

Total carbon analysis may overestimate organic carbon content of fresh waters in the presence of high dissolved inorganic carbon

Stuart Findlay^{1*}, William H. McDowell², David Fischer¹, Michael L. Pace³, Nina Caraco¹, Sujay S Kaushal⁴, and Kathleen C. Weathers¹

¹Cary Institute of Ecosystem Studies, PO Box AB, Millbrook, NY 12545

²Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824

³Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22904

⁴University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, Solomons, MD 20688

Abstract

Automated carbon analyzers often are configured to provide estimates of both total organic carbon (TOC) and nonpurgeable organic carbon (NPOC). We show there can be an overestimation of total carbon in the presence of moderate to large quantities of dissolved inorganic carbon. This leads to overestimates of TOC, which is measured as the difference between total carbon and inorganic carbon. Water samples were analyzed as both TOC and NPOC on a Shimadzu TC 5050 Carbon Analyzer. The difference between TOC and NPOC increased as a function of concentrations of dissolved inorganic carbon (DIC). Water samples spiked with DIC ranging from 0 to 100 mg DIC/L also reported increased TOC as large as 8 mg C/L. Our data suggest that the Shimadzu 5050 analyzer (and by analogy other instruments that estimate TOC by difference between TC and IC) overestimates total carbon (TC) when calibrated with an organic standard as recommended by the manufacturer. The magnitude of the overestimation varies both with the amount of DIC present in the sample and the extent to which measurement efficiency of the analyzer is less than 100%. The consequences will be most severe in analysis of samples from systems spanning a large range in DIC. Time series from individual systems are less likely to be affected because the necessary large change in DIC would be detected as changes in pH or other attributes well before any change in DOC. Systems with high DIC will, however, be susceptible to even small variations in measurement efficiency.

In the past two decades, there has been a large increase in use of automated dissolved carbon analyzers for measurements in surface waters. During this time period, initial questions about oxidation efficiency for various water samples and cross-laboratory comparability have been thoroughly addressed (Peltzer et al. 1996; Sharp et al. 2002). Renewed interest in the performance of analytical instruments for dissolved carbon analysis has stemmed, in part, from the realization that concentrations of dissolved organic carbon (DOC) are much more dynamic than earlier perceptions (Findlay 2003). In addition, for many systems, DOC has shown long-

term trends, as well as highly variable susceptibility to biotic metabolism (Evans et al. 2005, 2008; Tranvik and Bertilsson 1999). Moreover, with increases in atmospheric CO₂ levels, the carbon pools in surface fresh- and marine waters are almost guaranteed to change (Orr et al. 2005).

Most of the longer-term records of dissolved inorganic and organic carbon in fresh waters have been supported and maintained because of an interest in the quality of drinking water reservoirs or in the shifting sources of acidity in soils and freshwaters that influence DOC concentrations (Forsberg 1992; Driscoll et al. 2003; Burns et al. 2006; Monteith et al. 2007; Evans et al. 2008). More recently, there have been efforts to track changes in inorganic as well as organic carbon (Jones et al. 2003; Raymond and Cole 2003). There have been several recent reports of high quantities of volatile organic carbon (VOC) in the world's oceans representing a significant carbon flux to the atmosphere (e.g., Dachs et al 2005), and VOC is often found to be a contaminant in groundwaters (Williams et al. 2006).

*Corresponding author: E-mail: findlays@ecostudies.org

Acknowledgments

This research was supported by several grants from the Hudson River Foundation and the National Science Foundation (DEB 0414262, DEB 0423476, DEB 0454001, DBI-0521091, and DBI 0640300) and the US EPA (Connecticut River Airshed Watershed Consortium).

DOI 10:4319/lom.2010.8.196

Over the past several decades, methods for analyses of dissolved carbon pools in surface waters have shifted from benchtop wet chemistry to a variety of automated instruments. The instruments use some combination of acidification/sparging to remove inorganic carbon and catalyzed oxidation to convert organic carbon to CO_2 before detection with infrared absorbance or gas chromatography.

