QUALITY CONTROL FOR A NEW PERMANGANATE OXIDIZABLE C METHOD

Joel Gruver[1]

[1]Department of Soil Science, North Carolina State University, Box 7619, Raleigh, NC 27695. E-mail address: jgruv@hotmail.com

ABSTRACT

Duration of reaction and soil mass was evaluated as sources of experimental error in a new permanganate oxidizable C (POC) method. The method's short duration of reaction was more sensitive to variation in procedural timing than longer durations of reaction, but small coefficients of variation (< 5%) were achieved using the recommended timing. Sensitivity to management was evaluated using soil from two experiments. The method was found to be sensitive to tillage intensity and level of C inputs. Analysis of multiple soil masses revealed an asymptotic relationship between permanganate availability and reaction efficiency. A computational technique was developed to correct for the method's lack of linearity. Nine quality control protocols are proposed to reduce experimental error.

INTRODUCTION

Weil et al. (2003) recently proposed a permanganate oxidizable C method for evaluating soil management effects on soil quality. The method differs from previously described permanganate methods (Loginow et al., 1987; Blair et al., 1995; Moody et al., 1997; Bell et al., 1998; Blair et al., 2001) in the following substantive ways:

1. Reduced concentration of permanganate solution (easier to prepare, safer)
2. Reduced complexity (elimination of many steps including grinding, filtering and centrifugation).
3. Reduced cost (faster and requires less specialized lab equipment)
4. Increased sensitivity to management

The new method is sensitive to management (e.g. contrasting tillage systems and C input regimes) and correlated with biologically active C parameters (e.g. microbial biomass C, soluble carbohydrates, substrate induced respiration) that are more difficult to measure (Weil et al., 2003)

This paper evaluates sources of experimental error in the method and proposes specific quality control protocols.

MATERIALS AND METHODS

Soils from two cropping systems experiments (Tables 1a and b) and 3 non-experiment areas were used to evaluate the method.

Table 1a. Organic transition experiment.

Geographic location	Goldsboro, NC
Experiment station	Center for Environmental Farming Systems
Year of initiation	1999
Soils	Wickham sandy loam, Tarboro loamy sand
Systems	3 low C input regimes, 3 high C input regimes
Plots sampled	3 reps of all systems
Time of sampling	April 2003

Table 1b. Tillage system experiment.

Geographic location	Reidsville, NC
Experiment station	Upper Piedmont Research Station
Year of initiation	1984
Soil	Wedowee sandy loam
Systems	9 systems with contrasting tillage intensity
Plots sampled	4 reps of 2 systems (plow/disk and continuous no-till)
Time of sampling	June 2003

Experimental plots were sampled at 2 depths (0-7.5, 7.5-15 cm). Field moist cores were gently crumbled and spread on paper to air dry. Air-dry soil was passed through a sieve with 2-mm mesh.

Soil was collected in bulk from the sandy loam surface horizon of a general production area at the Center for Environmental Farming Systems. A dry sieving process was used to isolate a less than 0.5-mm fraction for long-term use as a low C experimental "standard".

Soil was collected in bulk from the sandy loam surface horizons of long-term sod sites at the Center for Environmental Farming Systems (CEFS) and the Upper Piedmont Research Station (UPRS). A dry sieving process was used to isolate a less than 0.5-mm fraction from the CEFS soil for long term use as a high C experimental "standard".

Permanganate oxidizable C analysis.
Permanganate oxidizable C levels (POC) were determined for the soils described above using the lab method proposed by Weil et al. (2003) as well as selected modifications (Table 2).

Table 2. Recommended and modified experimental parameters.

Procedural variable	Weil et al. value	Modified values
Mass of soil	5.0 g	0.25 – 9 g
Initial concentration of MnO_4^-	0.02 M	0.02 M
Volume of MnO_4^- solution	20 ml	20 ml
Duration of shaking	2 min	2, 5, 10 ,15, 18 min
Duration of settling	10 min	10, 30 min

Soil masses ranging from 0.25 to 9.00 g were reacted with 20.0 ml of 0.02 M permanganate solution in 50-ml screw top polycarbonate centrifuge tubes. The soil was added first followed by sequential aliquots of DI water (18.0 ml) and permanganate reagent (2.00 ml) using a mechanical repipetor and electronic pipet, respectively. The permanganate reagent contained 0.2 M $KMnO_4$, 1 M $CaCl_2$ and was adjusted to a pH of 7.2 using NaOH. The $CaCl_2$ was included to promote rapid flocculation of soil colloids. Weil et al. (2003) recommended raising the pH to 7.2 to increase reagent stability.

