols, three replicate analyses by each protocol were carried out on four samples of an Aura sandy loam from New Jersey. The four fields were sampled either in the fifth year of continuous crop production without any organic amendments, or in the first, second or third year of a hay in a hay/hay/hay/rye–vetch (*Vicia villosa*, Roth)/vegetables rotation with 10–15 Mg ha^{-1} of dry tree leaves plowed in before sowing the grass hay.

Laboratory sample exchange to evaluate repeatability of proposed field-kit method. To evaluate the consistency of the proposed method, nine air-dried, sieved (<2 mm) soil samples were obtained from the upper 15 cm of the no-till (NT), conventionally plow tilled (CT) and natural prairie plots in three replications of an experiment at Mandan, ND on a Wilton silt loam (Pachic Haplustolls). The tillage treatments had been in place for 17 years, and were confounded with cropping sequence. The CT treatment was under a spring wheat–fallow sequence, and the NT treatment was under a spring wheat–winter wheat–sunflower (*Helianthus annuus*) sequence. Subsamples of these soils were sent from North Dakota to Maryland. They were analyzed for active C by the proposed field-kit method (as described previously) in both the NRSC laboratory at Mandan and in the first author's laboratory in Maryland. These were the first samples ever run by the method in the Mandan lab. Results from the two labs were compared using linear regression.

Analysis of related soil quality properties and crop productivity

In addition to their analysis using the various $KMnO_4$-reactive C procedural variations described above, subsets of soils were also analyzed for total organic C [by LECO dry combustion or by wet acid chromate oxidation (Islam and Weil, 1998b)] and selected soil-quality properties that are thought to be related to active-fraction soil C. These were basal respiration (Islam and Weil, 2000), substrate-induced respiration (van de Werf and Verstrate, 1987), microbial biomass C (Islam and Weil, 1998a), anthrone-reactive C after microwaving [a measure of active C (Islam and Weil, 1997)], and aggregate stability [stability of 1–4 mm macroaggregates by a modification of Kemper and Rosenau (1986)]. Sets of diverse soil samples were used to investigate the relationships between 0.02 *M* $KMnO_4$-oxidizable active C and the just-listed soil-quality parameters by linear regression analysis (after analyzing the corresponding scatter plots to check for absence of curvilinear relationships).

A set of 18 samples from paired farm fields in Maryland, Pennsylvania and Virginia were used to evaluate the degree to which the various measures of soil C described above were able to distinguish (by paired *t*–test) between soils judged by farmers to be of higher or lower soil quality (Gruver, 1999). Soil samples from the conventional tillage and no-tillage treatments in the replicated experiment at Mandan, ND, described above, were compared by ANOVA to evaluate the relative ability of the proposed active C method and total organic C to detect a significant effect of management.

Corn biomass and grain yields were estimated by hand harvesting and weighing on 36 sampling plots (4 m × 2 m) in farm fields in the Lavanderos region in central Honduras. The relationships between soil C parameters and crop yields were investigated by linear regression techniques. SYSTAT version 9.0 (SPSS Inc., 1999) was used for all statistical analyses.

Results and Discussion

Effect of $KMnO_4$ solution molarity

When air-dry soil was reacted with 20 ml of $KMnO_4$ solution, increasing the concentration of the $KMnO_4$ solution from 0.005 to 0.05 *M* increased the amount of soil C oxidized, but there was little additional soil C oxidized when the concentration of the reacting solution was further increased to 0.1 *M* (Fig. 1). When the $KMnO_4$ concentration was increased beyond 0.1 *M* [to the 0.333 *M* of the Blair et al. (1995) method], the resulting solutions were too dark in color for absorbance readings to be obtained using the palm-top colorimeter proposed for the field-kit method. Increasing the $KMnO_4$ solution concentration beyond 0.025 *M* resulted in greater standard errors in the measurements and reduced ability of ANOVA to distinguish statistically between the two management

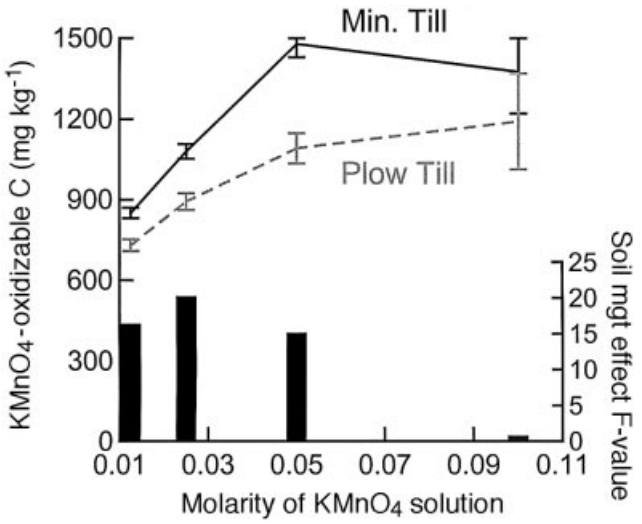

Figure 1. Effect of potassium permanganate concentration on the amount of soil C oxidized and on the ability of the oxidized C fraction to distinguish between soil management (tillage) treatments applied to a replicated experiment in Pennsylvania. Means and SE are shown for the C measurements. ANOVA *F*-values for soil management treatment effect are shown in the bar graph. Except for the solution concentration, the proposed field-kit protocol was followed.

treatments that had been imposed on the soils for 15 years in a replicated field plot experiment. The greatest treatment effect *F*-value was obtained with the 0.025 *M* $KMnO_4$ solution. In later studies using other soils (data not shown) we found that a concentration of 0.02 *M* was necessary to assure that readings could be obtained using the field-kit colorimeter for soils with essentially no organic matter. In these later studies, lower concentrations ($\leq$0.01 *M*) gave erratic results when soils were very high in organic matter (>5%) because all the $KMnO_4$ was consumed in the reaction, leaving no quantitative amount to be determined by absorbance. Therefore, a $KMnO_4$ solution concentration of 0.02 *M* was adopted as most suitable for the proposed active C method.