Many automated carbon analyzers determine organic carbon in a filtered water sample by two approaches. For instance, the Shimadzu® series instruments acidify and sparge a sample before analysis and any remaining carbon is then oxidized, detected, and is defined to be nonpurgeable organic carbon (NPOC). This approach is analogous to DOC measured by wet oxidation methods (e.g., McDowell et al. 1987). The alternate approach with the Shimadzu instruments is analysis for total carbon (TC) where an aliquot of the sample is oxidized, acidified, and sparged. The CO_2 measured represents the total produced from both dissolved inorganic carbon (DIC) and oxidized organic carbon (OC) (Fig. 1). A separate aliquot of the sample is then acidified and sparged without oxidation to measure the CO_2 derived solely from DIC, typically termed IC by instrument manufacturers. The difference between TC and IC is then taken as a measure of the CO_2 produced from total DOC, including both the nonvolatile and volatile components (Fig. 1), and this quantity is referred to as total organic carbon (TOC). The difference between TOC and NPOC is, in theory, VOC lost to the atmosphere during the open, acidified sparging step of the NPOC analysis. (In this paper, we refer to the difference in carbon between TOC and NPOC as “apparent” VOC [abbreviated aVOC]). Compounds lost to the atmosphere during this sparging would not necessarily be volatile under environmental conditions, since the acidification step lowers the pH to around 2, much lower than the vast majority of nat-

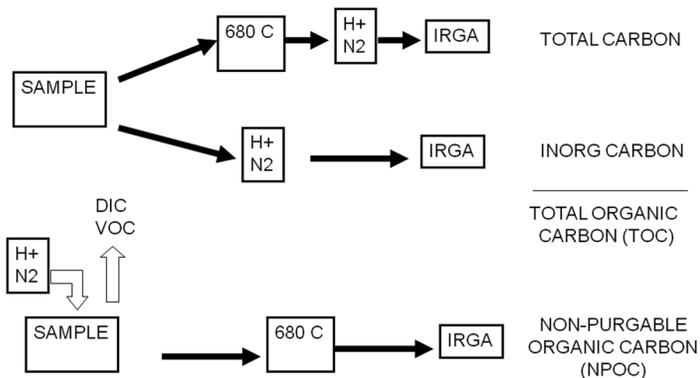


Fig. 1. Flow diagram of analytical scheme for Shimadzu 5050 Carbon Analyzer. In Total Carbon mode, the instrument estimates total organic carbon (TOC) as the difference between Total Carbon and Inorganic Carbon. In NPOC mode, IC is sparged from an acidified sample before the sample enters the instrument. The key difference is that the acidified sparging occurs in the sample train in the Total Carbon mode versus open to the atmosphere in the NPOC mode.

ural waters. Some instruments allow for a separate analysis and capture of CO_2 derived from both IC and DOC, which is useful in cases where both products are intended for further analyses such as determination of stable isotopes (see St-Jean 2003).

Over the course of many measurements of dissolved carbon pools from several lakes, streams, and one larger river, we have noted surprisingly large differences between TOC and NPOC. As we show below, this difference is largely (if not completely), a consequence of an overestimation of the TC term; we suggest that this “estimate” of aVOC should not be construed as a real pool of carbon.

In this article, we quantify the potential effect of DIC concentration on estimation of the total organic carbon pool during automated analyses and describe how measurement efficiency affects the magnitude of this DIC effect. We show the magnitude of aVOC for freshwater systems ranging from oligotrophic lakes to urban streams.

