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Bioavailability and size-fraction of dissolved organic carbon, nitrogen, and sulfur at the Arbutus Lake watershed, Adirondack Mountains, NY

Phil-Goo Kang · Myron J. Mitchell

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Abstract The heterogeneity of DOM is closely linked with the various sources and the diversity of biogeochemical processes. We studied the spatial and temporal patterns of the quantity (bioavailable [B-] and refractory concentrations by laboratory incubations) and quality ($\delta^{13}\text{C}$, aromaticity, and size-fraction by ultrafiltration [low molecular weight (LMW) < 1 kDa and high molecular weight (HMW) > 1 kDa]) of dissolved organic C (DOC), N (DON), and S (DOS) for surface waters (two upland streams, two wetland-affected streams, and lake outlet) over a 14-month period within the Arbutus Lake watershed in the Adirondack Mountains of New York State, USA. The % BDOC and % BDON of this watershed averages ranged from 6 to 18 % and from 12 to 43 %, respectively. The DOC and DON concentrations increased as water was transported through wetland areas of the Arbutus Lake watershed. DOC and DON constituents in the surface waters passing through a wetland were composed mostly of refractory HMW components (% HMWDOC: 55 %, % HMWDON: 60 %) with a higher level of aromaticity compared to upland streams (% HMWDOC:

approximately 35 %, % HMWDON: approximately 30 %). DOS was dominated by the refractory (% BDOS range 6–13 %) and LMW (% LMWDOS range 62–96 %) form and we suggest that bacterial dissimilatory sulfate reduction might play an important role in generating this distinct DOS biogeochemistry. The aromaticity was positively related to total DOC concentration, but negatively to % BDOC. Arbutus Lake DOM was dominated by bioavailable and LMW characteristics, compared to wetland-affected streams where refractory and HMW DOM fractions were more prevalent. Our study suggests the different variability of DOM characteristics among elements (C, N, S) using a “bioavailability-molecular size model” showing a diagenetic perspective due to the relative refractoriness of the LMW DOS. This study also highlights the importance of multiple approaches for understanding DOM biogeochemistry with respect to molecular size, bioavailability, aromaticity, stoichiometry, isotopic values, and elemental concentrations.

Keywords DOC · DON · DOS · Stable C isotope · Aromaticity (SUVA) · HMW vs. LMW · Bioavailability-molecular size model

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Introduction

DOM is a heterogeneous mixture that is derived from various sources and transformed by many biogeochemical

processes (Aitkenhead-Peterson et al. 2003; Bertilsson and Jones 2003). The bioavailability of DOM plays a critical role in understanding microbial activity and the cycling of nutrients (Findlay and Sinsabaugh 2003; Wetzel 2001). DOM bioavailability in aquatic ecosystems has been shown to play a central role in ecosystem function and structure serving as an important component of the microbial loop (Sinsabaugh and Foreman 2003). Elemental components of DOM, such as dissolved organic carbon (DOC), nitrogen (DON), and sulfur (DOS), each have unique biogeochemical roles. DOC is a major element fraction of DOM and often used to understand overall DOM biogeochemistry (i.e., Aitkenhead-Peterson et al. 2003). DON is linked to proteinaceous material derived from terrestrial and aquatic production and DOS may also contain a substantial amount of protein, but information on this DOM fraction is much less than that for DOC and DON (Schlegel, 1985; Abbt-Braun and Jahnel, 2001).

The introduction of a size-reactivity model with a diagenetic perspective by Amon and Benner (1996) shed light on the importance of high molecular weight (HMW) DOC as a bioavailable source and simultaneously gave an opportunity for extending the perspective on the role of DOM bioavailability. Previously the model of Saunders (1976) was widely accepted for describing DOM bioavailability and suggested that the degree of the recalcitrance of DOM to microbial breakdown increased as the proportion humic substances, polymeric molecules and higher molecular size increased (Cummins et al. 1972; Thurman 1985; Kaplan and Newbold 2003). Most of the studies of bioavailability versus molecular size of DOM have focused on DOC, but other dissolved organic elements such as those of N and S have limited information in spite of their ecological importance.

The bioavailability of DOM can be measured by bioassay experiments, which can be done under a wide range of experimental conditions including differences in incubation conditions (i.e., temperature and duration), whether the microbial assemblage includes only the natural community or also has microbial additions, and nutrient regimes ranging from sample background to the addition of nutrients (Benner 2003; McDowell et al. 2006). Although there are no standard protocols to date, the bioassay experiment may be a useful approach for estimating the bioavailable DOM (Benner 2003). In addition to evaluating bioavailability, various indices have been used to understand the heterogeneity of DOM including stoichiometry (C:N ratio; Qualls and

Haines 1991; 1992), SUVA (specific ultra violet absorbance at 254 nm; Weishaar et al. 2003), stable C isotopic values (Schiff et al. 1990) and size-fractions (including the size separation using tangential-flow ultrafiltration with 1 kDa cutoff membrane; Benner 2003). Each of these measurements provides some insight on DOM characteristics, but using a combination of these measurements provides further information on the physical and chemical attributes of DOM (i.e., Fellman et al. 2008; Inamdar et al. 2011).