Optimal absorption wavelength

The standard curves prepared with zero, 0.005, 0.010 and 0.020 *M* $KMnO_4$ solutions exhibited excellent linearity ($R^2 > 0.99$) regardless of the wavelength of light used to read absorbance. However, the regression lines for the standard curve varied with the different wavelengths used. If the standard curve is described by the equation for a straight line (molarity = $a + b \times$ absorbance), a lower slope (b) will facilitate the detection of small differences in active soil C that result in small changes in the $KMnO_4$ solution concentration and light absorbance. The standard curve slope using 550 nm light was significantly lower (0.039) than the slopes using the longer wavelengths (0.061, 0.062, 0.073 and 0.108 for 560, 565, 570 and 580 nm light).

Averaged across the molar concentrations of the $KMnO_4$ solutions, the absorption measured at 550 nm gave consistently higher readings (data not shown) and higher or equal R^2 than absorption of standard solutions measured at other wavelengths. The 550 nm wavelength was adopted for use in the proposed active C method because the use of this wavelength always resulted in a standard curve with the lowest slope and highest regression coefficient. Use of 550 nm, rather the 665 nm specified by Blair et al. (1995), should enhance the precision with which the amount of soil C oxidized may be determined. Fortunately, we were able to find a single-wavelength generic (not preprogrammed for a specific analysis) hand-held colorimeter available for 550 nm absorbance. Light of wavelength 550 nm was used for all determinations subsequently reported in this paper, and the palm-top colorimeter was used for all subsequent results described as using the 'field-kit method'. It should be noted that when the values for the molarity of the $KMnO_4$ remaining unreacted are converted to mg soil C reacted, the curve so obtained has a negative slope, as the greater the amount of C reacted, the lower the absorbance.

Effect of shaking time

The effect of shaking time on the amount of C oxidized by a 0.02 *M* $KMnO_4$ solution was investigated using soil samples from two adjacent fields on a Maryland grain farm. The farmer reported that, although the fields were alike with regard to soil type and management history, one field consistently produced greater crop yields than the other. The amount of C oxidized increased with the time the soil was shaken in the solution (Fig. 2). A shake time of 15 minutes (as per the method of Blair et al., 1995) was also tried (data not shown). In addition to being an onerously long time for hand shaking in a field-kit method, the 15-min shake time resulted in all the 0.02 *M* $KMnO_4$ being consumed for most soils, so no absorbance reading could be made. Furthermore, no results could be obtained from a treatment that used a 15-min shake time divided into two 7.5-min shaking periods with a 3-min standing time between shaking periods. Immediately on re-shaking, the permanganate purple color of the mixture suddenly disappeared. We subsequently observed that this sudden color disappearance usually occurred if a sample was disturbed after having been shaken and allowed to settle.

With 1-, 2- or 4-min shake times, the 0.02 *M* $KMnO_4$-reactive C was significantly higher in the field reported to be consistently higher yielding. However, the ANOVA *F*-ratios for the effect of field were 13, 14 and 6 for the 1-, 2- and 4-min shake times. The SE of the active C measurements did not change between 1 and 2 min of shake time, but did increase with longer shake time (Fig. 2). We therefore adopted a 2-min shake time for the proposed active C method. Because of the factors just discussed, the duration of shaking should be precisely timed and any further disturbance of the mixture after settling carefully avoided.

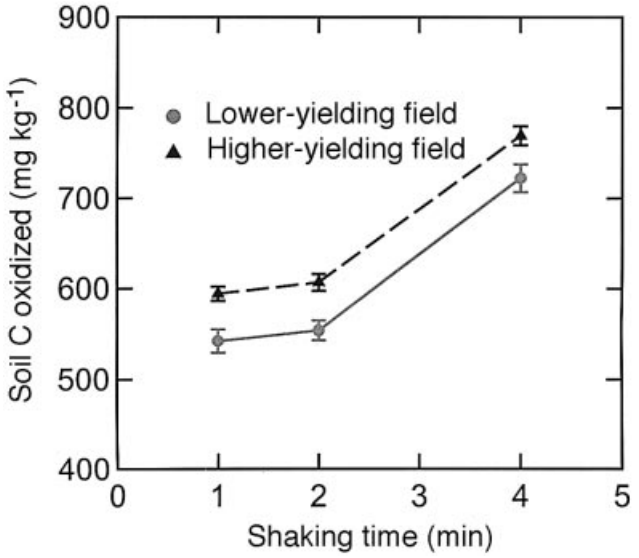

Figure 2. The effect of shaking time on the amount of C oxidized by a 0.02 *M* $KMnO_4$ solution in soil samples from two adjacent farm fields in Maryland with differing yield histories reported by the farmer. The data are means of eight samples from each field taken in spring. Bars indicate the standard errors of the means.

Table 1. Active soil C (mg kg^{-1}), as measured by oxidation with several concentrations of $KMnO_4$, as influenced by the use of flocculation with 0.1 *M* $CaCl_2$ or centrifugation to clear the supernatant solution. Means of four soils.

Clarification treatments	$KMnO_4$ concentration			
	0.005 *M*	0.01 *M*	0.025 *M*	*F*-ratio
Centrifugation	620	1110	1780	1900***
0.1 *M* $CaCl_2$	620	1130	1870	890***
t–test	ns	ns	ns	

*** Significant at the 0.001 level.

Method of supernatant clarification

The amount of oxidizable C measured in four soils did not vary significantly between subsamples using centrifugation/filtration to clear the supernatant (Blair et al., 1995) and subsamples for which the $KMnO_4$-reacting solutions were made up in 0.1 *M* $CaCl_2$. Increasing the ionic strength of a solution, especially with a divalent ion such as Ca^{2+}, is expected to stimulate the flocculation of soil particles and therefore hasten their settling out of suspension (Brady and Weil, 2002, p. 427–428). Using 0.1 *M* $CaCl_2$ in the $KMnO_4$ solution resulted in a clear supernatant solution within a few minutes of standing after shaking. For all three concentrations of $KMnO_4$ tried, there was no difference in the absorbance readings for centrifuged $KMnO_4$ without $CaCl_2$ compared to simply allowing 5 minutes for settling with 0.1 *M* $CaCl_2$ (Table 1). Therefore, use of a 0.02 *M*

$KMnO_4$ made with 0.1 *M* $CaCl_2$ was adopted for the field-kit protocol in the proposed active C method.