Tubes were capped and shaken end to end (240 oscillations per minute) for times ranging from 2 to 18 min. Tubes were prepared in sets of 25, with each set including 5 permanganate standards (2, 1.5, 1, 0.5, 0 ml of 0.2 M $KMnO_4$ reagent brought to 20 ml with DI water) and 2 tubes containing a standard soil.

After shaking, the suspensions were allowed to settle for either 10 or 30 min. An electronic pipette was used to transfer 1.0-2.0 ml aliquots of supernatant to clean tubes. The aliquots were diluted 10-20 fold with DI water followed by 5 seconds of orbital shaking with a vortex mixer. Absorbance was promptly measured at 565 nm using a Hitachi 100-60 spectrophotometer.

The following equation was used to calculate POC as a function of the quantity of permanganate reduced (Mn^{+7} -> Mn^{+4}) in each tube:

Equation 1:

POC (g/kg) = [0.02 - (a + b x absorbance)] x 9 x 0.02 / sm

where **0.02** is the intitial MnO_4^- concentration (mol/liter) in each tube, **a** and **b** are the intercept and slope of a standard curve, **9** is the mass (g) of C oxidized by 1 mol of MnO_4^-, **0.02** is the volume (l) of solution in each tube and **sm** is the mass (g) of soil added to each tube (Weil et al., 2003).

RESULTS AND DISCUSSION

Products of soil C:permanganate reaction.

Weil et al. (2003) reported that manganese is reduced from Mn^{+7} to Mn^{+2} during reaction with POC. While it is possible that some Mn^{+2} is produced, we believe that the primary manganese product is manganese dioxide (Mn^{+4}).

The accumulation of a dark brown layer was routinely observed in the tubes during the settling period. We also observed that lab equipment used for POC analysis developed a brownish discoloration that was insoluble in DI water and 0.1 M HCl but was quickly removed by a rinse with 0.1 M ascorbic acid. Manganese dioxide is an insoluble dark brown compound that readily accepts electrons from ascorbic acid (CRC, 1990).

We propose that the following redox half reactions and associated oxidation state transitions occur during the Weil et al. (2003) method.

Reduction half reaction

$MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2 + 4OH^-$ **$E^o = 0.60V$**

$Mn^{+7} \rightarrow Mn^{+4}$

Oxidation half reaction

$CH_20 + O_2 \rightarrow CO_2 + H_2O + 4e^-$

$C^0 \rightarrow C^{+4}$

These half reactions and oxidation state transitions are congruent with the stoichiometric relationship (0.75 mol C : 1 mol Mn) assumed in equation 1.

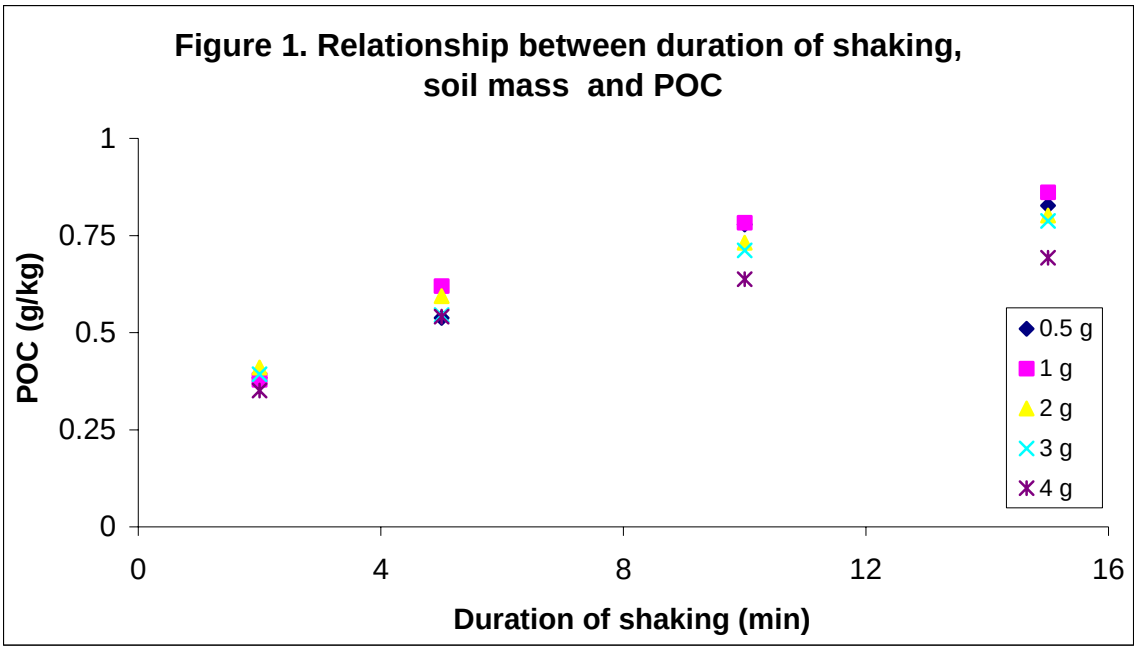
The permanganate reduction reaction listed above only occurs under alkaline conditions (CRC, 1990). Under acidic conditions, the following two reactions occur (CRC, 1990):