The Arbutus Lake watershed in the Adirondack Mountains of New York State has been the site of a wide range of hydrobiogeochemical studies including some evaluations of DOC and DON that have focused on the mass budgets of both DOC (Piatek et al. 2009) and DON (McHale et al. 2000; Mitchell et al. 2001; Park et al. 2003). Previous studies have also shown how changes in hydrological conditions (Inamdar et al. 2004; Park et al. 2005; Mitchell et al. 2001) affect the DOC and DON dynamics within this watershed. In addition, Ito et al. (2005) found substantial differences in the relationships between DOC and DON concentrations for 52 lake/watersheds in the Adirondacks including the Arbutus lake/watershed. There have been some studies of S biogeochemistry (Campbell et al. 2006; Mitchell et al. 2006; 2008) that suggested the importance of bacterial dissimilatory sulfate reduction (BSR), but none of these earlier studies analyzed DOS although other studies have suggested BSR can contribute to DOS production (Giesler et al. 2009; Werne et al. 2004).

Our current study focused on quantifying the characteristics of DOC, DON, and DOS using a range of approaches for determining bioavailable and refractory concentrations, size-fractionated concentrations with 1 kDa cutoff, stoichiometry, stable C isotopic ($\delta^{13}\text{C}$) values, and SUVA measurements. Our objectives were to evaluate spatial and temporal changes of DOM quantity and quality focusing on how the patterns of DOC, DON and DOS concentrations and their characteristics vary over space and time within the Arbutus watershed. This information should provide insight on what features in the watershed are linked to these patterns of DOC, DON and DOS.

Site description

The Arbutus Lake watershed (43°58'48"N, 74°13'48"W) is located within the Huntington Wildlife Forest in the

Adirondack Mountains of New York State. The Arbutus Lake watershed has an area of 352 ha, the elevations range from 513 to 748 m (Park et al. 2005). The annual temperature averaged 4.8 °C and total annual precipitation averaged 1,080 mm from 1981 to 2000 (Park et al. 2005). The Archer Creek subcatchment (135 ha) represents the major inlet of water (45 %) to Arbutus Lake (McHale et al. 2000) (Fig. 1). The overstory vegetation in the upper slopes consists of mixed northern hardwoods including American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), yellow birch (*Betula alleghaniensis*), and white pine (*Pinus strobus*). Lower slopes close to the lake are often dominated by conifer stands and include eastern hemlock (*Tsuga canadensis*), red spruce (*Picea rubens*), and balsam fir (*Abies balsamea*) (Park et al. 2005). Soils consist of primarily spodosols of the Becket-Mundal series (coarse-loamy, mixed, frigid, Typic Haplorthods) (de Warrd and Walton, 1967; Campbell et al. 2006). The surficial geology consists of thin to medium deposits of glacial till with a high sand content; the bedrock geology is largely composed of igneous rocks with some calcium-rich minerals (Christopher et al. 2008). Two upland subcatchments (S14 and S15) in the Archer Creek catchment that are almost adjacent and have similar topographical features, but different soil and vegetation characteristics (Christopher et al. 2006) were also evaluated in our study. The S14 subcatchment has higher soil Ca^{2+} , NO_3^- , and pH levels than the S15 subcatchment. The S14 subcatchment also contains a relatively higher basal area of base-rich indicator tree species [e.g., sugar maple, American basswood (*Tilia americana*), eastern hophornbeam (*Ostrya virginiana*)] than S15. The stream chemistry of these two subcatchments reflects the difference of the soil chemistry, i.e., S14 having greater pH and higher concentrations of Ca^{2+} and NO_3^- than S15. Christopher et al. (2008) suggested that these upland streams were dominated by deep and shallow groundwaters. Within the Archer Creek Catchment there are a series of wetlands, including a palustrine peatland [Greenwood Mucky peats: Dysic, frigid Typic Haplohemists] that covers 4 % of the Archer Creek catchment (Bischoff et al. 2001). Arbutus Lake has a surface area of 50 ha, an average depth of 3.0 m and a maximum depth of 8.4 m (Driscoll and van Dreaseon 1993). The lake has been classified as a medium till drainage lake (Newton and Driscoll 1990) with a lake trophic status between

oligotrophic and mesotrophic (Owen et al. 1999). The lake also has been known to be a relatively insensitive to acidic changes compared to some other surface water in the Adirondack and Catskill regions, NY (Chen et al. 2004).

Materials and methods

Sampling was performed 12 times over a 14-month period for five surface water (S14, S15, wetland, inlet, and outlet) locations (Fig. 1). Surface waters were collected at the outlets of the S14 and S15 subcatchments, the wetland, and the inlet (also known as the outlet of the Archer Creek catchment) and outlet of Arbutus Lake. Water samples were collected in polyethylene bottles that had been precleaned with ultraclean deionized water kept on ice when transported to the Biogeochemistry Laboratory in State University of New York-College of Environmental Science and Forestry (SUNY-ESF) in Syracuse, NY. Samples were stored in the dark at 4 °C until further analyses for a maximum of three days. Sampling events and the discharge at the inlet and the outlet are presented in Fig. 2.