Soil drying

Although air drying and sieving is standard procedure for most laboratory measurements of soil C, we compared different degrees of soil drying in an attempt to simplify and streamline the protocol for the field-kit version of the proposed active C method. The effect of soil sample drying time on C oxidized by a 0.02 *M* $KMnO_4$ solution is shown in Figure 3. Means and SE are shown for samples from various treatment plots in field experiments on two soils.

Increased soil dryness increased the amount of C reacting with the $KMnO_4$ solution, probably because of the higher redox potential that usually accompanies increased dryness (Bartlett and James, 1993), although no attempt was made to measure soil Eh. The difference in soil water content among the drying treatments was negligible in terms of affecting dilution of the reagent (<1 ml compared to 20 ml of reagent), especially since the greatest oxidation differences occurred among the drier treatments. Readings on fresh, moist soils were more variable than on dried soils, but even moderate drying (spreading soil in a thin layer for 15–30 min in the sun) resulted in good repeatability and discrimination between soil management treatments (data not shown). We concluded that long-term air drying is the preferred method of sample preparation for maximum accuracy, but that, for comparisons in the field, 15 min of drying in the sun should be adequate if a black tray is used. Of course, ambient humidity and temperature can affect the degree of soil dryness achieved, and should be taken into account when comparing soils dried under differing conditions. The main consideration is that all samples being compared should be of equal dryness, since the dryness of the soil does influence the results.

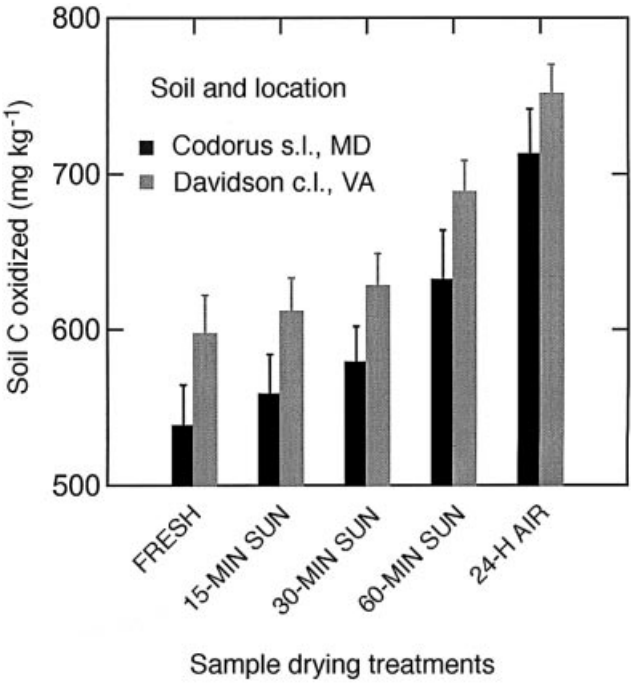

Figure 3. Effect of sample drying time on C oxidized by 0.02 *M* $KMnO_4$ solution for two soils. Soil moisture content was near field capacity at time of sampling. Air temperature was 25°C in full sun. Means and SE for each soil are shown. Samples were collected from four blocks of two treatments on each soil.

Soil grinding

Some degree of soil grinding or aggregate crushing is normal practice in preparing soil samples for most laboratory analyses (Wollum, 1994). The main purpose of grinding and sieving soils prior to analysis is to assist in homogenizing the sample, so a representative subsample can be taken. To assure an unbiased representation of the soil sample, the small subsample that is actually subjected to the analysis should contain a large number (>1000) of soil particles or aggregates. Including a large number of individual particles in a subsample is made possible either by using a large sample size or by reducing the size of the individual particles. Many procedures for soil C analysis call for the soil to be ground to pass a 0.5 mm sieve because of the small (<1 g) sample size used. Aside from these considerations of subsampling error, crushing and sieving a soil sample may affect the determination of soil C because the process may expose some C, especially particulate organic matter that was occluded inside the larger soil aggregates.

Table 2 shows that crushing aggregates from a maximum diameter of 2 mm to a maximum diameter of 0.1 mm increased the amount of active C by 7–12%, as determined by reaction with 0.025 *M* $KMnO_4$ (this study was done before 0.02 *M* was chosen as the $KMnO_4$ concentration in

Table 2. Effect of soil grinding on oxidation of organic C by neutral 0.025 *M* $KMnO_4$ in soil samples from adjacent plots with differential management histories. Means of eight on-farm plots on a Glenelg silt loam (Typic Hapludults) in Lancaster Co., PA.

	Oxidizable soil C (mg kg^{-1})		
Years in no-till management	Uncrushed aggregates (<2 mm)	Crushed aggregates (<0.1 mm)	ANOVA *F*-ratio for aggregate size
3	1780	1980	12.42*
17	2700	2910	20.65*
ANOVA *F*-ratio for management	285***	356***	

* Indicates that the effect of crushing was significant at the 0.05 level.

*** Indicates that the effect of management history was significant at the 0.001 level.

the proposed method). In addition, the precision of the determination improved slightly when using crushed soils, as indicated by the greater ANOVA *F*-value for management. However, the *F*-values for both crushed and uncrushed soils were highly significant, indicating a high level of consistency in these particular field plots as well as in the active C analysis. For the laboratory protocol of the proposed active C method, we adopted the use of <0.5 mm ground and sieved samples. However, for a field-kit protocol, such fine sieving would be impractical, and crumbling finely by hand is called for, with the caution that differences in aggregate strength may influence the degree to which aggregates are disrupted by this sample preparation technique. In addition, in the field we found it more convenient to measure out analytical subsamples using a standard 5 ml scoop instead of a balance. Although this method of subsampling based on volume has it merits, a high degree of reproducibility is not one of them. Nevertheless, we found that for samples of similar textures, crumbling soil and leveling it off in a 5 ml scoop measured out 4.86 g of soil with a coefficient of variation of 6%. We therefore consider the 5 ml scoop to be an acceptable alternative for the use of a portable balance weighing to 0.1 g for work in the field when the objective is to obtain comparative values among similar soils.