$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O$ **E^o = 1.68V**
$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$ **E^o = 1.51V**

We have not evaluated the sensitivity of the POC method to pH, but the contrasting reactions and electron potentials presented above suggest that the kinetics and stoichiometry of reactions in acid and alkaline solutions will differ.

Duration of shaking.
Weil et al. (2003) evaluated durations of shaking ranging from 1 to 15 min and reported that 2 min of shaking resulted in the best combination of analytical precision, experimental convenience and sensitivity to management. They emphasized that "the duration of shaking should be precisely timed and any further disturbance of the mixture after settling carefully avoided".

We evaluated durations of shaking ranging from 2 to 18 min using different combinations of shake time, pre-shake time, settling time, and mass of soil so that interactions could be identified. An asymptotic relationship was observed between POC and duration of shaking for different masses of the low C standard soil (Fig. 1). The precise duration of shaking emphasized by Weil et al. (2003) appears to decrease in importance as the duration of shaking increases. The divergence of results for different soil masses as the duration of shaking was increased was probably related to changes in reaction efficiency, a source of experimental error that will be discussed later.



We evaluated the impact of duration of shaking on sensitivity of POC to management using soils from contrasting management systems in 2 experiments (See tables 1a and 1b for an overview of the experiments and tables 3a and 3b for experimental results).

Table 3a. Effect of tillage system on POC.

Tillage regime	2-min shake	5-min shake	18-min shake
		POC (g/kg)	
Continuous no till	0.53	0.65	1.05
Fall Plow/spring disk	0.06	0.13	0.30
F value	595.30	82.70	73.40

Table 3b. Effect of C input regime on POC.

Carbon input regime	C inputs* (kg/ha)	2-min shake POC (g/kg)	15-min shake POC (g/kg)
High C systems	6990	0.52	0.59
Low C systems	2030	0.43	0.44
F value		12.00	15.10

* Cover crop, manure, and compost applied from 1999-2001.

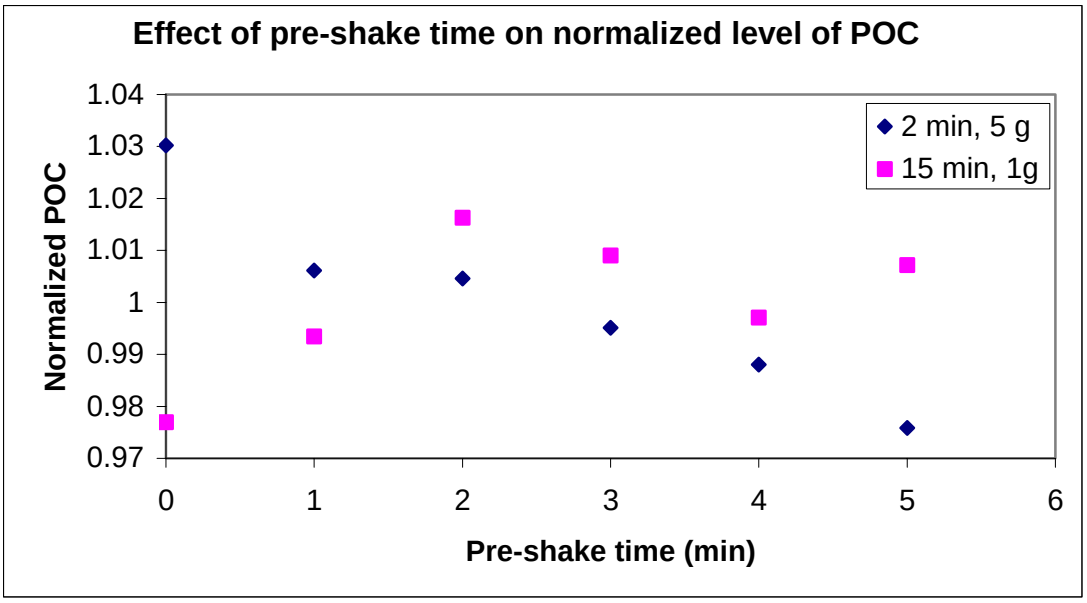
All durations of shaking (2, 5 and 18 min) resulted in POC levels that varied significantly between the fall plow/spring disk and continuous no-till systems but the 2-min duration of shaking produced the most divergent (largest F value) levels of POC (Table 2a).