Materials and procedures

Sites—Data on TOC and NPOC were derived from six groups of sites that spanned urban to rural land covers, and Michigan to Baltimore, MD, USA. The groups included: (1) multiple locations in the tidal freshwater Hudson River, NY, USA (abbreviated HR); (2) streams draining suburban and urban watersheds associated with the Baltimore Ecosystem Study Long Term Ecological Research (BES LTER) site, MD, USA; (3) a set of 16 small (second to fourth order) streams in Dutchess County, NY, USA (DUT); (4) multiple locations in the Walkill River drainage, NY, USA (WAL); (5) a set of 15 lakes located at the University of Notre Dame Environmental Research Center in northern Michigan, USA (CAS), and (6) multiple tributaries that flow into Lake Sunapee in NH, USA (SUN). Timing of collection ranged from multiple years and seasons for the Hudson River to a single summer sampling of the Walkill River.

Analysis—Samples were analyzed on a Shimadzu 5050 TC Analyzer at the Cary Institute of Ecosystem Studies, Millbrook, NY, USA. All water samples were filtered (either Gelman 934AH or Whatman GF/F) and stored cold before carbon analysis.

With each analytical run of the Shimadzu, there were three interspersed sets of organic carbon standards (potassium phthalate, 2.5, 5, 10, and 20 mg C L^{-1}) and blanks of Nanopure water. Samples were first run in Total Organic Carbon (TC-IC) mode and then reanalyzed with the instrument in NPOC mode. For measurement of both TC and NPOC, the phthalate standard curve was used. For measurement of IC, a mixed carbonate/bicarbonate inorganic carbon standard was used, as recommended by the manufacturer.

Several checks on Carbon Analyzer performance were suggested by large differences between TOC and NPOC. We analyzed standards containing only inorganic carbon as well as additions of inorganic carbon to natural water samples or phthalate standards.

For most data presentations, we show only the mean for each freshwater system rather than all the data because the number of observations varies greatly among systems (see legend for Fig. 2). Model 2 regressions were calculated using the Geometric Mean approach described by Prepas in Downing and Rigler (1984).

Assessment

Concentrations for TOC and NPOC ranged broadly across sites with means from 4-14 mg C/L (Fig. 2), which spans the median concentration for global surface waters (see Mulholland 2003). Whereas TOC and NPOC were clearly positively associated, the points were all below the 1:1 line. Lack of a 1:1 relationship suggests these two analytical approaches are not equivalent and explicit statement of which was used becomes crucial for interpreting data reported in the literature. The difference between TOC and NPOC (aVOC for brevity) was positively correlated with ambient DIC (Fig. 3) across a DIC range from 3 to 30 mg/L. With a slope of 0.095, a range of 10 mg DIC/L would be associated with a reported difference of nearly 1 mg/L between analytical approaches. The difference ranged from about 1 mg C/L to almost 3.5 mg C/L across samples and sites, and these values represent 14%-57% (SUN, BES respectively) of TOC. These estimates of aVOC are large relative to other estimates of volatile carbon pools. For example, Thurman (1985) suggested that VOC in unpolluted waters is generally less than 0.05 mg/L although heavily contaminated sites may have concentrations in excess of 10 mg/L (c.f. Williams et al. 2006).