Comparison of results using laboratory and field-kit protocols

We have developed two versions of the proposed active C method, one for precise work in the laboratory (referred to herein as the laboratory protocol), and one for use in a field setting, where some precision may be sacrificed for simplicity, speed, convenience and low cost. To compare the results from these two protocols, we subjected duplicate subsamples of 59 soils to active C analysis using both versions of the proposed method. Except where indicated otherwise, we used air-dried (>24 h), sieved soils for both protocols, and weighed out the subsample for analysis using a portable balance. The field-kit protocol differed from the laboratory protocol in these ways:

1. the use of a Hach® palm-top single-wavelength colorimeter (550 nm) instead of a laboratory spectrophotometer;
2. the inclusion of 0.1 *M* $CaCl_2$ in the 0.02 *M* $KMnO_4$ solution and a 5-min settling period instead of using centrifugation to enhance settling and clarify the supernatant;
3. the use of 1 ml graduated disposable bulb pipettes instead of laboratory-grade pipettes to measure out volumes of the reacting solution and supernatant aliquots; and
4. the use of hand shaking instead of a mechanical orbital shaker.

As illustrated by the data in Figure 4, results from the two protocols of the proposed active C method were highly correlated ($R^2 = 0.98$) over a wide range of soils. Also shown in Figure 4 are data for 16 soils sampled from southern Brazil and analyzed using a prototype portable kit in the field. These analyses were performed using a 5 ml scoop to measure hand-crumbled soil that had been only partially air-dried under cool (8–12°C), cloudy, humid winter conditions. The laboratory determinations were performed on air-dried, sieved subsamples 1 month after transporting the samples from Brazil to Maryland. The added variability due to inconsistent drying and imprecise subsampling probably account for the greater degree of discrepancy between the laboratory and field determinations in this set of samples.

Repeatability of proposed method

Given that the proposed active C method is designed to measure an operationally defined fraction of soil C, there may be no good way to estimate the *accuracy* of the method. However, we estimated the precision and repeatability of the method in several ways. Table 3 shows the results for soils from four New Jersey farm fields, either in the fifth year of continuous crop production without organic amendments, or in the first, second or fourth year of hay in a leaves/hay/hay/hay/rye–vetch/vegetables rotation. With one unexplained exception, the laboratory protocol and the field protocols gave very similar CVs, in the range of 1–4%, certainly within an acceptable range for most soil C analyses. Based on the LSD values shown in Table 3, both

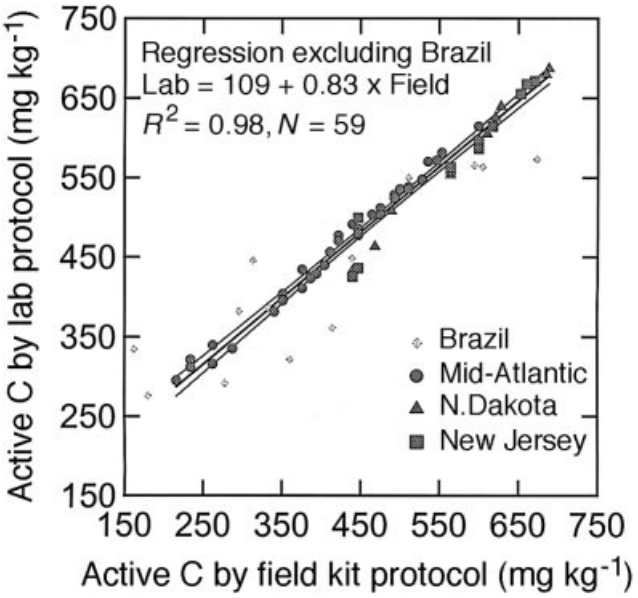

Figure 4. A comparison between the laboratory and field-kit protocols for the proposed active C method. All determinations, except those for the Brazil field-kit protocol, used sieved, air-dry soil samples. The Brazil field-kit determinations were made under poor conditions for soil drying (cold, damp weather), using a scoop to measure hand-crumbled soil. The regression comparing these Brazil field determinations to the laboratory protocol was: Laboratory = 158 + 0.7 × field, $R^2 = 0.76$, $N = 16$.

versions of the method distinguished between soils that were in the hay and leaves rotation and those that were not.

The repeatability of the proposed method was also investigated through a sample exchange in which nine well-homogenized subsamples of air-dried soil from a replicated field experiment in North Dakota were analyzed in laboratories at the University of Maryland and the USDA/NRCS in Mandan, ND. The Maryland laboratory is that of the senior author and the personnel there were already well experienced with the method. The personnel at the North Dakota laboratory were unfamiliar with the procedure prior to the time these samples were analyzed. The field-kit protocol was used, as described previously. The soil samples came from conventionally tilled cropped plots, no-till managed cropped plots, and lightly grazed native prairie plots. Figure 5 shows that the two labs produced very similar active C results.

Table 3. Variance in 0.02 *M* $KMnO_4$-oxidizable C results using the laboratory and field-kit protocols to measure active C in 0–15 cm samples of an Aura sandy loam (coarse-loamy, siliceous, semiactive, mesic Typic Fragiudults) from four New Jersey farm fields with different organic matter management histories. Means and CVs for triplicate determinations.

	Laboratory method		Field-kit method	
Experimental treatments	**Mean (mg kg^{-1})**	**CV (%)**	**Mean (mg kg^{-1})**	**CV (%)**
Continuous cropping	453.6	8.8	444.6	0.9
H H V L H[1]	600.5	2.1	605.7	1.7
H V L H H	569.8	2.5	576.1	3.6
V L H H H	664.8	1.3	661.4	1.4
LSD ($P < 0.001$)	93.1		54.4	

[1] H = hay, L = fallowed with ~15 Mg ha^{-1} municipal tree leaves incorporated, V = vegetables. Underlined last symbol on the right indicates rotation phase when sampled.