Both durations of shaking (2 and 15 min) resulted in POC levels that varied significantly between high and low C input systems but duration of shaking had little impact on sensitivity (similar F values) (Table 2b).

Reaction time.

When soils are analyzed in batches, tubes receiving aliquots of permanganate reagent earlier in a batch have greater reaction time than tubes receiving aliquots later in the batch. The difference in pre-shake time between the first and last tube is typically 4 min for a batch of 25 tubes (~ 10 sec per tube). Difference in pre-shake time was observed to be a small but statistically significant source of error when 5 g of soil was analyzed with a 2-min duration of shaking but not when 1 g of the same soil was analyzed with a 15-min duration of shaking (Fig. 2). Smaller soil sub-samples (1 g vs. 5 g) were observed to be less representative (Fig. 2).

Permanganate oxidizable C is also sensitive to duration of settling, as the relative effect of increasing duration of settling from 10 to 30 min was greater when duration of shaking was 2 min as compared to 15 min (data not shown).



Analytical accuracy.

Assessing the accuracy of a method that measures a compositionally diverse pool of electron donors (assumed to be C compounds) by quantifying abundance of the electron recipient (permanaganate) is problematic.

The Weil et al. (2003) method's sensitivity to management and high correlation with standard biologically active C parameters indicate that it is a measure of the intended analyte (i.e. a management sensitive biologically active soil C pool) but the method's range of linearity has not been established.

One option for evaluating linearity would be to spike soils with differing amounts of a specific permanganate oxidizable C compound (e.g. simple carbohydrates, amino acids, amino and amide sugars (Weil et al. (2003)). Another option would be to use different masses of the same soil. We chose the latter option and observed an asymptotic relationship between soil mass and permanganate reduction (Fig. 3 and 4). Soil masses of the high and low C standard soils, ranging from 0.25 to 9 g, were analyzed for POC. Permanganate oxidizable C values were greatest when permanganate availability was high and decreased asymptotically as permanganate availability decreased.

Figure 3. Relationship between soil mass and fraction of permanganate reduced for a high and low C soil