Incubation for bioavailability of DOM

We refer to bioavailable DOM as the DOM utilized by heterotrophic bacteria through uptake of C, N, and S to obtain energy (Benner 2003; Tranvik 1988a, b), and the extent of the uptake (shown as % bioavailability of DOM) may represent a turnover rate of each element (Kirchman 2003) under our experimental condition. Samples were filtered through a precombusted GF/F filter (a nominal pore size of 0.7 μm , Whatman®) at 550 °C for 2 h. The sample filtrates (100 mL each) were poured into a 120 mL precombusted (550 °C, 2 h) glass bottle closed with aluminum foil, covered with acid-washed caps coated with Teflon and incubated in duplicate at 20 °C for 30 days in the dark to investigate the changes in the bioavailable (labile) and refractory DOM concentrations of DOC, DON, and DOS constituents (i.e., Hopkinson et al. 2002; McDowell 2003). The incubated bottles were shaken daily two–three times. The DOM concentration of samples incubated for 30 days was operationally defined as the refractory (R-) DOM and the difference between the initial (0-day) and the 30-day DOM concentrations was defined the bioavailable (B-) DOM. For example, for C total DOC

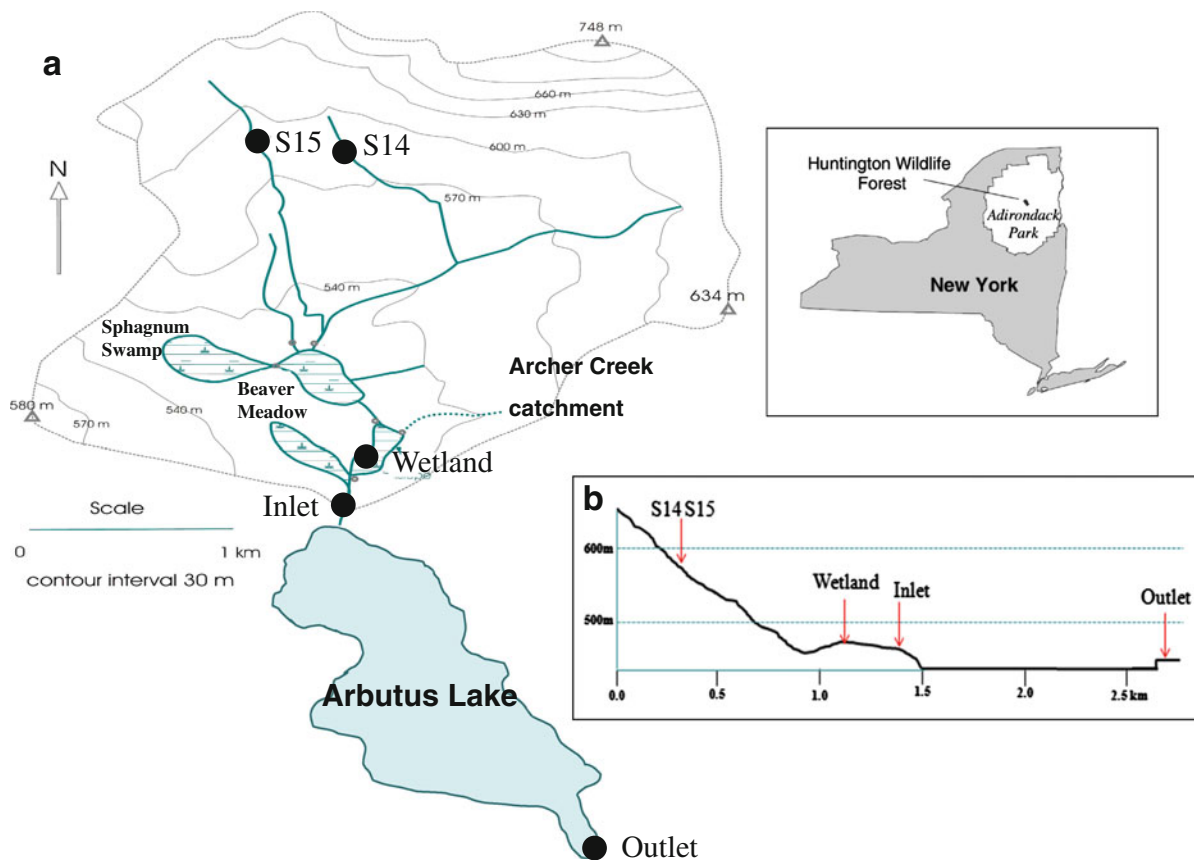


Fig. 1 Site map (a) and profile (b) showing the location of groundwaters and surface waters in the Arbutus Lake watershed, Huntington Wildlife Forest, New York

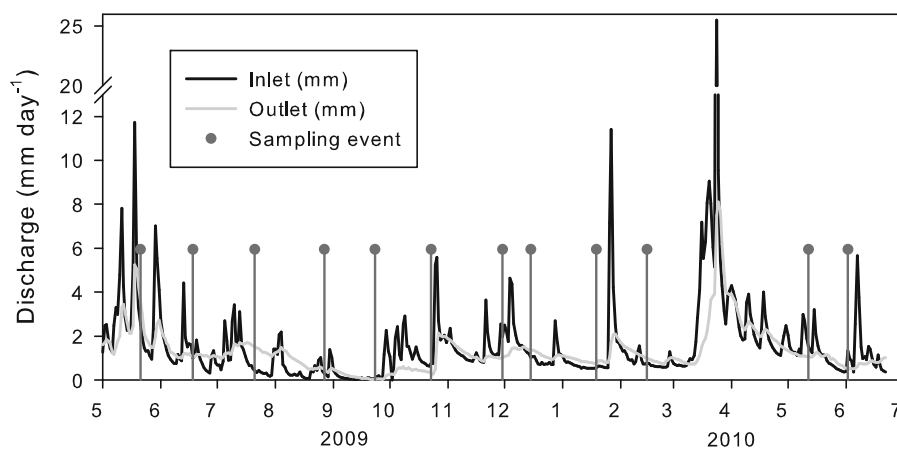


Fig. 2 Daily discharge at the inlet and outlet sites and sampling events ($n = 12$) at the Arbutus Lake watershed from May 2009 to June 2010