Usefulness of the proposed active C method for assessing soil quality

A repeatable, easy-to-use method for estimating active soil C will be helpful in assessing soil quality only to the extent that the C fraction measured is sensitive to changes in soil quality and allows the investigator to detect these changes consistently. Furthermore, to be meaningful as an estimate of the size of the active C pool, the results of the proposed method should exhibit significant relationships with soil microbial processes and other soil-quality indicators. Here we present data to show that the proposed method is both more sensitive to management-induced soil

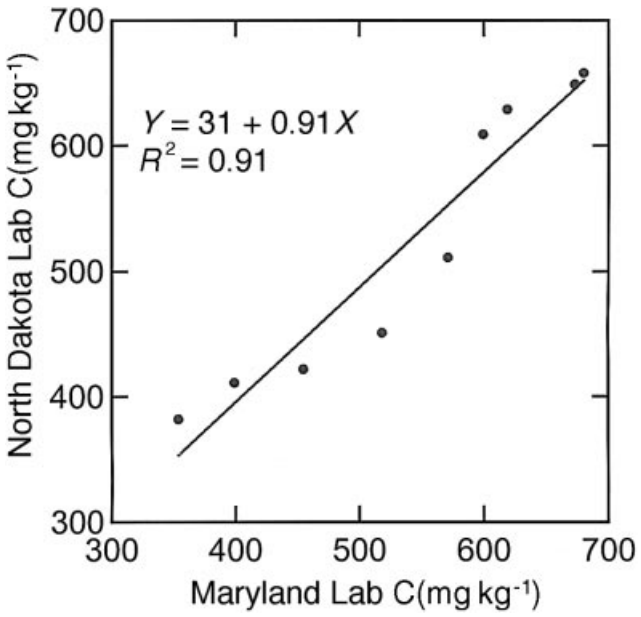

Figure 5. A comparison of active C measured by two different labs using subsamples from the same nine North Dakota soil samples. The protocol used was that of the field-kit method described herein. The North Dakota laboratory had no prior experience with the method.

Table 4. The relationship between soil quality (SQ) rating by farmers and selected fractions of soil carbon as determined by various methods in 19 pairs of soils in the mid-Atlantic region.

	Carbon conc. (mg kg^{-1})[1]		Paired *t*-test	
Soil C analysis method	**Higher SQ**	**Lower SQ**	***t*-value**	**Probability**
0.02 *M* $KMnO_4$ oxidizable C, field-kit version	455.6	384.7	6.25	0.000007
0.02 *M* $KMnO_4$ oxidizable C, laboratory version	493.2	430.2	6.20	0.000008
Total organic C (LECO)	24,900.0	17,300.0	5.02	0.0001
0.333 *M* $KMnO_4$ oxidizable C (Blair et al., 1995)	5450.0	3960.0	4.63	0.0002
Anthrone-sulfuric acid reactive carbohydrate C	97.2	78.1	3.65	0.002
p–Hydroxybenzoic acid hydrazide reactive C	31.1	26.8	1.83	0.08

[1] Pairs of similar soils judged by farmers as being higher or lower in soil quality, based on the relative yield histories, workability, erodibility and other farmer observations (Gruver, 1999).

changes and more closely related to biologically mediated soil properties than are other measures of soil C, including total soil organic C and C oxidizable by the 0.333 *M* $KMnO_4$ method of Blair et al. (1995).

Management effects on active C

Farmers in several mid-Atlantic States identified pairs of fields that, in their experience, exhibited contrasting soil quality, usually because of past management (Gruver, 1999). The soils in each pair were very similar pedologically and were generally mapped as the same or similar soil series and phase. Table 4 shows how six measures of soil organic C differed between the soils grouped by farmers as higher and lower in soil quality. The sensitivity of the C parameter to the perceived differences in soil quality can be judged by the *t*-value, a statistic indicating with how much certainty the two populations of soils were different by a paired *t*–test. Four of the five C parameters differed significantly between the lower and higher soil-quality fields. However, the *t*-values for the laboratory and field-kit protocols of the proposed active C method were greater that those for total organic C or 0.333 *M* $KMnO_4$-oxidizable C by the Blair et al. (1995) method. Therefore, even though the latter two measures exhibited greater relative differences between the lower- and higher-quality soil groups, the proposed active C method exhibited a more consistent difference between the two soil-quality categories.

Figure 6 presents two measures of soil organic C found in experimental plots subjected to either conventional tillage or no-tillage management in a wheat-based rotation at Mandan, North Dakota. Based on the *F*-values from analysis of variance, the two treatments differed significantly with regard to active C as measured by the proposed method (ANOVA *F*-ratio = 34), but were not significantly different in total organic C (ANOVA *F*-ratio = 4). Such results are in agreement with the theoretical expectation and common observation that the active C pool is more rapidly increased or decreased by changes in soil management and cropping systems than is the mainly recalcitrant total soil organic C pool (Paustian et al., 1997).

Relationship between active C and other soil quality indicators

Table 5 shows the correlations among five soil organic C fractions and four microbial soil properties in surface soil samples collected from 18 mid-Atlantic farm fields representing a wide range of textures, organic matter contents and cropping systems. Active C by the proposed 0.02 *M* $KMnO_4$ method was more closely correlated than was the Blair et al. (1995) method with each of the four measures of microbial activity (substrate-induced respiration, basal respiration, microbial biomass and soluble carbohydrates). On the other hand, the Blair et al. (1995) method gave results more closely correlated to total organic C than those from the proposed method. Both methods for permanganate-oxidizable C gave results that were weakly

