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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

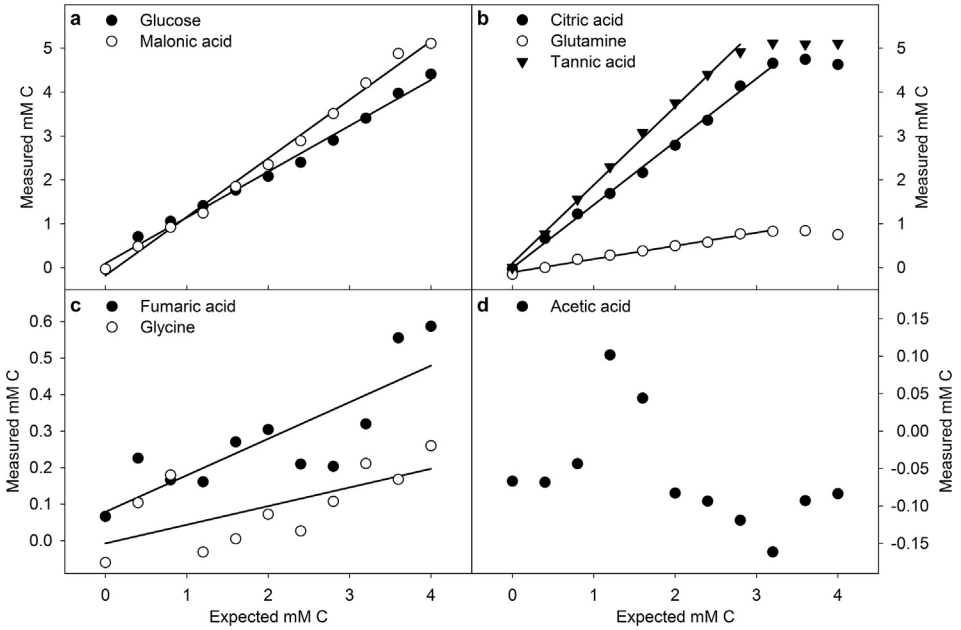

Fig. 1. Linear relationship between measured and expected C concentrations of eight common organic compounds estimated using a calibration curve based on oxalic acid. Regression coefficients and statistics are presented in Table 1. Only regressions statistically significant at $P < 0.05$ are shown.