concentration (TDOC) was the sum of bioavailable DOC concentration (BDOC) and refractory DOC concentration (RDOC). All of the 30-day incubated

samples were kept in an incubator at 4 °C until chemical analyses and all analyses were completed within three months. No bacterial inoculum was used, but natural

microbial assemblages were allowed to grow in the incubator vessels because most of the natural bacterial community would be able to pass through the GF/F filter used in our determinations (Benner 2003; del Giorgio and Davis 2003; Tranvik 1988a). We assumed that BDOM was the amount of the DOM utilized for microbial uptake. Oxygen depletion during the incubation could affect the results of our study. However, we monitored nitrate concentration as a measure of the decrease in alternate electron acceptors associated with oxygen depletion and found no significant change in nitrate concentration. DOC decomposition during incubation might contribute to increased CO₂ concentrations in the solution and decreasing pH values in the bottle. We did not measure pH values before and after the incubation, but incubation studies have not reported adverse effects of increasing CO₂ concentration on bacterial growth in the case of either using a bacterial inoculum and over much longer incubation periods than used in our study (e.g., Benner 2003; del Giorgio and Davis 2003; Hopkinson et al. 2002; McDowell et al. 2006; Tranvik 1988a, b).

Size fractionation of DOM

For determining size fractionations, water samples were filtered through a precombusted GF/F filter (Whatman®). The filtrate was then passed using a tangential flow ultrafiltration with a 1 kDa cutoff (Millipore Pellicon 2® cassette filter). High-molecular-weight (HMW) was defined as DOM > 1 kDa (operationally defined as the material retained using tangential flow ultrafiltration), and low-molecular-weight (LMW) defined as < 1 kDa (operationally defined as the material permeated) (Giesler et al. 2009). For further chemical analyses, HMW materials with both a relative volumes <300 mL and a high concentration compared to an original sample were diluted by clean deionized water (10×). In order to check the hydraulic recovery rate $(=(\text{HMW} + \text{LMW})/\text{Initial})$ during the size-fraction experiment, we measured water volumes of the total, the HMW and the LMW and the hydraulic recovery rate averaged 99 %.

Chemical analyses and SUVA

The Biogeochemistry Laboratory (SUNY-ESF), which is a participant in the USGS QA/QC program, analyzed

various solutes for the current study. DOC was analyzed via UV oxidation and sodium persulfate using the Tekmar–Dohrmann Phoenix 8000 TOC® analyzer (detection limit, DL: 0.1 μmol L⁻¹) and analytical precision (AP) during the experiment period was ±2.6 % of a CV. Ion chromatography using a Dionex IC® was utilized to measure nitrate (NO₃⁻) and sulfate (SO₄²⁻) with AP of ±3.9 % (DL 1.1 μmol L⁻¹) and ±2.0 % (DL 0.25 μmol L⁻¹), respectively. Ammonium (NH₄⁺) was measured with an autoanalyzer using the Berthelot Reaction followed by colorimetric analysis (DL 1.4 μmol L⁻¹) and the AP was ±3.2 %. Total dissolved nitrogen (TDN) concentration was obtained using the persulfate oxidation procedure followed by the colorimetric analysis on the autoanalyzer (DL 1.4 μmol L⁻¹; AP: ±4.2 %). Total dissolved sulfur (TDS) was analyzed using the inductively coupled plasma spectrometry (model: Perkin Elmer Optima 3300DV) and the AP was ±3.9 %. DON and DOS were determined by subtracting inorganic N and S (DIN–NH₄⁺ and NO₃⁻; DIS–SO₄²⁻) from total dissolved N and S (TDN, TDS), respectively.

The calculated errors for DON and DOS concentrations were ±6.6 % (square root of the sum of the squared analytical precision of TDN, NH₄⁺ and NO₃⁻) and ±4.4 % (square root of the sum of the squared analytical precision of TDS and SO₄²⁻) (Fellman et al. 2008). Units of concentrations and elemental ratios were given on a molar basis. The relative aromaticity of DOM was provided by SUVA (specific UV absorbance, unit: L mg C⁻¹ m⁻¹) (Weishaar et al. 2003), which was calculated by dividing the UV absorbance at 254 nm (cm⁻¹) by the concentration of DOC (mg C L⁻¹) (Hood et al. 2005).

