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Short communication

Correction factors for dissolved organic carbon extracted from soil, measured using the Mn(III)-pyrophosphate colorimetric method adapted for a microplate reader

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ABSTRACT

Oxidizable dissolved organic carbon (DOC) is regularly measured in environmental samples using a colorimetric method with Mn(III)-pyrophosphate as the oxidizing agent. It is simpler to use and has a much higher throughput than the commonly used dichromate oxidation and combustion methods. Here, we demonstrate that the method often leads to an underestimation or overestimation of the concentration of common organic compounds in solutions. To our knowledge, no published study has taken this fact into account when analyzing DOC data. Hence, we compared Mn(III)-pyrophosphate-based results with measurements performed with a total organic carbon combustion analyzer for samples of organic and mineral soil horizons of two temperate deciduous forests, of organic soil horizon of a primary-growth hemlock stand, and of a peatland located in New England, USA. The Mn(III)-pyrophosphate method consistently underestimated DOC concentration in soil extracts. We present correction factors for the different types of soil studied. By employing correction factors, we find the method can be an inexpensive, accurate, and high throughput tool to measure DOC in environmental samples.

A colorimetric method developed by Bartlett and Ross (1988), using Mn(III)-pyrophosphate as the oxidizing agent, has been widely used to measure oxidizable dissolved organic carbon (DOC) in rain, stream, and soil water in ecosystems ranging from Arctic tundra to peatlands, tropical forests, and a variety of agricultural systems (e.g., Xu et al., 2005; Gomes et al., 2012; Lipson et al., 2013; Martins Bezerra et al., 2013; Turner et al., 2013). Coupled with chloroform fumigation-extraction (Vance et al., 1987), it has also been used extensively to estimate microbial biomass carbon in soils (e.g., da Silva et al., 2012; Gomes et al., 2012). Some organic compounds such as glycine and acetic acid resist oxidation (Bartlett and Ross, 1988), raising the concern that DOC concentration in environmental samples may be underestimated. In an extensive review of the literature, we searched the Web of Science database and Google Scholar on July 15, 2014, for all publications citing the original methods paper by Bartlett and Ross (1988). We found 110 journal articles and theses in which the Mn(III)-pyrophosphate method was used on environmental samples and found none where authors mentioned any verification or correction for a possible underestimation of DOC concentration (Supplementary Table 1). In this study, we adapted the Mn(III)-pyrophosphate colorimetric method to use on a microplate reader and assessed its validity for soil extracts by comparing results to DOC measurements made with a total organic carbon (TOC) combustion analyzer.

We collected soil samples from three locations in northeastern United States. Organic horizon (OH) and mineral soil (MS) samples were collected at the Hubbard Brook Experimental Forest, New Hampshire (43°56′N, 71°45′W) and Harvard Forest, Massachusetts (42°32′N, 72°11′W) in April, May, August, and October of 2012 and 2013. At Hubbard Brook, samples were collected in a mature sugar maple–yellow birch stand; soils were base-poor spodosols developed on glacial till (Comerford et al., 2013). Harvard Forest samples were collected in a mature red oak–red maple stand on a mixed mesic Typic Dystrochrept soil (Melillo et al., 2002). Samples were 10 × 10 cm OH monoliths and 5 cm-diameter MS cores from the top 15 cm of the mineral soil. At Harvard Forest, OH samples were also collected in a primary-growth hemlock stand in August 2013 (Hadley and Schedlbauer, 2002).

Peat samples were removed from the Central Unit of Caribou Bog, a 2200-ha peatland complex near Bangor, Maine (44°56′N, 68°46′W). The Central Unit is an eccentric raised bog underlain

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with glacio-marine clay–silt mineral soils (Comas et al., 2011). We collected 5 cm-diameter peat cores from the top 20 cm of the dead moss layer in May, August, and October 2013 in sites dominated by (1) *Sphagnum* lawn, bryophytes, and sedges, (2) *Sphagnum* lawn and low ericaceous shrubs (*Kalmia polifolia* Wangenh., *Rhododendron groenlandicum* (Oeder) Kron & Judd) and (3) ericaceous shrubs and black spruce (*Picea mariana* (Mill.) B.S.P.). Soil characteristics are presented in Supplementary Table 2.

Samples were immediately brought back to the laboratory and stored at 4 °C. OH and MS samples were homogenized by sieving through 2-mm mesh (4-mm mesh for hemlock OH) and removing rocks, roots, and woody debris. Roots were removed from peat samples without sieving. Five grams each of OH and peat sample or 10 g of MS sample were placed in 50-mL centrifuge tubes. 40 mL of 0.5 M K_2SO_4 were added to each tube. Slurries were shaken for 1 h on an oscillating table and filtered through a Whatman #1 paper filter. The extracts were frozen at −20 °C until DOC concentration was measured. Time between sample collection and freezing of the extracts was kept under 48 h.