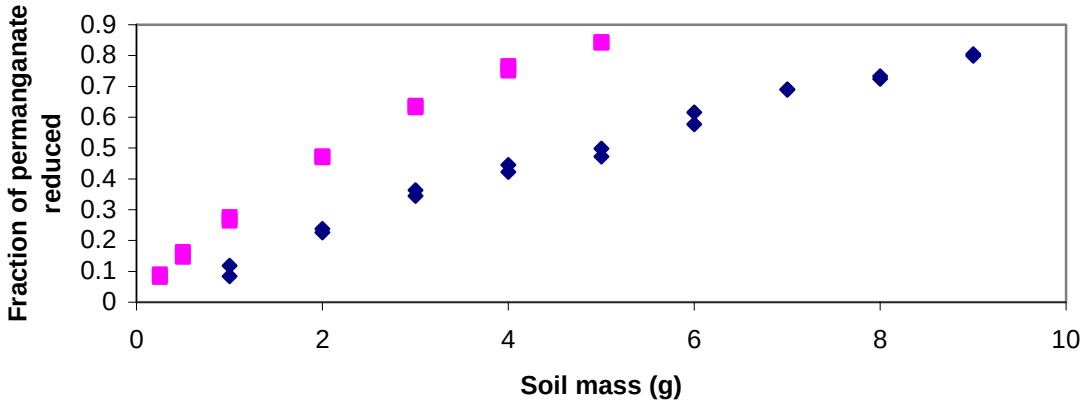
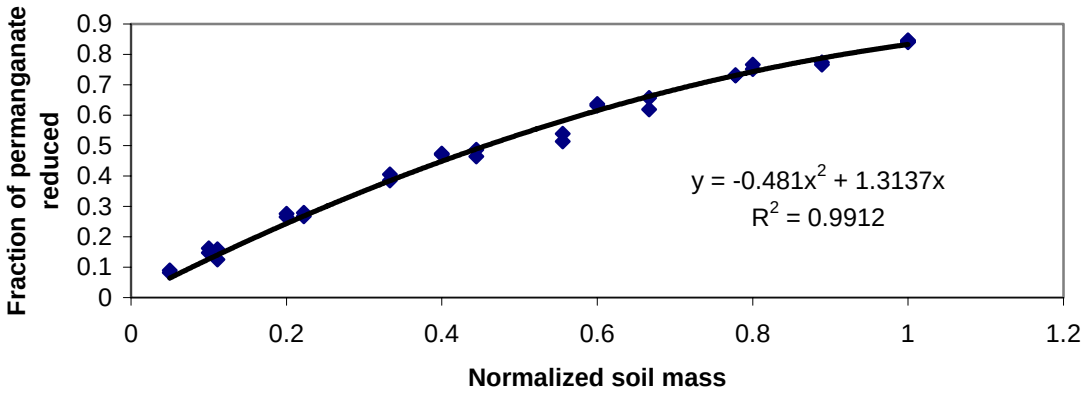


Figure 4. Relationship between fraction of permanganate reduced and normalized soil mass



The excellent fit obtained with the combination of the high and low C soil data sets (Fig. 4) indicates that permanganate availability rather than soil:solution ratio controls reaction efficiency.

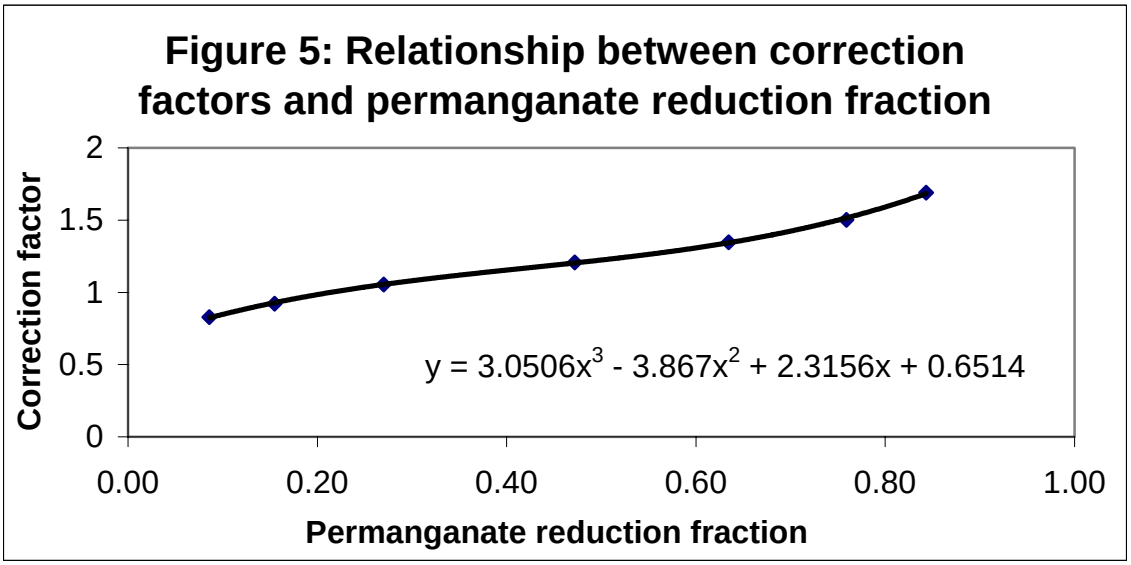
Table 4. Corrected POC results for the high C standard soil.