Isotopic analyses

Filtrates were freeze-dried, and then sent to the Isotope Science Laboratory at the University of Calgary, Alberta, Canada where stable C isotopic ratios (δ¹³C values) of DOC in filtrate, HMW and LMW were measured using a Costech ECS 4010 elemental analyzer coupled to a Thermo-Finnigan Delta XL Plus® isotopic ratio mass spectrometer (IRMS). The freeze-dried materials were weighed into tin cups, and then acidified with dilute HCL for 48 h in order to remove inorganic C in an acid-fume desiccator, which can affect overall stable C isotopic values of samples (Teece and Fogel 2004). The isotope ratios were

expressed in the conventional ‘delta (δ) notation’ in per mil (‰):

$$\delta^{13}\text{C} (\text{‰}) = \left[(R_{\text{sample}}/R_{\text{standard}}) - 1 \right] \times 10^3$$

where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ ratios of the sample and the standard (Vienna Pee Dee Belemnite), respectively. Accuracy and precision of measurement was independently verified using United States Geological Survey (USGS) 40 L-glutamic acid ($\delta^{13}\text{C} = -26.4 \pm 0.1$ SD ‰ [$n = 30$]) and USGS 41 L-glutamic acid ($\delta^{13}\text{C} = +37.6 \pm 0.2$ SD ‰ [$n = 30$]). Daily precision of the instrument was verified by repeated analyses of internal laboratory standards using caffeine (Sigma-Aldrich®) (-37.0 ± 0.2 SD ‰ [$n = 412$]), which were analyzed every six samples during the sample runs.

Statistical analyses

We used a one-way analysis of variance (ANOVA) in conjunction with either Dunnett’s comparisons to compare average stoichiometry (C:N, C:S and N:S), SUVA, and stable C isotope values of the total form (control) with other forms, i.e., labile, refractory, HMW, and LMW forms, of each site (family error rate = 0.05) or Tukey’s pairwise difference test to elucidate average DOM concentration, SUVA values, and stable C isotope ratios of each form across sites at $\alpha = 0.05$ level using Minitab® (Version 16, Minitab, Inc.). Pearson correlation between various forms of DOM concentrations was analyzed for significance levels of $p < 0.05$ using Minitab® (Version 16, Minitab, Inc.). Also, linear regression models were used to analyze relationships among DOM concentrations in various forms, SUVA, and stable C isotope ratios at $\alpha = 0.05$ level using SigmaPlot (Version 11.0, Systat Software, Inc.). The analysis of covariance (ANCOVA) was used to compare the slopes of linear regression lines based on $\alpha = 0.05$ using Minitab (Version 16, Minitab, Inc.).

Results

DOM concentrations and the change of bioavailability and size

DOC

The refractory form was dominant (>82 %) compared to the bioavailable form in TDOC of DOM (Table 1).

RDOC concentration increased significantly from upland streams (S14 and S15) to the wetland site ($p < 0.05$) (Table 1). The patterns of mean BDOC concentrations across sites showed similar values compared to that of RDOC, and therefore, % BDOC tended to decrease along with increasing RDOC concentrations as water moved from upland streams through the wetland. The proportion of HMWDOC in upland streams was lower than that of LMWDOC, and the % HMWDOC increased in the wetland, inlet and outlet.

The most notable seasonal pattern in TDOC concentrations across sites was the increase in concentrations from Sept through Oct at the inlet and wetland (Fig. 3) where the variation of TDOC was significantly ($p < 0.05$) related to the changes of RDOC and HMWDOC (Table 2). The % BDOC in upland streams increased from Sept to Dec with a subsequent decrease and relatively low values in subsequent months. We found that the change of % BDOC versus TDOC depended on the catchment characteristic (Fig. 4) in that, DOM bioavailability was higher in upland streams than in the wetland and inlet and then slightly increased in the lake. The variation of TDOC in wetland and inlet was more related to the HMWDOC (wetland: $r = 0.79$, inlet: $r = 0.95$; Table 2) than LMWDOC (wetland: no significance, inlet: $r = 0.60$; Table 2), but in the outlet, TDOC showed significant relationship with LMWDOC ($r = 0.80$) (Table 2). The seasonal pattern between inlet and outlet was also different.

DON

The refractory component was a larger component (>57 %) of TDON in DOM than the bioavailable component, but DON (ranged from 12 to 43 % of mean % BDONs) showed a higher bioavailability than DOC (ranged from 6 to 18 % of mean % BDOCs) (Table 1). Concentrations of TDON, RDON and HMWDON were significantly greater ($p < 0.05$) in the wetland than upland streams (S14 and S15) (Table 1) as shown in the change in DOC concentrations. Average values for DON concentrations increased from the inlet to the outlet, and this pattern differed from that of DOC (Table 1).

Overall the distribution of % BDON and % LMW-DON among sampling sites was more variable compared to DOC components. In upland streams, the

Table 1 Average (SD) of total (T-), refractory (R-), bioavailable (B-), high-molecular-weight (HMW-; >1,000 Da) and low-molecular-weight (LMW-; <1,000 Da) concentrations ($\mu\text{mol L}^{-1}$) of DOC, DON, and DOS and the contribution