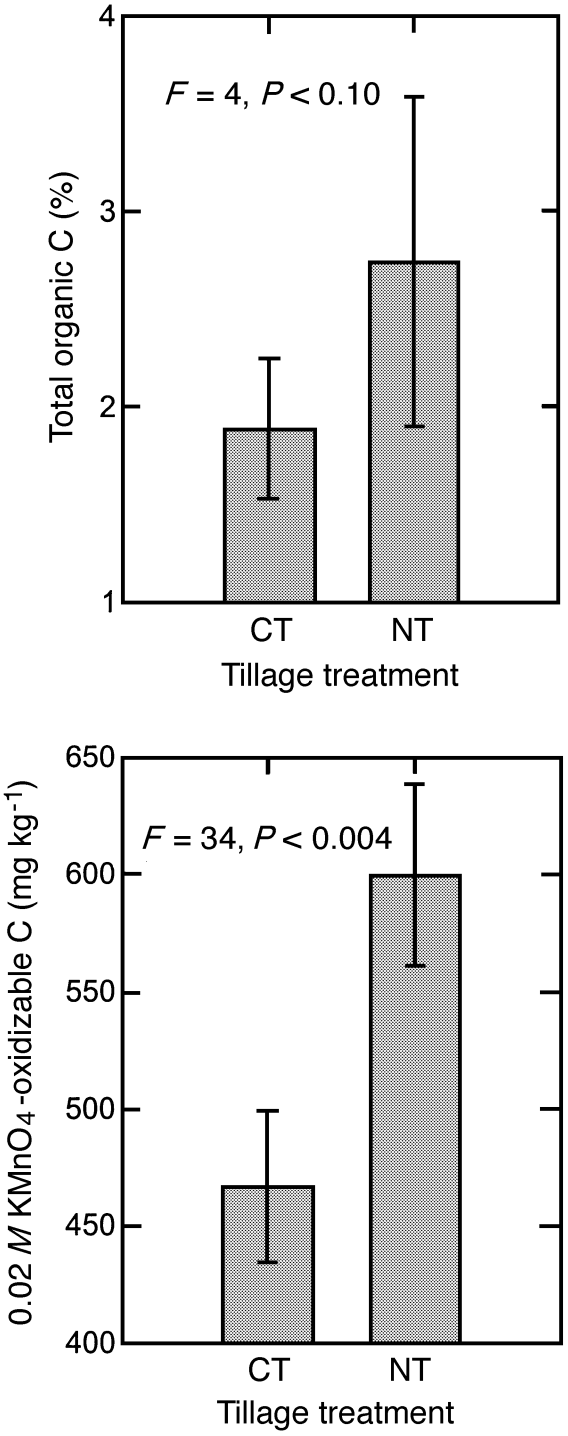

Figure 6. Comparative sensitivity of total organic C and active C by the proposed method in distinguishing between long-term tillage treatments (CT = conventional plow tillage, NT = no-till) in a replicated wheat-based rotation experiment at Mandan, ND. The total C levels were not significantly different between the treatments, but a highly significant difference was observed in the active C levels (*F* ratio and probability from ANOVA shown). The soil was a Wilton silt loam (fine-silty, mixed, superactive frigid Pachic Haplustolls).

Table 5. Linear correlations (*r*) between selected measures of microbial activity and various fractions of soil C in samples from 18 mid-Atlantic farm fields.

	Total organic C[1]	0.5 *M* K_2SO_4 extractable C	Soluble carbohydrate C[2]	0.333 *M* $KMnO_4$ oxidizable C[3]	0.02 *M* $KMnO_4$ oxidizable C[4]
Substrate-induced resp.	0.55*	0.73***	0.48*	0.60**	0.74***
Basal respiration	0.45^{NS}	0.07^{NS}	0.56*	0.46^{NS}	0.56*
Microbial biomass C	0.60**	0.37^{NS}	0.96***	0.79***	0.85***
Soluble carbohydrate C[3]	0.56*	0.33^{NS}	1.00	0.68**	0.84***
Total organic C[1]	1.00	0.58**	0.56*	0.77***	0.69**
K_2SO_4-extractable C	0.58**	1.00	0.30^{NS}	0.51*	0.51*

[1] By LECO high temperature combustion.
[2] Glucose equivalents reactive with anthrone after microwave irradiation.
[3] By method of Blair et al. (1995).
[4] By proposed active C method, laboratory protocol.
*, **, *** Indicate significance at the 0.05, 0.01 and 0.001 probability levels.
NS indicates no significant correlation at the 0.05 probability level.

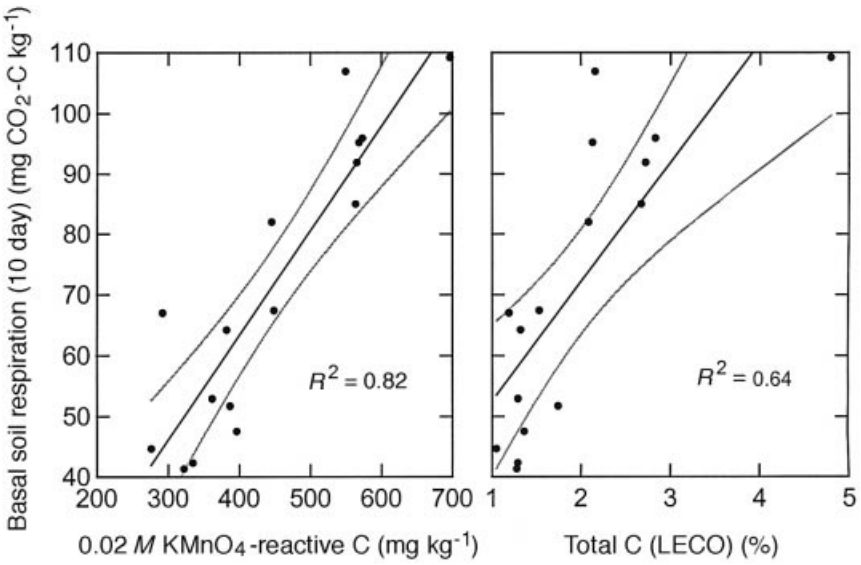

Figure 7. The relationships between basal microbial respiration and two fractions of soil organic C, total and active (as determined by the laboratory protocol of the proposed method) in 16 soil samples from farm fields in southern Brazil.