Soil Mass (g)	Percent MnO_4^- Reduction	Equation 1 g POC / kg soil	Correction factor	Corrected g POC / kg soil
0.25	8.6	1.237	0.824	1.019
0.5	15.5	1.115	0.928	1.035
1.0	27.0	0.973	1.055	1.026
2.0	47.2	0.849	1.203	1.022
3.0	63.4	0.761	1.343	1.022
4.0	75.9	0.683	1.515	1.035
5.0	84.3	0.607	1.683	1.021

The corrected POC values presented in Table 4 were derived as follows:

The linear component of the second order polynomial presented in Fig. 4 was chosen to define the correct permanganate reduction fraction. Consequently, the product of the correction factor and the measured permanganate reduction fractions equaled the chosen linear model.

A third order polynomial was fit to the relationship between correction factors and measured permanganate reduction fractions (Fig. 5):



This third order polynomial was used to generate the correction factors presented in Table 4. Permanganate oxidizable C values calculated using Equation 1 were then corrected by the respective correction factors. The correction technique was evaluated using results from a soil under long-term sod at the UPRS (Table 5).

Table 5. Validation of correction model.

Soil Mass (g)	Percent MnO_4^- Reduction	Equation 1 g POC / kg soil	Correction factor	Corrected g POC / kg soil
0.5	15.6	1.12	0.930	1.04
1.0	28.3	1.01	1.066	1.07
2.0	48.4	0.87	1.211	1.05
3.0	68.7	0.82	1.406	1.15
4.0	82.5	0.74	1.642	1.21

The corrected values were more consistent than those calculated using Equation 1, but there was a small over-correction for the greater masses of soil.

The application of a correction factor (to account for lower reaction efficiency when greater amounts of C are oxidized) would be expected to increase the sensitivity of POC to management-induced differences. Increased sensitivity was observed when the correction factor was applied to data from the long-term tillage system experiment at the UPRS (data not shown).

Proposed quality control protocols.

We have found that adherence to the following quality control protocols reduces experimental error when performing the Weil et al. (2003) method.

1. Always use clean, dry centrifuge tubes. Contamination with dust will result in the reduction of permanganate. Periodically rinse centrifuge tubes and glassware (including cuvettes) with dilute ascorbic acid to remove manganese dioxide precipitate. Rinse tubes and glassware thoroughly to remove all residual ascorbic acid.

2. Include standard soils at the beginning and end of each analytical batch. Standard soils should be selected that are representative of the range of POC that is likely to be found in unknown soils. Standard soils should be pulverized so that they will pass through a sieve with 0.5-mm (or smaller) openings.

3. Replicates of unknown samples should be analyzed in separate analytical batches. It is of some value to include replicates in the same batch but this type of replication is not appropriate for determining true experimental error.

4. Standardize each batch of permanganate reagent by titration of a known mass of sodium oxalate ($Na_2C_2O_4$) - See Appendix A. Standardize reagent again if absorbance values for the standard curve change.

5. Include four or more standards for a standard curve in each analytical batch.

6. Standards can be prepared by adding 0, 0.5, .0, 1.5 and 2.0 ml of permanganate reagent to centrifuge tubes using a high quality electronic pipette and then dispensing an 18.0-ml aliquot of DI water into each tube. Cap and shake tubes as part of a 25-tube analytical batch. The concentrations will be: 0, 0.00541, 0.001053, 0.01538 and 0.02 M. We routinely obtain R-squared values greater than 0.999.

7. Dilute aliquots of supernatant so that absorbance readings have maximum resolution. Appropriate dilution factors will depend on the spectrophotometer. We have had good success diluting 10 to20 fold.

8. Maintain consistent procedural timing (i.e. durations of pre-shake, shaking and settling)

9. Analysis of small sample masses (< 5 g) requires a proportionately greater level of sample homogenization to obtain representative sampling.

CONCLUSIONS

We concur with Weil et al. (2003) that the pool of soil C oxidized during 2 min of shaking in 0.02 M permanganate is a sensitive indicator of management effects on soil quality.

The method's short duration of reaction (pre-shake + shake + settling) is more sensitive to variation in procedural timing than when longer durations of reaction are used, but analytical precision (CV < 5%) can be achieved if the quality control protocols listed above are followed.

Our biggest concern about the Weil et al. (2003) method is its apparent non-linearity. Results from three soils differing in taxonomy and C content (Fig. 3 and 4, Tables 4 and 5) showed similar asymptotic loss of reaction efficiency over the method's entire range of reaction. This non-linearity may be inconsequential for some routine applications (e.g. use of POC as a general indicator of soil quality or response to improved OM management) but correction seems desirable for research

applications. We have proposed a correction technique and recommend this technique rather than the specific third order polynomial (Fig. 5) that was derived from a limited number of soils.