(%) of the refractory, the bioavailable, the HMW, and the LMW to the total in DOC, DON and DOS in the S14, S15, wetland, inlet, and outlet

| | S14 | S15 | Wetland | Inlet | Outlet |
|-----------------------------------|--------------|--------------|---------------|---------------|--------------|
| TDOC ($\mu\text{mol L}^{-1}$) | 166.1 (61.6) | 129.3 (64.0) | 420.4 (100.2) | 444.7 (142.7) | 414.8 (70.0) |
| RDOC ($\mu\text{mol L}^{-1}$) | 123.3 (27.6) | 90.8 (16.8) | 388.1 (94.2) | 403.1 (130.7) | 379.0 (55.7) |
| BDOC ($\mu\text{mol L}^{-1}$) | 38.0 (50.6) | 26.1 (34.8) | 32.3 (25.5) | 30.2 (39.9) | 39.9 (54.1) |
| HMWDOC ($\mu\text{mol L}^{-1}$) | 51.1 (14.8) | 42.8 (15.2) | 227.8 (109.5) | 258.9 (129.3) | 223.3 (44.2) |
| LMWDOC ($\mu\text{mol L}^{-1}$) | 96.6 (43.1) | 68.6 (21.6) | 142.5 (47.0) | 146.8 (30.6) | 156.7 (40.1) |
| % RDOC | 82 (20) | 84 (16) | 93 (5) | 94 (9) | 91 (10) |
| % BDOC | 18 (20) | 17 (16) | 7 (5) | 6 (9) | 9 (10) |
| % HMWDOC | 33 (12) | 36 (11) | 55 (21) | 56 (13) | 55 (12) |
| % LMWDOC | 58 (16) | 59 (19) | 37 (16) | 35 (10) | 38 (6) |
| TDON ($\mu\text{mol L}^{-1}$) | 4.5 (2.0) | 5.0 (3.0) | 8.5 (4.1) | 9.4 (2.2) | 11.2 (4.6) |
| RDON ($\mu\text{mol L}^{-1}$) | 2.7 (1.8) | 2.8 (2.1) | 7.7 (4.2) | 8.2 (2.2) | 8.9 (2.2) |
| BDON ($\mu\text{mol L}^{-1}$) | 1.8 (0.9) | 2.2 (2.2) | 0.8 (0.8) | 1.2 (0.9) | 2.2 (4.0) |
| HMWDON ($\mu\text{mol L}^{-1}$) | 1.2 (0.7) | 1.1 (0.7) | 4.3 (1.2) | 4.5 (2) | 5.3 (1.5) |
| LMWDON ($\mu\text{mol L}^{-1}$) | 2.9 (1.6) | 2.7 (1.9) | 2.9 (1.7) | 4.8 (2.5) | 5.6 (5.3) |
| % RDON | 57 (20) | 58 (31) | 88 (16) | 83 (9) | 85 (18) |
| % BDON | 43 (20) | 42 (31) | 12 (16) | 13 (9) | 15 (18) |
| % HMWDON | 29 (20) | 31 (27) | 60 (21) | 48 (15) | 52 (19) |
| % LMWDON | 63 (25) | 59 (30) | 38 (21) | 53 (23) | 45 (27) |
| TDOS ($\mu\text{mol L}^{-1}$) | 21.8 (2.5) | 12.6 (2.7) | 8.3 (1.1) | 8.0 (1.5) | 6.0 (1.1) |
| RDOS ($\mu\text{mol L}^{-1}$) | 20.5 (2.5) | 11.7 (3.1) | 7.6 (1.5) | 6.9 (1.5) | 5.6 (1.1) |
| BDOS ($\mu\text{mol L}^{-1}$) | 1.3 (1.0) | 0.8 (0.9) | 0.7 (1.0) | 1.1 (1.0) | 0.4 (0.4) |
| HMWDOS ($\mu\text{mol L}^{-1}$) | 0.9 (0.4) | 0.7 (0.6) | 1.4 (0.6) | 1.7 (0.7) | 1.7 (0.7) |
| LMWDOS ($\mu\text{mol L}^{-1}$) | 19.8 (2.8) | 12.1 (3.1) | 6.3 (1.2) | 5.4 (2.6) | 3.7 (1.1) |
| % RDOS | 94 (4) | 93 (8) | 92 (12) | 87 (14) | 93 (7) |
| % BDOS | 6 (4) | 7 (8) | 8 (12) | 13 (14) | 7 (7) |
| % HMWDOS | 4 (2) | 6 (7) | 17 (7) | 21 (11) | 28 (12) |
| % LMWDOS | 91 (10) | 96 (15) | 76 (11) | 68 (25) | 62 (21) |

Note that the sum of either the labile and the refractory or the HMW and the LMW might not be equal to the total DOM due to some missing values during the incubation experiment or the size-fraction determinations due to the lack of volume needed for the respective measurements

temporal TDON pattern was similar to TDOC, but % BDON showed opposite patterns with % LDOC, i.e., decrease of % BDON in S14 from Oct and in S15 from Sept to early Dec, 2009. The outlet on Dec, 2009 showed a peak in % BDON as shown also for % BDOC. The seasonal pattern of TDON in S14, wetland, and inlet was more related to the RDON (S14: $r = 0.88$, wetland: $r = 0.98$, inlet: $r = 0.92$) than LDON (Table 2), but in the outlet, BDON ($r = 0.88$) and LMWDOC ($r = 0.87$) showed a significantly ($p < 0.05$) positive relationship with TDOC (Table 2).