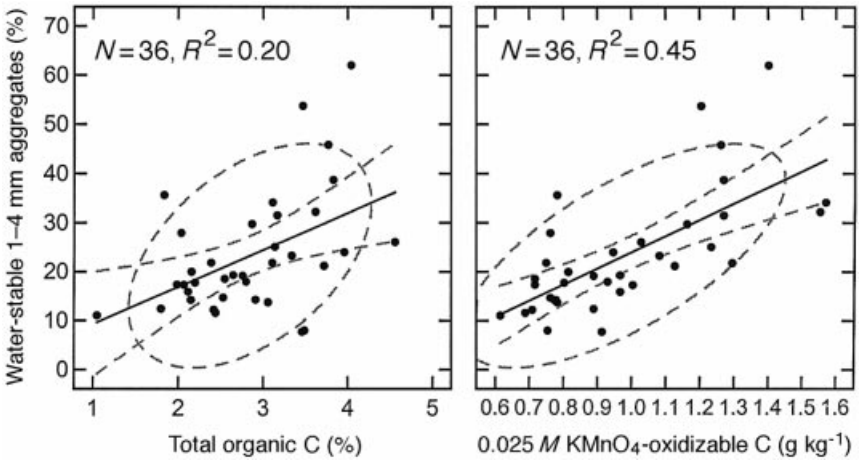

Figure 8. The relationships between the stability of macroaggregates and the content of active C or total organic C in soils from hillside farmers' fields in the Lavanderos region of Honduras. The active C in these samples was determined using the laboratory protocol of the proposed method, but with 0.025 *M* rather than 0.02 *M* $KMnO_4$ solution.

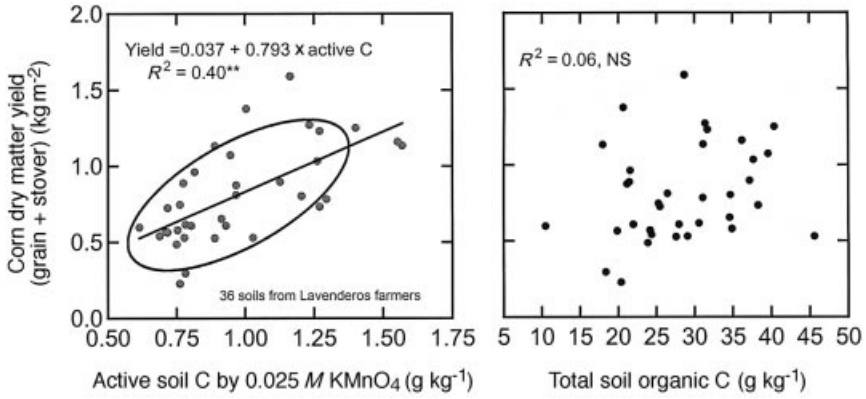

Figure 9. The relationship between above-ground dry matter yield of corn (*Zea mays* L.) in 36 on-farm plots in Lavanderos Honduras and the soil content of active C (left) or total C (right).

and equally correlated ($r = 0.51$) with soluble C (by K_2SO_4 extraction). Soluble C was not correlated to any of the microbial activity measures, except substrate-induced respiration. Soluble carbohydrates were very closely correlated ($r = 0.96$) to microbial biomass C. This is not surprising as the carbohydrates were measured after the same microwave irradiation treatment applied to lyse cells in determining microbial biomass.

In several other sets of soils investigated, active carbon by the proposed method was consistently more closely related to other soil-quality properties than was total organic C (Figs 7–9). For example, for the 16 soils sampled from farms in southern Brazil, basal respiration was more closely related to active C than to total C (Fig. 7). For a set of 36 soil samples from hillside farms in central Honduras, the aggregate stability varied more closely with active C than with total organic C (Fig. 8). On these same on-farm plots in Honduras, active C, but not total C, exhibited a significant linear relationship with the crop biomass produced (Fig. 9).

Conclusions

We have shown that a dilute (0.02 *M*) solution of slightly alkaline $KMnO_4$ can be used to react with diverse soils to estimate a biologically active soil C pool. We developed a highly simplified method in which dilute $KMnO_4$ reacts with the most readily oxidizable (active) forms of soil C, converting Mn(VII) to Mn(II), and proportionally lowering absorbance of 550 nm light. A 0.02 *M* $KMnO_4$ solution concentration, air-dry soil (or 15 min of sun drying in the field), and 2 min of shaking provided optimum ease, consistency and sensitivity of results to management effects using laboratory equipment or a field kit with a palm-size colorimeter. Addition of 0.1 *M* $CaCl_2$ to the permanganate reagent provided for rapid settling of the soil after shaking, eliminating the need for centrifugation or filtration in the field kit.

Results from the laboratory and field kit were very similar ($R^2 = 0.98$), as were those from an inter-laboratory sample exchange ($R^2 = 0.91$). Compared to total organic C, the active soil C measured by the new procedure was more sensitive to management effects, and more closely related to soil productivity and biologically mediated soil properties, such as respiration, microbial biomass and aggregation. The new procedure presents several distinct advantages over the 0.333 *M* $KMnO_4$ procedure of Blair et al. (1995). These include the dilute reagent which is easier to work with and less hazardous, the elimination of centrifugation and filtration steps, a simpler, streamlined protocol suitable for field as well as laboratory use, and measurement of a soil C fraction that is more closely related to microbial and soil-quality properties. Although we did not attempt to do this, it should be possible to use the new method in calculating a C management index such as that proposed by Blair et al (1995).

Based on the results just described, we assembled a field kit using a 0.02 *M* $KMnO_4$ solution made with 0.1 *M* $CaCl_2$; a palm-sized single (550 nm) wavelength spectrometer; plastic, screw-top, conical centrifuge tubes for hand-shaking; disposable 1.0 ml graduated bulb pipettes; and a 5 cm^3 soil scoop. The entire kit can fit into a 16 × 15 × 20 cm plastic carrying case and is suitable for use in the field.

The field kit has potential as a tool for farmer education in the field, as well as for soil-quality research. We suggest that it might be a suitable addition to the NRCS soil-quality test kit, as that kit currently includes no soil organic matter test. Current work is focusing on calibrating the new method for routine use in soil-testing programs to help advise on the need for improved soil organic matter management.

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Appendix

Field-kit method for $KMnO_4$-oxidizable C to estimate active organic carbon in soils[1]

Materials.