To measure DOC, we adapted Bartlett and Ross's (1988) method to use with a 96-well microplate reader. On each microplate, we pipetted duplicate 100-μL aliquots of eight oxalic acid standards with concentrations ranging from 0- to 4-mM C and triplicate 100-μL aliquots of 24 soil extracts. We added 50 μL of 10 mM Mn(III)-pyrophosphate solution and 50 μL of concentrated sulfuric acid to each well. The 96-well microplates were incubated in the dark at room temperature for 18 h before measuring absorbance at 495 nm on a microplate reader (VersaMax, Molecular Devices, Sunnyvale, California, USA). For each microplate, soil extract absorbance was converted to C concentration using a linear calibration curve based on the oxalic acid standards. We also measured total organic carbon content of the same soil extracts using an Apollo 9000 TOC Analyzer with autosampler (Teledyne Tekmar, Mason, Ohio, USA). Arginine was used as a standard. Regression analyses were conducted in Matlab version 7.11.0 (MathWorks, Natick, Massachusetts, USA).

As a preliminary test, we assayed solutions of known concentration (0- to 4-mM C) of several organic compounds to determine if the colorimetric method measured all C present (Fig. 1, Table 1). Of the eight substances tested, only glucose and malonic acid showed a stable recovery rate throughout the concentration range (Fig. 1a). The recovery rate of citric acid, glutamine, and tannic acid was stable up to ~3-mM C after which the Mn(III)-pyrophosphate had lost all its color and no further change in absorbance could be detected (Fig. 1b). In four of these five cases, the slope of the linear regression between the measured and expected C concentrations was greater than 1 (Table 1), indicating the method overestimated the amount of C present. The recovery rate of glutamine was less than 1; in this case C concentration was underestimated. Fumaric acid and glycine also had a recovery rate smaller than 1, and the linear regressions explained a much lower proportion of the variability (Table 1, Fig. 1c). Finally, acetic acid recovery rate was near zero and the linear regression was not statistically significant (Table 1, Fig. 1d). Given the high variability in the recovery rate of the various organic compounds and the great difficulty in measuring the relative abundance of these and other substances in environmental samples, it is highly probable that reported DOC concentrations using the Bartlett and Ross (1988) colorimetric method are biased high or low depending on the particular mix of DOC compounds present.

Table 1
Regression coefficients of the linear relationships, shown on Fig. 1, between C concentration of a few common organic compounds measured using the Mn(III)-pyrophosphate method and the expected C concentration.

Organic compound	Slope	Intercept	r^2	Concentration range applicable (mM C)
Acetic acid	Not significant at $P < 0.05$			…
Citric acid	1.439	−0.008	0.997	0–3.2
Fumaric acid	0.100	0.079	0.682	0–4
Glucose	1.045	0.098	0.991	0–4
Glutamine	0.300	−0.106	0.989	0–3.2
Glycine	0.051	−0.008	0.431	0–4
Malonic acid	1.335	−0.180	0.993	0–4
Tannic acid	1.784	0.098	0.997	0–2.8

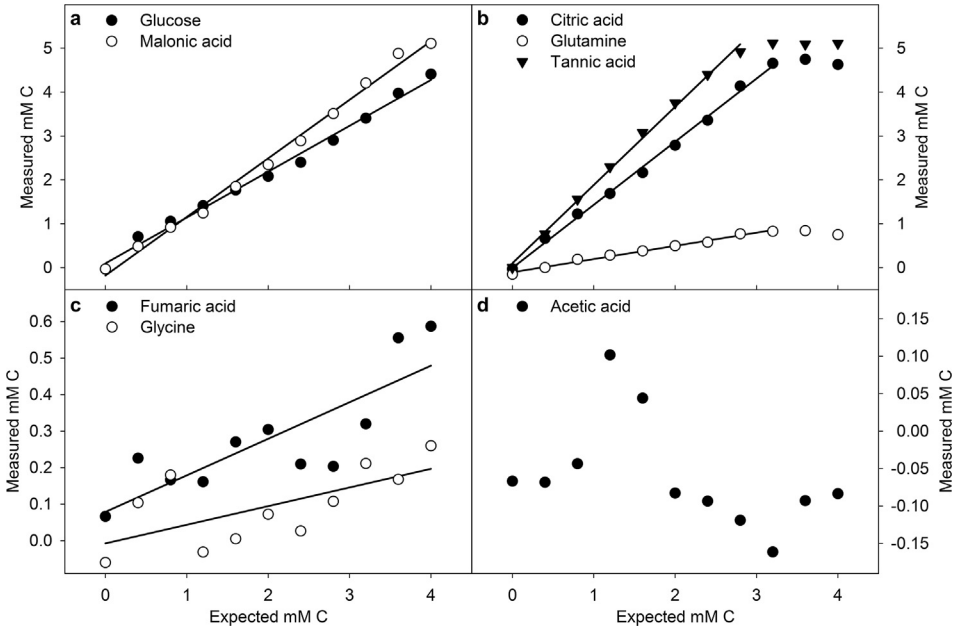

Fig. 1. Linear relationship between measured and expected C concentrations of eight common organic compounds estimated using a calibration curve based on oxalic acid. Regression coefficients and statistics are presented in Table 1. Only regressions statistically significant at $P < 0.05$ are shown.

