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Long-term storage of riverine dissolved organic carbon

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Abstract

The effect of long-term (2 years) storage on dissolved organic carbon concentrations of filtered river water was investigated. Replicate samples were stored at 3 temperatures 20°C, 4°C or -20°C, either acidified or without acidification. The effects of sample volume were tested by storing 4 ml or 20ml samples at each temperature and again acidified or non-acidified. Only frozen, acidified samples were stable for up to 1 year and none of the treatments were suitable for storing samples for 2 years. The volume of sample stored did not influence DOC measurements.

Introduction

Approximately 1% of terrestrial primary production is exported annually by rivers to the coastal ocean as dissolved organic carbon (DOC), and this DOC flux is closely correlated with river discharge (Harrison et al., 2005; Cole et al., 2007; Worrall and Burt, 2007). Over recent decades there is growing evidence of rising DOC concentrations in central and northern Europe and North American rivers and lakes (Freeman et al., 2001; Evans et al, 2005; Skjelkvåle et al., 2005; Gedney et al., 2006; Dawson et al., 2009; Hruška et al., 2009). The reasons for these increases are subject to debate but are linked to primary productivity changes in catchments as a result of elevated atmospheric CO_2 (Freeman et al., 2004), increasing mobility of DOC within soils (Evans et al., 2006), as well as organic carbon loss from top soils (Bellamy et al., 2005). Changes in DOC may also be explained by changes in deposition chemistry and/or sensitivity to catchment acidity (Monteith et al., 2007; Dawson et al., 2009; Hruška et al., 2009). Whatever the causes of such large-scale changes, they not only have implications to long-term regional carbon balance (Cole et al., 2007), but also for the dynamics and biogeochemical cycling of the dissolved organic matter (DOM) pool and inorganic nutrients during their transit from catchment to coast (Søndergaard and Thomas, 2003; Mattsson et al., 2009). Clearly reliable DOC measurements are crucial to this debate and the establishment of reliable long-term data sets.

The DOC pool is made up of both labile and refractory material. The refractory component can remain within aquatic systems for decades, hundreds or even thousands of years (Battin et al., 2008), whereas the labile material is degraded both photo-chemically and biologically over much shorter time periods of days or even hours (Wangersky, 1993; Battin et al., 2008). Given the potential for rapid change in labile DOC composition, even samples analyzed within hours of collection are potentially vulnerable to a loss of DOC, and reliable preservation methods are required to minimize losses. Ideally analysis of DOC samples should occur immediately after sample collection; however, during extended fieldwork campaigns or seasonal sampling programs this is not always possible. For these and other reasons storage conditions that will reliably preserve samples for longer than a few months is vital. Although there has been a considerable effort to refine the analytical methods associated with DOC analysis over the past 20

years, to our knowledge only 3 publications deal with the key issue of long-term storage of DOC samples up to 5 months studies (Sugimura and Suzuki, 1988; Peltzer and Brewer, 1994; Tupas et al., 1994) and these were within seawater samples. Here we report a brief study that addressed the issue of long-term storage (up to 2 years) of DOC samples, and investigated the merits of acid preservation *vs* non-acidified storage, under ambient laboratory temperatures, cold storage and freezing temperatures. We also compared the relative merits of different sample volumes for the suitability of long-term storage.

Materials and Procedures

Sampling was conducted on 30 January 2008 in the River Conwy, North Wales. Samples were collected by immersing a 2L acid cleaned high density polyethylene bottle approximately 20 cm below the water surface, after rinsing the bottle 3 times with sample water. The samples were returned to the laboratory within 0.5 h.

All samples were filtered directly into either 4 mL borosilicate vials or 20 mL ampoules (both pre-combusted at 500°C, 3 hours) through disposable syringe filters (Whatman® GD/X, pore size 0.45 µm) using an acid-cleaned plastic syringe. It is realised that with a pore size of 0.45 µm these filters do not exclude all bacteria, but routinely DOC concentrations in the literature are reported from filtrations using pre-combusted glass fibre filters (typically Whatman®GF/F) of effective pore sizes greater than 0.6 µm (Tipping et al., 1988; Curtis and Adams, 1995; Hessen et al., 1997; Schindler et al., 1997; Gergel et al., 1999; Pace and Cole, 2002; Pastor et al., 2003; Xenopoulis et al., 2003; Spencer et al., 2007) and so it was realistic to work with such samples treated in this manner. Additionally the contribution of sub-micrometre (< 1 µm) particles to DOC has been estimated to be only approximately 10% (Isao et al., 1990, and references therein).

Replicate samples were prepared for DOC analysis (completed within 4 h of collection): 5 replicates were acidified with ultrapure HP_3O_4, and 5 with of 37% HCl. The DOC concentrations were not significantly different at 422 ± 3 µmol L^{-1} and 419 ± 3 µmol L^{-1} respectively. Therefore in subsequent acidification of samples within this study HP_3O_4 was used.

Two set of samples were prepared: The first was acidified to a pH of 2.0 and the second was left non-acidified. The vials were then sealed with Teflon-lined screw caps, and the ampoules flame sealed.

Replicate acidified and non-acidified vials and ampoules were then stored in the dark at the following 3 storage temperatures:

1) Ambient laboratory (20 ± 5 °C)
2) Cold storage (4 ± 2 °C),
3) Frozen (-20 ± 2 °C).