DOS

The refractory form was significantly greater ($p < 0.05$) fraction of TDOS in DOM than the bioavailable from (Table 1). TDOS concentrations in upland streams were significantly ($p < 0.05$) higher than those for the wetland, inlet and outlet sites (Table 1) unlike the patterns of DOC and DON concentrations. In upland streams, S14 showed significantly (each paired T test results: $p < 0.05$) higher concentrations of RDOS and LMWDOS than S15. In the outlet, all means of the DOS

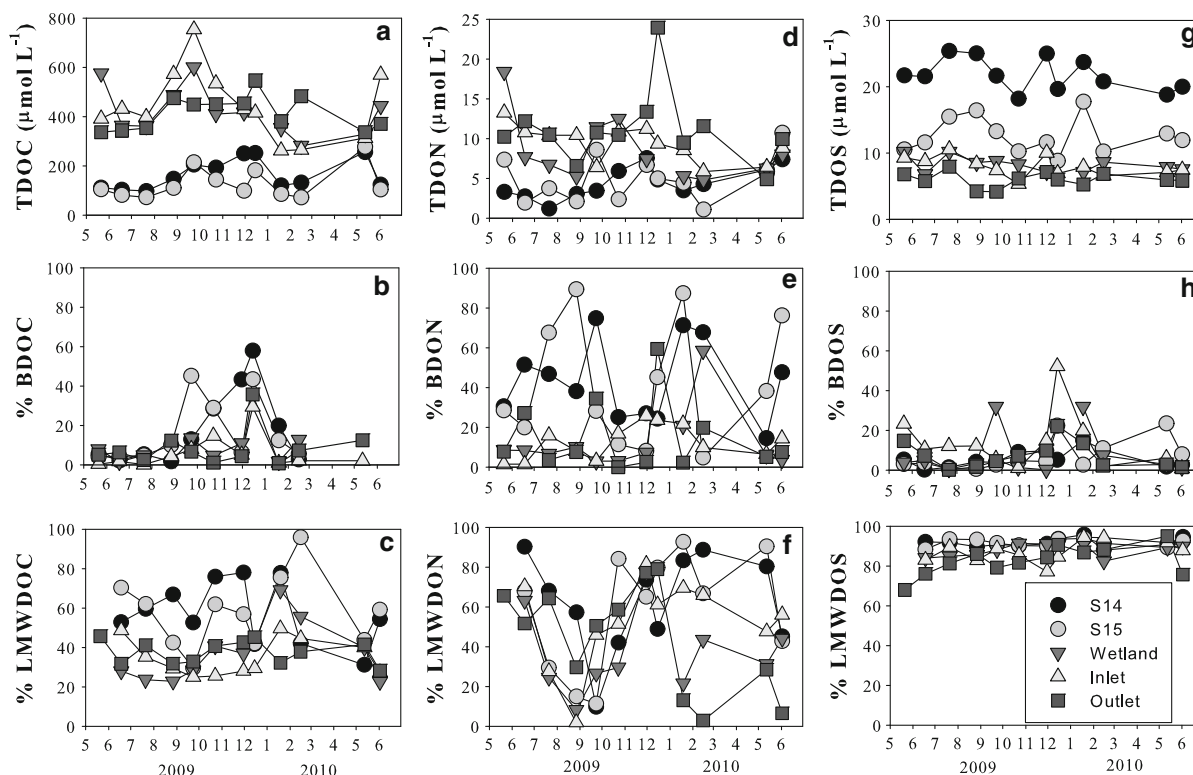


Fig. 3 The temporal variation of total (T-) DOC, DON and DOS concentrations ($\mu\text{mol L}^{-1}$) and each percent (%) of the bioavailable (B-) and the low-molecular-weight DOM (LMW-)

to the total form of each element in the S14, S15, wetland, inlet, and outlet from May 2009 to June 2010

forms except HMW forms decreased from the inlet (Table 1). The high dominance of LMW forms compared to HMW forms in DOS differed than that observed for the DOC and DON.

Overall, the temporal patterns of RDOS and LMW-DOS reflected the temporal pattern of the TDOS due to their predominance in TDOS compared to BDOS (Table 2). The upland streams TDOS increased from August to September and this seasonal pattern was different with DOC and DON patterns observed. In the wetland and inlet, the temporal variation of TDOS concentrations showed no consistent patterns. Outlet TDOS concentrations were lowest ($4.2 \mu\text{mol L}^{-1}$) in August and September. The weak seasonal patterns in TDOS concentrations and its components differed from the stronger seasonal patterns found for DOC and DON.

Stoichiometry

The molar total C:N ratios tended to increase from upland streams (S14: mean $42 \pm \text{SD } 17$; S15: 34 ± 19)

to the wetland (55 ± 16) with a subsequent decrease in inlet (50 ± 23) and outlet (41 ± 15) (Fig. 5), but there was no statistically significant difference among these ratios due to large variation within each sampling location. The distinct DOS concentration patterns compared to those of DOC and DON influenced the pattern of the ratios of C:S and N:S with respect to bioavailable and HMW forms. Total C:S ratios in upland streams (S14: mean $8 \pm \text{SD } 3$, S15: 11 ± 6) were significantly lower (one-way ANOVA with Tukey's pairwise comparison: $F_{4,53} = 41.11$, $p < 0.001$) than those in the latter three sites (wetland: 51 ± 11 , inlet: 56 ± 24 , outlet: 72 ± 21). We observed higher C:S ratios in bioavailable and HMW forms than in the total form of each site (Fig. 5). The LMW C:S ratio of each site was significantly lower (each paired *T* test results: $p < 0.05$) than the total C:S ratio, indicating S-rich characteristics of the LMW DOM compared to the total DOM. The total N:S ratio showed a significant increase (one-way ANOVA with Tukey's pairwise comparison: $F_{4,54} = 27.66$, $p < 0.001$) from