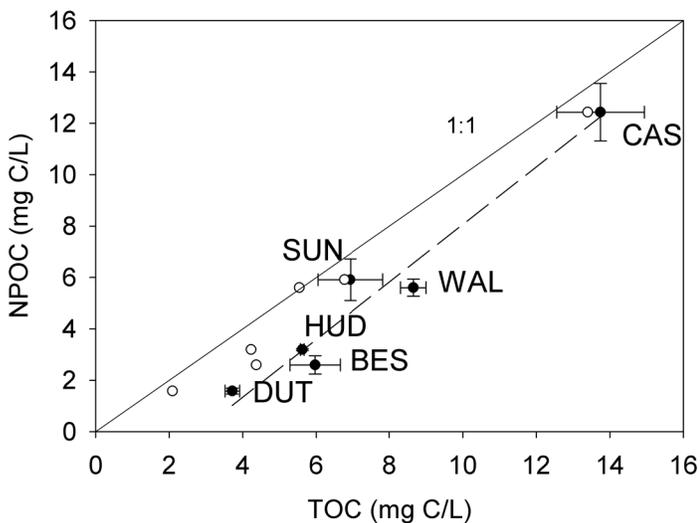


Fig. 2. Bi-plot of mean (\pm SE) TOC and NPOC for six different groups of systems (see text for description). Number of observations ranges from 310 for the Hudson to 25 for SUN. DUT = streams in Dutchess County, NY, USA; HUD is the Hudson River, USA; BES is the Baltimore LTER, USA; SUN is Sunapee Lake, NH, USA; WAL is the Walkill River in NY, USA, and CAS is a set of lakes in Michigan, USA. Open circles to the left of filled circles show TOC “adjusted” for ambient DIC (see text). The solid line is 1:1 and the dashed line is Model 2 Regression ($y = -3.14 + 1.12 [x]$, see text).

To directly examine DIC effects, we spiked twelve natural water samples from multiple sites with a range of sodium carbonate/bicarbonate ranging from 0 to 100 mg C/L and analyzed them for TOC and NPOC concentrations. Estimated values of TOC (TC-IC) increased with increasing DIC (2 of the 12 examples are shown in Fig. 4). Slopes of these relationships were ~ 0.08 suggesting that the difference between TOC and NPOC (or aVOC) would increase up to approximately 8 mg C/L across the 100 mg C/L range of our DIC spikes.

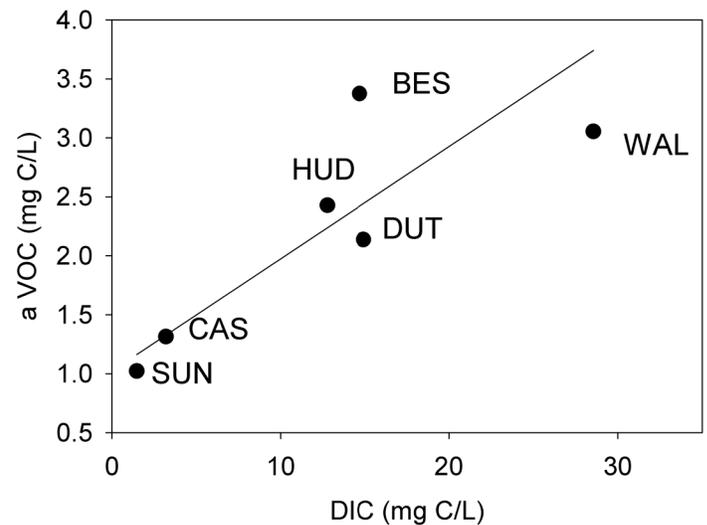


Fig. 3. Difference between TOC and NPOC (“Apparent VOC”) in samples from 6 groups of aquatic systems from central to eastern North America, shown relative to ambient DIC. Values are means, number of individual measurements, and site legends as in Fig. 2. The line is Model 2 Regression $y = 1.024 + 0.095 [x]$.

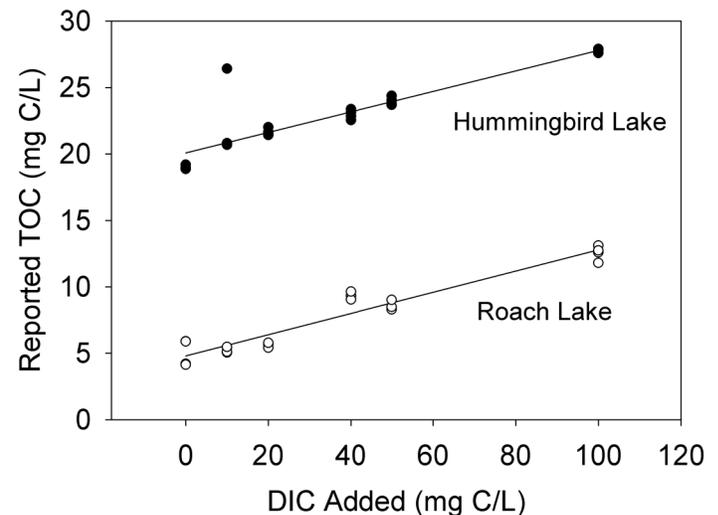


Fig. 4. Effect of DIC addition on TOC values reported for two lakes, Hummingbird and Roach) from northern Michigan, USA. Slopes (r^2) of Model 1 regressions are 0.077 (81%) and 0.08 (92%), respectively. Shown are three replicates for each level of DIC addition.