Additional research is needed to confirm that asymptotic loss of reaction efficiency is a general attribute of the Weil et al. (2003) method. We plan to investigate this phenomena using a much broader set of soils using both the multiple mass and the matrix spike approach previously described. We also plan to investigate the sensitivity of the method to variation in solution pH.

REFERENCES

Bell, M.J., P.W. Moody, R.D. Connolly, and B.J. Bridge. 1998. The role of active fractions of soil organic matter in physical and chemical fertility of Ferrosols. Australian J. Soil Res. 36:809-819.

Blair, G.J., R.D.B. Lefroy, and L. Lise. 1995. Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. Australian J. Agric. Res. 46:1459-1466.

Blair, G.J., R. Lefroy, A. Whitbread, N. Blair, and A. Conteh. 2001. The development of the KMnO4 oxidation technique to determine labile carbon in soil and its use in a carbon management index. In R. Lal, J. Kimble, R. Follet, and B. Stewart (eds.). Assessment Methods for Soil Carbon. Lewis Publishers, Boca Raton, FL. p. 323-337.

CRC. 1990. Handbook of Chemistry and Physics, seventy-first edition. D.L. Lide (editor). CRC Press, Boca Raton, FL.

Loginow, W., W. Wisniewski, S.S. Gonet, and B. Ciescinska. 1987. Fractionation of organic carbon based on susceptibility to oxidation. Polish J. Soil Sci. 20:47-52.

Moody, P.W., S.A. Yo, and R.L. Aitken. 1997. Soil organic carbon, permanganate fractions, and the chemical properties of acidic soils. Australian J. Soil Res. 35:1301-1308.

Weil R.W., Islam K.R., Stine, M., Gruver J.B. and S.E. Samson-Liebig. 2003. Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. American J. Alt. Ag. 18 (1):3 -17.

Appendix A

Modified from **http://onsager.bd.psu.edu/aronne/labsynfes033.pdf**

Standardizing a permanganate solution with a known mass of sodium oxalate ($Na_2C_2O_4$). The titration reaction is:

$$5\ C_2O_4^{2-}(aq) + 2\ MnO_4^{-}(aq) + 16\ H^{+}(aq) \rightarrow 10\ CO_2(g) + 2\ Mn^{2+}(aq) + 8\ H_2O(l)$$

1. Weigh approximately 0.1200 g of sodium oxalate and transfer to a 250 mL Erlenmeyer flask. Add 10 mL of 6 M H_2SO_4 and 65 ml of DI water to the flask.
2. Fill a clean buret with the $KMnO_4$ solution to be standardized. Note that the solution is a very dark purple color so volume readings should be taken from the top edge of the liquid instead of the bottom of the meniscus.
3. Heat the sodium oxalate solution to 80-90 ^{o}C. When you remove the thermometer to perform the titration, be sure to rinse the thermometer into the flask since you do not want to lose any of the sodium oxalate.

4. Record the initial reading on the buret, to the nearest 0.01 mL and begin to add the $KMnO_4$ solution to the flask but do not add too rapidly and be sure to swirl the solution. You should observe that the purple solution loses its colour as it falls into the hot solution.
5. If you add the $KMnO_4$ solution too rapidly, or do not swirl well, you may find you have some brown colouration in your solution. This is due to the formation of manganese dioxide (MnO_2). If you have not added any more $KMnO_4$ than needed to reach the endpoint, the excess oxalate should reduce the MnO_2 momentarily. However, if you fail to swirl the sample and overshoot the endpoint while MnO_2 is formed, the titration is ruined and must be performed again.
6. You should start to notice that as you are nearing the endpoint of your titration that the decolouration of the $KMnO_4$ takes longer and longer. At this time you should add the $KMnO_4$ more slowly, preferably drop by drop. When you have reached the endpoint, there will be a faint colour that persists in the solution
7. It is useful to run a blank for this titration since the sulphuric acid solution may contain some impurities that react with the potassium permanganate and introduce error. Add 10 mL of 6 M H_2SO_4 and 65 mL of DI water to an Erlenmeyer flask and heat it to 80-90 ^{o}C. Titrate until you have a persistent faint pink colouration.
8. Subtract this volume from the volume of $KMnO_4$ used in the titration of the sodium oxalate sample.
9. Molarity of permanganate solution = g of sodium oxalate *2.98507 / mls of permanganate to reach endpoint.