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Microbial use of low molecular weight DOM in filtered and unfiltered freshwater: Role of ultra-small microorganisms and implications for water quality monitoring

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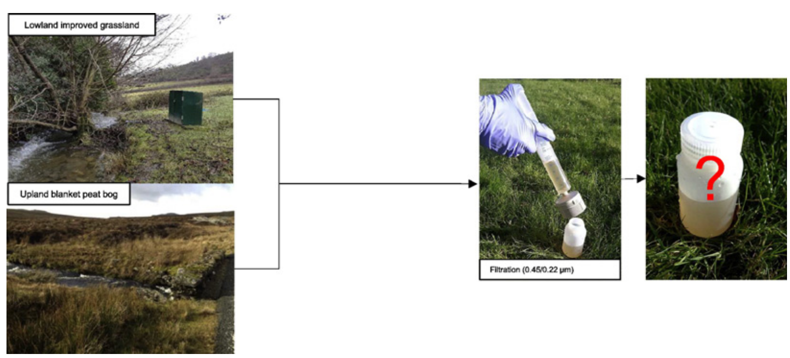
HIGHLIGHTS

- DOM cycling is important, but poorly understood, in freshwater systems.
- Current sampling methods for DOM characterisation are insufficient.
- Filtration did not halt nutrient depletion, particularly at high temperatures.
- Evidence suggests that ultra-small microorganisms were involved in DOM cycling.
- The role of ultra-small microorganisms in sample integrity should be considered.

GRAPHICAL ABSTRACT



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ABSTRACT

Dissolved organic matter (DOM) plays a central role in regulating productivity and nutrient cycling in freshwaters. It is therefore vital that we can representatively sample and preserve DOM in freshwaters for subsequent analysis. Here we investigated the effect of filtration, temperature (5 and 25 °C) and acidification (HCl) on the persistence of low molecular weight (MW) dissolved organic carbon (DOC), nitrogen (DON) and orthophosphate in oligotrophic and eutrophic freshwater environments. Our results showed the rapid loss of isotopically-labelled glucose and amino acids from both filtered (0.22 and 0.45 μm) and unfiltered waters. We ascribe this substrate depletion in filtered samples to the activity of ultra-small (<0.45 μm) microorganisms (bacteria and archaea) present in the water. As expected, the rate of C, N and P loss was much greater at higher temperatures and was repressed by the addition of HCl. Based on our results and an evaluation of the protocols used in recently published studies, we conclude that current techniques used to sample water for low MW DOM characterisation are frequently inadequate and lack proper validation. In contrast to the high degree of analytical precision and rigorous statistical analysis of most studies, we argue that insufficient consideration is still given to the presence of ultra-small microorganisms and potential changes that can occur in the low MW fraction of DOM prior to analysis.

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1. Introduction

Dissolved organic matter (DOM) represents a key source of nutrients and energy for plants and microorganisms living in pristine low nutrient status waters (Gardner et al., 1989; Lindell et al., 1996; Bernot et al., 2010; Durand et al., 2011; Stutter and Cains, 2015). Conversely, DOM can also be seen as undesirable in freshwaters due to its potential to make pollutants more bioavailable, its ability to affect the hormone balance of freshwater organisms, its ability to generate significant reductions in dissolved oxygen concentrations owing to its uptake by microbial populations, and its potential to lead to the formation of carcinogens during chlorination of drinking water (Steinberg et al., 2008; Durand et al., 2011; McIntyre and Gueguen, 2013). Understanding the origin, behaviour and fate of DOM in aquatic ecosystems is therefore important for predicting how it will influence primary productivity and overall water quality. It is clear from recent studies that DOM is composed of thousands of individual compounds which can be biologically processed within the river network leading to significant changes in the quality and quantity of DOM during passage from catchment to coast (Battin et al., 2003; Lusk and Toor, 2016). While some high molecular weight (MW) compounds (>1000 daltons (Da); Kujawinski, 2011) may be relatively recalcitrant to microbial breakdown, some low MW compounds are highly labile, making representative sampling difficult due to potential losses during transport and storage prior to analysis.

DOM is operationally defined as C-containing compounds that can pass through a 0.45 μm filter (Thurman, 1985; Nimptsch et al., 2014), this limit being historically linked to the microbiological standard for drinking water (Goetz and Tsuneishi, 1951). This filtering process is designed to remove microorganisms and organic debris from the sample, although the passage of nano-particulate DOM is inevitable. It is now well established, however, that freshwaters contain a range of ultra-small organisms (e.g. viruses, bacteria, archaea) which can also readily pass through a 0.45 μm apertures (Fig. 1; Comolli et al., 2009; Maranger and Bird, 1995). While viruses can be considered to be biologically inert from a DOM standpoint, the remaining ultra-small bacteria and archaea are thought to be physiologically active in a planktonic state (Baker et al., 2010; Luef et al., 2015). Currently, the ecological significance of these nano-organisms in nutrient cycling and DOM processing in natural freshwaters remains unknown. In addition, they also have the potential to compromise the quality of DOM in filtered samples destined for laboratory analysis.