Slopes for the increase in TC measurement as a function of added DIC for all 12 water types ranged from 0.99 to 1.25 (mean = 1.069 ± 0.094 SD). Slopes for IC ranged from 0.94 to 1.05 (mean 0.96 ± 0.063) and, most importantly, were always lower than the corresponding TC slope (t-test $P < 0.001$). Thus, a unit addition of IC is reported by the instrument as a bigger increase in TC than was actually added. The net result is that as inorganic carbon content increases in a sample, calculated TOC (TC minus IC) also increases even without change in organic carbon.

We emphasize that our data do not show a problem with direct analysis of inorganic carbon with inorganic carbon standards, or the analysis of DOC by the NPOC (acidify and sparge) method. Analysis of DOC as NPOC is completely unaffected by added DIC, and variation in NPOC is not correlated with variation in DIC (data not shown). Insensitivity of NPOC to DIC has been observed repeatedly by analysts at both the Cary Institute and University of Hampshire, and is central to the success of this approach to measuring DOC in seawater samples (e.g., Sharp et al. 2002), which contain vastly more DIC than the values seen in our samples. We also confirmed that the sparging step (common to the TC and NPOC mode of analysis) quantitatively removes inorganic carbon. Inadequate IC removal would cause our observed pattern of increasing TOC with higher IC concentrations but we have found no evidence that this is occurring. Remeasurement of DIC in previously sparged samples consistently shows complete DIC removal.

Assessing the source of the difference between TOC and NPOC generated solely by additional DIC is not straightforward, given the mixed analysis of organic and inorganic carbon that occurs in the TC mode of instrument operation. The fundamental challenge is that the analysis of TC requires simultaneous measurement of both organic and inorganic forms of carbon with equal efficiency. Three factors (oxidation efficiency, delivery, and detection) might affect the accurate measurement of organic carbon in TC samples whereas only two (delivery and detection) would affect the accurate measurement of inorganic carbon. The standard curves prepared from potassium phthalate for use in both NPOC and TC analyses include the oxidation efficiency of the organic carbon. When the instrument estimates TC, this same aggregate efficiency is applied to the inorganic as well as the organic fractions, even though there is no possibility for inefficient oxidation of inorganic carbon. Therefore, in a mixture of organic and inorganic carbon, the total carbon will often be overestimated because an inappropriate adjustment for oxidation efficiency has been applied to the inorganic fraction. Samples with greater proportions of inorganic carbon in the mixture will result in greater overestimation.

Even small inefficiencies in TC oxidation and delivery to the detector can lead to significant overestimation under certain circumstances. For example, in a hypothetical sample of 45 mg/L IC and 5 mg/L OC and an overall measurement efficiency of 97.5%, TC will be overreported by 1.15 mg/L. Con-

sequently, the TOC fraction would be reported as 6.15 rather than the true 5 mg/L, a 23% overestimation of TOC. Thus, in the presence of high but not unrealistic concentrations of DIC, overreporting of TC is likely to cause an overestimate of TOC in absolute terms and relative to NPOC.

Discussion

Dissolved organic carbon in natural waters is an operationally defined entity made up of diverse and largely uncharacterized compounds (McKnight et al. 2003). A wide variety of approaches has been used to measure these compounds, but most commercially available analytical instruments typically include direct measurement of NPOC, as well as an attempt to capture "total" DOC using the TC minus IC approach. The total DOC or TOC analysis typically requires subtraction of an inorganic fraction (measured against an inorganic standard) from total carbon (organic and inorganic) measured against an organic standard. This approach is only appropriate for waters of relatively low DIC due to inherent limitations in measuring an analyte by difference and has been widely applied in freshwaters where DIC may be 80% of total carbon. In high DIC samples, the open acidifying/sparging technique is mandatory. Because these approaches do not yield equivalent concentrations of the organic fraction, lack of a clear statement of the analytical approach used makes it difficult to evaluate or compare many data sets reported in the literature.