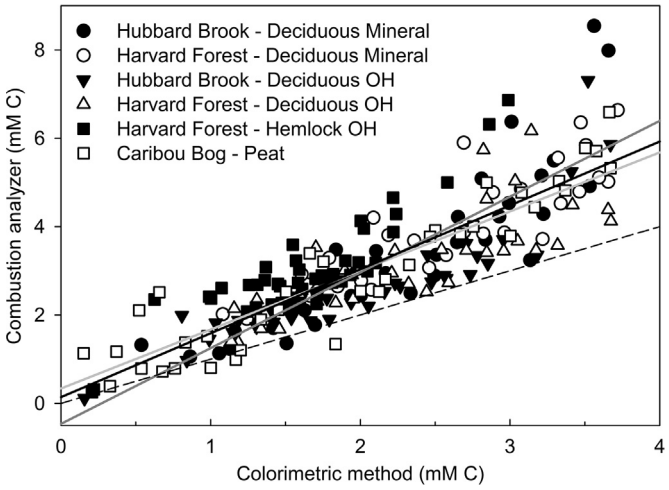

Fig. 2. Linear relationship between DOC concentration of soil extracts measured using the Mn(III)-pyrophosphate method and a TOC combustion analyzer. The regressions for OH and peat samples, MS samples, and all samples aggregated are shown as light gray, dark gray, and black solid lines, respectively. The dashed line represents the 1:1 slope. Regression coefficients and statistics are presented in Table 2.

To determine the magnitude of bias in soil samples collected in New England ecosystems, we compared the DOC content of OH, MS, and peat samples measured using the colorimetric method and a TOC combustion analyzer (Fig. 2). Combustion methods are considered to yield absolute values of TOC and are often used as a benchmark to evaluate the efficiency of other methods (Nelson and Sommers, 1996). Linear regressions between colorimetric and combustion measurements explained most of the variation ($r^2 = 0.62–0.87$; Table 2). The colorimetric method underestimated DOC concentration by 19–91% depending on the type and provenance of samples (Table 2). The underestimation differed between sites and types of soil, presumably reflecting varying proportions of the different organic C compounds present, which in turn reflect differences in microbial populations and soil characteristics and processes.

A potassium dichromate colorimetric method is also widely used to measure DOC in soil extracts (Nelson and Sommers, 1996). There is no need to apply a correction factor to that method if samples are heated for long enough because all C gets oxidized.

Table 2
Regression coefficients of the linear relationships between DOC concentration of soil extracts measured using the Mn(III)-pyrophosphate method and a TOC combustion analyzer for each site and type of soil. Mn(III)-pyrophosphate-based measurements can be corrected using the equation $DOC_{corrected}$ = slope × $DOC_{Mn(III)\text{-}pyrophosphate}$ + intercept. The correction is applicable for concentrations of 0.08–4 mM C, the range over which the Mn(III)-pyrophosphate method gives valid results.

Site	Forest type	Soil type	Slope	Intercept	r^2
Hubbard Brook	Sugar maple–yellow birch	Organic	1.393	−0.151	0.78
Hubbard Brook	Sugar maple–yellow birch	Mineral	1.906	−0.938	0.77
Harvard Forest	Red oak–red maple	Organic	1.190	0.428	0.62
Harvard Forest	Red oak–red maple	Mineral	1.481	0.153	0.79
Harvard Forest	Hemlock	Organic	1.793	0.027	0.81
Caribou Bog	Raised bog	Peat	1.449	0.135	0.87
		All organic and peat	1.336	0.338	0.73
		All mineral	1.715	−0.463	0.77
		All samples	1.447	0.140	0.75

This technique is more complicated to use, requires some specialized equipment and more reagents, and has substantially lower throughput than the Mn(III)-pyrophosphate method. DOC analysis by combustion yields the most accurate measurements of organic C (Nelson and Sommers, 1996), but it requires an expensive analyzer that also requires frequent maintenance and troubleshooting.

Our results suggest that DOC in soil extracts can be successfully measured using the Mn(III)-pyrophosphate colorimetric method as long as a correction for the bias is applied. When adapted to be run on an absorbance microplate reader, the Mn(III)-pyrophosphate colorimetric method becomes an easy, inexpensive high-throughput technique allowing processing of several hundreds of samples per day. Because the correction factor varies depending on the forest and type of soil studied, we recommend that a subset of the samples also be run on a combustion analyzer to determine the correction factor applicable. If this is impossible, at a minimum a general correction factor, such as the ones we present for New England soils, should be used. We suggest the Mn(III)-pyrophosphate colorimetric method could also be used on other matrices containing unknown amounts of C-containing compounds, such as rain and stream water, as long as a specific correction factor is estimated.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.08.011.

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