1. Stock solution: 0.2 *M* $KMnO_4$ in 1 *M* $CaCl_2$ (pH 7.2). Adjust pH to 7.2 using 0.1 *M* NaOH. This solution pH is important for maintaining stability of the stock solution for 3–6 months. The pH-adjusted 0.2 *M* $KMnO_4$ stock solution should be kept in a dark bottle.
2. Standard solutions: 0.005, 0.01 and 0.02 *M* $KMnO_4$ in 0.1 *M* $CaCl_2$. Make by adding 1.25, 2.50 or 5.00 ml of the 0.2 *M* $KMnO_4$ stock solution to respective centrifuge tubes and diluting to the 50 ml mark with distilled water.
3. Hand-held colorimeter (generic 550 nm colorimeter, Hach® Company, Boulder, CO).
4. Optically matched glass cuvettes; laboratory tissues for wiping cuvettes.
5. Plastic lab ware: nine graduated polypropylene conical centrifuge tubes (50 ml); two plastic disposable 1 ml graduated bulb pipettes, for stock and for dilute solutions; scoop (calibrated to 5 ml); plastic cup (50 ml); rack to hold centrifuge conical tubes in upright position.
6. Distilled water in sealable squeeze bottle.

Procedure.

Most conveniently carried out with batches of three samples.

1. If sampling moist soil in the field, take a small representative subsample of field-moist soil (approximately 20 g or five scoops), crumble gently and spread thinly on a piece of black paper to air-dry for 15 min (preferably in direct sunlight). Mix the crumbled soil two or three times during air-drying.
2. Using a disposable bulb pipette, place 2.0 ml of the 0.2 *M* $KMnO_4$ in a clean 50 ml graduated polypropylene conical centrifuge tube. Add distilled water to the 20 ml mark and cap the tube. Swirl the tube to mix the solution thoroughly. Add one level scoop (or weigh 5.0 g) of uniformly dry soil to the tube and cap it tightly.
3. Shake vigorously (about 100 strokes/min) for 2 min, and then stand the tube in a rack for 5–10 min to allow soil to settle. Protect the tube from direct sunlight. The $CaCl_2$ in the solution will cause the soil to flocculate and rapidly settle, clearing the upper portion of the solution.
4. The settling time may be used for making a standard curve as follows.
   (a) Fill a clean[2] glass cuvette with distilled water; wipe the outside of the vial with a tissue and place the vial in the colorimeter well. Put the cover in place and press the 'zero' button. After a few seconds, the LED should read '0.00'. Remove the cuvette.
   (b) Add about 45 ml of distilled water to a clean graduated centrifuge tube. Using the disposable bulb pipette, add 0.50 ml of the 0.005 *M* $KMnO_4$ standard solution to the tube, then fill and empty the pipette with the diluted solution several times to insure that all the solution is delivered. Then add distilled water to the 50 ml mark, cap and shake to mix. Pour about 15 ml of this diluted standard into a clean 20 ml glass cuvette; wipe the outside with a tissue and place in the colorimeter well. Put the cover in place and press the 'read' button. Record the absorbance displayed.
   (c) Repeat these steps (4b) using 0.50 ml of the 0.01 *M* and 0.02 *M* $KMnO_4$ standard solutions. Record the absorbance for each standard solution. Construct a standard curve with absorbance on the *x*-axis and concentration on the *y*-axis.
5. After measuring the absorbance of the standard solutions, add approximately 45 ml distilled water to a clean, graduated centrifuge tube. Use a clean bulb pipette to take 0.50 ml of liquid from the upper 1 cm of the soil–$KMnO_4$ suspension (avoid floating debris) and transfer this to the tube of distilled water. Wash out the residual $KMnO_4$ solution in the pipette by filling and emptying it three times with the diluted solution. Then add distilled water up to the 50 ml mark, cap, and shake. Pour about 15 ml of this diluted solution into a clean 20 ml glass cuvette. Wipe the outside of the cuvette with a tissue and place it in the colorimeter well. Put the cover in

place and press 'read'. Record the absorbance for the sample solution[3].

Calculation[4]. The bleaching of the purple $KMnO_4$ color (reduction in absorbance) is proportional to the amount of oxidizable C in soil. In other words, the greater the $KMnO_4$ color *loss* (the lower the absorbance reading), the greater the amount of oxidizable C in the soil. To estimate the amount of C oxidized, use the assumption of Blair et al. (1995) that 1 mol MnO_4 is consumed (reduced from Mn^{7+} to Mn^{4+}) in the oxidation of 0.75 mol (9000 mg) of C:

$$\text{Active C (mg kg}^{-1}) = [0.02\ \text{mol/l} - (a + b \times \text{absorbance})] \times (9000\ \text{mg C/mol}) \times (0.021\ \text{solution}/0.005\ \text{kg soil})$$

where 0.02 mol/l is the initial solution concentration, a is the intercept and b is the slope of the standard curve, 9000 is mg C (0.75 mol) oxidized by 1 mol of MnO_4 changing from Mn^{7+} to Mn^{4+}, 0.021 is the volume of $KMnO_4$ solution reacted, and 0.005 is the kg of soil used.

[1] To increase precision and convenience when working in a laboratory, precise weighing of 5.0 g air-dry, <1 mm sieved soil can be substituted for the 5 ml of crumbled soil, a horizontal shaker at 120 rpm can be used instead of hand shaking, a standard laboratory spectrophotometer set to read 550 nm light can be used in place of the portable colorimeter, and an auto-pipettor can be used instead of disposable bulb pipettes.

[2] If blank absorbance readings increase after 10–20 determinations, it may be necessary to clean the glass cuvette vials with 10% bleach solution to remove sorbed permanganate.

[3] If absorbance is <0.01, repeat steps 2, 3 and 5 using half as much soil (2.5 g) and adjust the soil weight accordingly in the calculation given below.

[4] For reference, typical absorbance readings obtained by the authors for the standard solutions using a HACH 550 nm colorimeter are: 0, 0.21, 0.44 and 0.84, which give the standard curve equation: conc. = –0.0005 + 0.0252 × abs. Therefore a typical calculation (within rounding error) for active C would be: active C (mg/kg) = [(0.02) – (–0.0005+0.0252 × Abs)] × 36000.