For investigators interested in assessing the total dissolved organic matter of a water sample on a routine basis, no single method is entirely appropriate. The acidified sparging measurement excludes volatile organics, which may be of importance in freshwater and marine systems (Dachs et al. 2005). The measurement of total organic carbon by difference has inherent analytical difficulties associated with variability in measurement efficiency of DOC that may preclude accurate measurement of a mixed organic/inorganic carbon sample, as outlined in this paper.

Therefore, some uncertainty in absolute concentration of samples and variability across methods is to be expected, unless a common set of samples has been compared across analysts (*see* Peltzer et al. 1996). Analysts working with marine or other high DIC samples have little choice but to acidify and sparge before analysis and so will not be subject to the same problem. Differences between a TOC value and NPOC from analytical methods commonly used in laboratories for the analysis of freshwaters may be significant. These differences can affect results and thus could alter conclusions drawn from comparing data among studies. TOC, as routinely measured in freshwaters, appears sensitive to error, but NPOC undoubtedly underrecovers some (potentially important) compounds. Investigators should be clear in describing which analytical approach has been taken to ensure that appropriate and robust comparisons have been—and can be—made.

Given that DIC concentration influences reported values for TOC, we can estimate how large this effect is across sys-

tems, or over time within systems. However, we DO NOT propose that the approach shown here can be used as a simple or universal “correction” for the magnitude of the artifact. The difference between TOC (from TC-IC) and NPOC determined from our spike experiments was about 0.1 mg “aVOC” per mg DIC/L, based on the difference in mean slopes given above. If this quantity is subtracted from the estimates of ambient TOC in the multiple water bodies we analyzed, an “adjusted” TOC can be generated (Fig. 2 open circles). With this adjustment, points move closer to the 1:1 line but they do not evenly straddle the line, thus bias still exists. The actual quantities of “apparent” VOC range from 0 to 1.8 mg C/L. Before adjustment, the VOC estimates ranged from 10% to 57% of TOC and post-adjustment concentrations were 0% to 48% of TOC with the largest change being a decline from 35 to 0% for samples from the Walkill River.

The other component of the overestimation is the measurement efficiency and, with moderate to high values of DIC, even fairly small differences in efficiency may be significant. For example, in a sample where DIC is 75% of total carbon, an efficiency of 0.95 will lead to an overreporting of TOC by nearly 16%. This artifact can generate temporal trends in data series. For instance, if the measurement efficiency for the hypothetical example with a “true” OC content of 5 mg C/L declines from 95% to 90% over the course of a year, reported TOC would appear to increase from 5.8 to 6.7 mg C/L.

For actual data sets on DOC trends, artifacts may arise due to changes in DIC or changes in measurement efficiency. Below we calculate the magnitude of these changes required to cause the observed DOC trend in the Hudson River (Findlay 2005). TOC in the Hudson has been reported to have increased from ~3 to ~6 mg C/L over the past 16 years, although DOC measured as NPOC has remained unchanged (Findlay 2005). Given the relationship between TOC and added DIC determined in spike experiments on Hudson River water, an increase of 75 mg DIC/L would be necessary to account for the measured change in TOC. However, DIC in the Hudson has only actually increased by about 3.3 mg/L (Cole and Caraco unpubl. data), and this would explain less than 5% of the increase in TOC in the Hudson River. Therefore, for the Hudson and probably many other aquatic systems, very large changes in DIC would be required to cause a substantial “artifactual” change in TOC. The changes in DIC would be so great as to be hard to miss and would probably be detected well before there was evidence of a change in DOC concentration. Thus, it is unlikely an investigator would erroneously report a trend in DOC driven by increases in DIC.