One of the main approaches for assessing DOM concentrations in water is via manual grab sampling, during which samples are 0.45 μm filtered in situ or ex situ prior to storage in pre-washed bottles. Alternatively, automatic sampling systems may be employed to reduce the amount of time and resources required (Cassidy and Jordan, 2011). However, automatically collected samples present challenges as they are not filtered after collection and are rarely recovered from site on a daily basis; therefore samples may be subject to significant periods of storage during which DOM biodegradation can occur. In addition, the samples may be exposed to higher temperatures than those of the river, potentially increasing the rate of microbial activity and loss or transformation of DOM (Ahad et al., 2006; Johnston et al., 2009). Although preservatives can be used to minimise nutrient transformations, these may interfere with subsequent metabolomics, biochemical and microbiological analysis and are frequently not used (Ferguson, 1994; Kotlash and Chessman, 1998).

The three most commonly measured macronutrients that contribute to the molecular structure of DOM, and are key water quality parameters are C, N and P. Although the exact composition of all the dissolved organic C, N and P compounds in the aquatic environment is largely undefined, DOM can be divided into a high and low MW DOM fraction. The low MW DOM (<1000 Da) fraction includes a wide range of common metabolites in either a monomer or oligomer form (e.g. amino acids, peptides, sugars, organic acids; Helms et al., 2008). As these compounds may be typically present at very low concentrations (<500 nM), particularly in low nutrient-status waters, their significance is frequently overlooked relative to the more stable high MW humic DOM fraction (Kujawinski, 2011). However, when their rapid rate of formation and turnover are considered, the overall flux of low MW DOM through the aquatic biota may be significant (Meon and Amon, 2004). As these compounds are likely to have a quick rate of turnover in the aquatic environment, their detection can be challenging especially in non-sterile samples. The aim of this study was therefore to: (1) compare the rate of microbial uptake of three low MW DOM components over time in unfiltered (whole microbial community) and filtered (ultra-small microbial community) water samples; (2) determine the impact of temperature on the microbial utilization of low MW DOM; and (3) establish whether sample acidification provides an effective preservative for low MW DOM. The results of the study will be used to evaluate the significance of ultra-small microorganisms in low MW DOM turnover and also to devise potential strategies to representatively sample this DOM fraction.

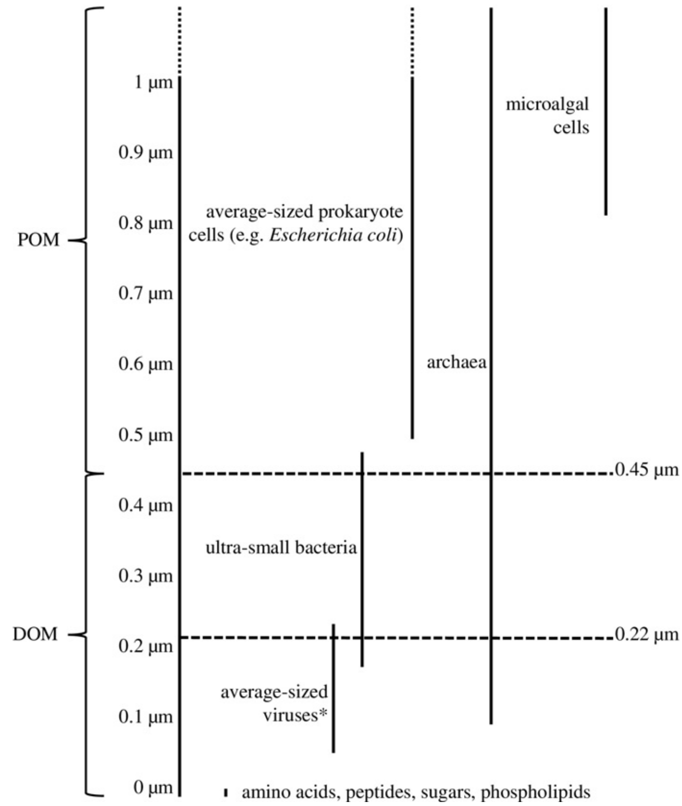

Fig. 1. Relative size of dissolved organic matter (DOM) and particulate organic matter (POM) components in comparison to bacteria, archaea and viruses. POM > 0.45 μm > DOM. 0.45/0.22 μm filter cut-offs indicated by a dashed line. * Some giant viruses > 1 μm exist.

2. Materials and methods

2.1. Field site and sampling

Samples were collected from two contrasting sub-catchments within the Conwy catchment, North Wales (Fig. 2; Supplementary Fig. S2). The Hiraethlyn sub-catchment is an area of primarily lowland improved grassland used predominantly for agricultural livestock production (Cooper et al., 2014; Jones et al., 2016). It has an average elevation of 56 m a.s.l., an annual air temperature of 8.57 ± 0.04 °C and an annual rainfall of up to 1000 mm (Emmett et al., 2016). The Migneint sub-catchment is an area of upland blanket peat bog supporting acid heathland vegetation and low intensity sheep production. It has an