In the case of the ambient samples only acidified samples were stored. N.B. all sub-sampling was completed within 3 hours of collection

Two subsequent sets of analyses were completed in January 2009 and January 2010. The samples stored in vials were directly used (after shaking) in the auto-sampler of the DOC analyser. The ampoules were thoroughly mixed before opening, and a sub-sample removed from each one and transferred to pre-combusted 4 mL vials. All non-acidified samples were acidified with HP_3O_4 prior to DOC analyses.

DOC was measured using high temperature combustion on an MQ 1001 TOC Analyser (Qian and Mopper, 1996). Certified reference material (CRM) of deep Florida Strait water and low carbon water (5–10 µmol L^{-1}) from the Hansell Laboratory, University of Miami, Rosenstiel School of Marine and Atmospheric Science are run on the machine daily during routine operation. Three batches of CRM were used during this experimental period with certified DOC concentrations of 47-48 µmol, 41-44 µmol and 41-43 µmol L^{-1}. The respective DOC concentrations measured from

these standards batches were 48 ±3 µmol L^{-1} (n=92), 44 ±6 µmol L^{-1}, (n=64) and 44 ±4 µmol L^{-1} (n=52). The detection limit of the instrument was 10 µmol L^{-1}.

Discussion

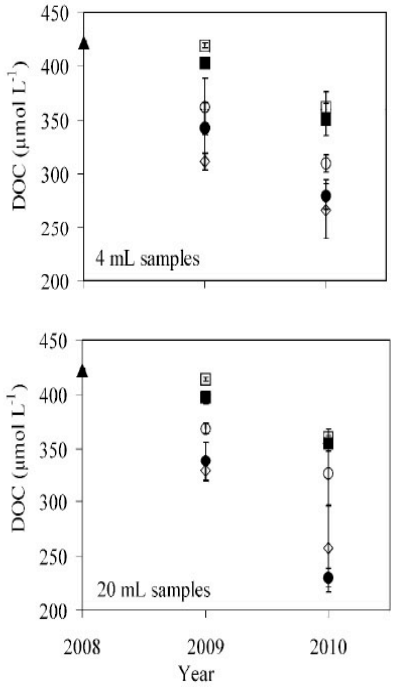

Figure 1 Changes in DOC concentration during a 2 year storage experiment using samples stored in 4 mL vials or 20 mL ampoules, and acidified with HP_3O_4 or left non-acidified. Samples were stored at 20 ± 5 °C (◊), 4 ± 2 °C, acidified (○), non-acidified (●), or -20 ± 2 °C, acidified (□), non-acidified (■). t = 0 (▲) was the initial measured DOC concentration of 422 ± 3 µmol L^{-1} (n=5).

After 1 year of storage the only samples that did not show a significant decrease in DOC concentration compared to t=0 samples were the frozen acidified samples at -20°C (Fig. 1). These losses were 1 and 2% in both the acidified vials and ampoules, respectively, and were not significantly different (tested with a using 2 sample T-Test) from the t=0 DOC concentration. There were decreases in DOC concentrations of 5 and 6% in the frozen non-acidified vials and ampoules, and although small these were statistically significantly different ($p = < 0.001$ in both cases) from the initial measured concentrations (Fig. 1). All other samples stored at ambient or 4°C exhibited too great a loss of DOC to be considered further.

After two years of storage there were significant decreases in DOC concentration in all frozen samples, regardless of whether acid had been added or not. Although these losses were not as great as those observed in the ambient and cold stored samples, the average losses were 14 and 15% in the acidified vials and ampoules, respectively, and 17 and 16% in the non-acidified vials and ampoules, respectively (Fig 1). The fact that this loss happened in both acidified and non-acidified samples would suggest that these losses are not due to bacteria activity since at pH 2 it seems highly unlikely that acidophilic bacteria would be active considering the sample water and low temperatures.

Sugimura and Suzuki (1988) reported a 15 to 20% decrease in DOC concentration in frozen acidified and non-acidified seawater samples over 7 days. Much of this loss occurred during the first hour of storage, after which the DOC concentrations remained relatively constant. In contrast, Tupas et al. (1994) analysed frozen, non-acidified samples up to 5 months after collection and reported that the DOC concentrations measured were not significantly different from those measured in the initial samples. Wangersky (1993) suggests that the loss of DOC observed by Sugimura and Suzuki (1988) may have occurred during sample preparation and freezing, indicating that rapid preservation is required to minimize this effect. The small (5 to 6%) but significant decrease in DOC concentration measured after one year in non-acidified, frozen samples in this study indicates that, as demonstrated by Tupas et al. (1994), that samples may remain stable for a few months, but thereafter reliability of results may become questionable.

There were no significant differences in DOC concentration between samples stored in

either 4 ml vials or 20 ml ampoules, suggesting that the storage sample volume should be chosen primarily to minimize the risk of contamination. These results are in contrast to those of Tupas et al. (1994) who reported consistently elevated DOC concentrations in samples (frozen) stored in 2 mL ampoules compared to those stored in 10 mL ampoules. They concluded that despite both sets of ampoule being flame sealed, the sealing may have contaminated the 2 mL samples.

This study has shown that long-term storage of riverine DOC samples (up to 1 year) is possible, but we recommend that samples are filtered and acidified prior to storage at -20°C, and that these procedures are carried out as soon as possible after sample collection to minimize the loss of labile DOC.

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