We can also consider what change in measurement efficiency over time would be necessary to generate a 3 mg C/L trend for Hudson River water. For Hudson River water with a mean DIC of about 14 mg C/L, a decrease in efficiency from 100% to 70% would yield a 3 mg C/L increase. We examined the area counts reported for a 20 mgC/L phthalate standard for 5 dates from July 2002 to July 2006 and found a mean of

52,965 with a standard deviation of 3,399 or 6.4% of the mean and no directional trend. Given these observations of instrument performance a 30% drop in efficiency should be readily detected so long as the operator tracks instrument performance. Waters with a greater proportion of DIC would be more sensitive to shifts (either directional or simply random) in measurement efficiency. In these instances, more rigorous tracking of measurement efficiency through routine analysis of a known standard (preferably different from the calibration standard) or by actual infrared gas analysis (IRGA) response to a known concentration would be advisable.

The other pattern to consider when trying to identify the likelihood of erroneous trends is whether it is conceivable that actual trends in DIC could generate “artifactual” trends in DOC. There have been reports of increasing DIC concentrations in some river systems (e.g., Raymond and Cole 2003), and it seems likely that these within-system changes in DIC are too small to cause meaningful changes in measured organic carbon. For the Mississippi River, DIC has increased by ~4 mg C/L; this magnitude of increase would lead to a change of only 0.16 in estimated TOC.

Our analysis suggests that the greatest chance for incorrect conclusions due to “aVOC” is in cross-system comparisons where the range in DIC can exceed tens of mg C/L. The systems we studied were not selected to necessarily span the entire natural range in DIC for surface waters (roughly 100 mg C/L; Wetzel 2001), yet we found a 10-fold range in aVOC (0.1 to > 1 mg C/L) related to differences in ambient DIC (~1–30 mg DIC/L). Samples with a high proportion of TC as DIC will also be sensitive to even a few percentage points change in measurement efficiency. Thus, it may be advisable to employ an acidification/sparging step for the analysis of some high DIC freshwaters.

Recommendations

1. All papers reporting any form of DOC analysis should clearly indicate the analytical approach (NPOC, or TOC = TC-IC). The NPOC value generated from the instrument is often reported as a TOC measurement, but this designation is not always made explicit in the methods description of manuscripts or published papers.

2. A concerted, community-wide effort should be made to assess which historic data sets were measured directly following an acidification/sparging approach, and which were measured as the difference between TC and IC

3. We need a set of standards or at least common analytes to track measurement efficiency and variation among laboratories. The International Humic Substances Society has a Suwanee River humic standard (<http://ihss.gatech.edu/ihss2/>) that may be suitable as a common reference material although a true standard for a mixed pool of compounds may not be feasible. Peyton (1993) discusses oxidation of various organic compounds by persulfate in wet-chemistry analyses and when other solutes may interfere with oxidation efficiency.

References

- Burns, D. A., M. R. McHale, C. T. Driscoll, and K. Roy. 2006. Response of surface water chemistry to reduced levels of acid precipitation: A comparison of trends in two regions of New York. *Hydrol. Process.* 20:1611-1627 [doi:10.1002/hyp.5961].
- Dachs, J., and others. 2005. High atmosphere-ocean exchange of organic carbon in the NE subtropical Atlantic. *Geophys. Res. Lett.* 32(21) [doi:10.1029/2005GL023799]. Article nr L21807.
- Downing, J. A., and F. H. Rigler. 1984. A manual on methods for the assessment of secondary production in fresh waters, 2nd ed. Blackwell Scientific.
- Driscoll, C. T., K. M. Driscoll, K. M. Roy, and M. J. Mitchell. 2003. Chemical response of lakes in the Adirondack Region of New York to declines in acidic deposition. *Environ. Sci. Technol.* 37:2036-2042 [doi:10.1021/es020924h].
- Evans, C. D., D. T. Monteith, and D. M. Cooper. 2005. Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. *Environ. Pollut.* 137:55-71 [doi:10.1016/j.envpol.2004.12.031].
- , and others. 2008. Does elevated nitrogen deposition or ecosystem recovery from acidification drive increased dissolved organic carbon loss from upland soil? A review of evidence from field nitrogen addition experiments. *Biogeochemistry* 91:13-35 [doi:10.1007/s10533-008-9256-x].
- Findlay, S. E. G. 2003. Bacterial response to variation in dissolved organic matter, p. 363-382. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems – Interactivity of dissolved organic matter*. Academic Press.
- . 2005. Increased carbon transport in the Hudson River, NY—Unexpected consequence of nitrogen deposition? *Front. Ecol. Evol.* 3:133-137 [doi:10.1890/1540-9295(2005)003[0133:ICTITH]2.0.CO;2].
- Forsberg, C. 1992. Will an increased greenhouse impact in Fennoscandia give rise to more humic and colored lake? *Hydrobiologia* 229:51-58.
- Jones, J. B., E. H. Stanley, and P. J. Mulholland. 2003. Long-term decline in carbon dioxide supersaturation in rivers across the contiguous United States. *Geophys. Res. Lett.* 30(10):Art. No. 1495 [doi:10.1029/2003GL017056].
- McDowell, W. H., J. J. Cole, and C. T. Driscoll. 1987. Simplified version of the ampoule persulfate method for determination of dissolved organic carbon. *Can. J. Fish. Aquat. Sci.* 44:214-218.
- McKnight, D. M., E. Hood, and L. Klapper. 2003. Trace organic moieties of dissolved organic material in natural waters, p. 71-96. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems – Interactivity of dissolved organic matter*. Academic Press.
- Monteith, D. T., and others. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450:537-U9 [doi:10.1038/nature06316].
- Mulholland, P. J. 2003. Large-scale patterns in dissolved organic carbon concentration, flux, and sources, p. 139-160. *In* S. E. G. Findlay and R. L. Sinsabaugh [eds.], *Aquatic ecosystems—Interactivity of dissolved organic matter*. Academic Press.
- Orr, J. C., and others. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681-686 [doi:10.1038/nature04095].
- Peltzer, E. T., B. Fry, P. H. Doering, J. H. McKenna, B. Nonman, and U. L. Zweifel. 1996. A comparison of methods for the measurement of dissolved organic carbon in natural waters. *Mar. Chem.* 54:85-96.
- Peyton, G. R. 1993. The free-radical chemistry of persulfate-based total organic-carbon analyzers. *Mar. Chem.* 41:91-103.
- Raymond, P. A., and J. J. Cole. 2003. Increase in the export of alkalinity from North America's largest river. *Science* 301:88-91 [doi:10.1126/science.1083788].
- Sharp, J. H., C. A. Carlson, E. T. Peltzer, D. M. Castle-Ward, K. B. Savidge, and K. R. Rinker. 2002. Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. *Mar. Chem.* 77:39-253 [doi:10.1016/S0304-4203(02)00002-6].
- St-Jean, G. 2003. Automated quantitative and isotopic (C-13) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser. *Rapid Commun. Mass Spec.* 17:419-428 [doi:10.1002/rcm.926].
- Thurman, E. M. 1985. *Organic geochemistry of natural waters*. Kluwer Academic.
- Tranvik, L., and S. Bertilsson. 1999. Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecol. Lett.* 4:458-463 [doi:10.1046/j.1461-0248.2001.00245.x].
- Wetzel, R. G. 2001. *Limnology: Lake and river systems*, 3rd ed. Academic Press.
- Williams, S. D., W. J. Wolfe, and J. J. Farmer. 2006. Sampling strategies for volatile organic compounds at Three Karst Springs in Tennessee. *Ground Water Monit. Remed.* 26:53-62 [doi:10.1111/j.1745-6592.2006.00044.x].

Submitted 26 June 2009

Revised 17 February 2010

Accepted 16 March 2010