Table 2 Pearson correlation coefficients ($p < 0.05$ and $r > 0.7$) for DOM concentrations for surface waters

| Category/no | Correlation | S14 (11) ^a | S15 (11) | Wetland (8) | Inlet (11) | Outlet (11) |
|-------------|---------------|-----------------------|-------------|-------------|-------------|-------------|
| General 1 | TDOS–LWMDOS | 0.98 | 1.00 | 0.95 | 0.94 | 0.88 |
| 2 | TDOS–RDOS | 0.91 | 0.97 | 0.78 | 0.76 | 0.93 |
| 3 | RDOS–LWMDOS | 0.88 | 0.97 | | 0.77 | 0.84 |
| Upland 1 | TDOC–BDOC | 0.89 | 0.97 | 0.74 | | |
| 2 | BDOC–HWMDON | 0.86 | | | | |
| 3 | BDOC–RDON | 0.76 | | | | |
| 4 | RDON–LWMDON | 0.72 | | | | |
| 5 | LWMDOC–BDOS | | 0.84 | | | |
| 6 | HWMDOC–HWMDON | | 0.74 | | | |
| 7 | BDOC–HWMDOC | | 0.73 | | | |
| Wetland 1 | TDOC–RDOC | | 0.87 | 0.98 | 0.96 | |
| 2 | TDON–RDON | 0.88 | | 0.98 | 0.92 | |
| 3 | RDOC–RDON | | | 0.89 | | |
| 4 | RDOC–TDON | | | 0.85 | | |
| 5 | RDOC–HWMDON | | | 0.84 | | |
| 6 | TDOC–RDON | 0.72 | | 0.74 | | |
| 7 | TDOC–TDON | | | 0.70 | | |
| Inlet 1 | RDOC–HWMDOC | | 0.93 | 0.76 | 0.96 | |
| 2 | TDOC–HWMDOC | | | 0.79 | 0.95 | |
| 3 | TDON–HWMDON | | | | 0.79 | |
| 4 | RDON–HWMDON | | | | 0.78 | |
| Outlet 1 | BDOC–BDON | | | | | 0.92 |
| 2 | TDON–BDON | | 0.71 | | | 0.88 |
| 3 | TDON–LWMDON | 0.74 | | | | 0.87 |
| 4 | TDOC–LWMDOC | | 0.77 | | | 0.80 |
| 5 | BDON–LWMDON | | | | | 0.77 |
| 6 | LWMDOC–LWMDON | | | | | 0.74 |
| 7 | BDOC–TDON | 0.71 | | | | 0.74 |
| 8 | LWMDOC–TDON | | | | | 0.71 |
| 9 | TDON–BDOS | | | | | 0.71 |
| 10 | BDOC–LWMDON | | | | | 0.70 |

Italic and bold values indicate the highest r value in each row and the $r > 0.8$, respectively. Categories were the same as sites (except S14 and S15 combined together as “Upland”) with the highest r value in each row, and the number was in order of the highest r values from highest to lowest in the same category. “General” in the category means overall strong correlation through the watershed

^a number of samples used for statistics

the upland streams (S14: mean $0.2 \pm \text{SD } 0.1$; S15: 0.4 ± 0.3) to the wetland (1.0 ± 0.4) and inlet (1.2 ± 0.3), following the significant increase to the outlet (1.9 ± 0.8). We found greater N:S ratios in the bioavailable and HMW forms compared to the total form of each site (Fig. 5).

SUVA

SUVA has been used for evaluating aromaticity and hence the presence of humic-like compounds in DOC (Inamdar et al. 2011). As water drained through the wetland there was a significant two-fold increase

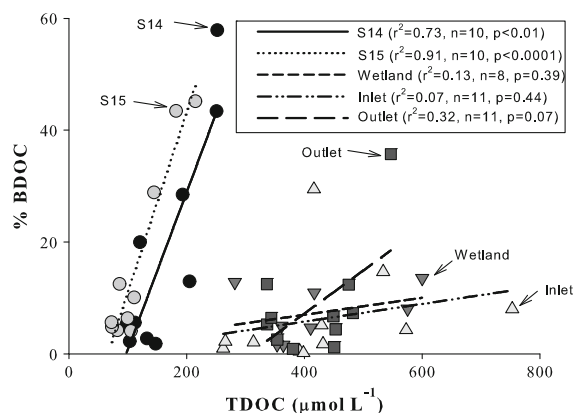


Fig. 4 Linear regression showing relationships between TDOC concentration and % BDOC in the S14, S15, wetland, inlet, and outlet from May 2009 to June 2010

(one-way ANOVA with Tukey's pairwise comparison: $F_{4,53} = 47.20$, $p < 0.001$) of SUVA values in the total DOM compared to the upland streams (S14 and S15) with concomitant significant increases of HMW ($F_{4,50} = 28.91$, $p < 0.001$) and refractory ($F_{4,47} = 39.44$, $p < 0.001$) SUVA values (Table 3). In the outlet, the values of HMW, refractory and total SUVA significantly decreased from the inlet ($p < 0.001$). The HMW SUVA values in upland streams were significantly higher than the total SUVA (Table 3), but there was no significant difference in the later three sites. We observed lower SUVA values in LMWDOM than in the HMWDOM and total DOM of each site (Table 3). In addition, along with BDOC increasing, our refractory SUVA values appeared to increase compared to total

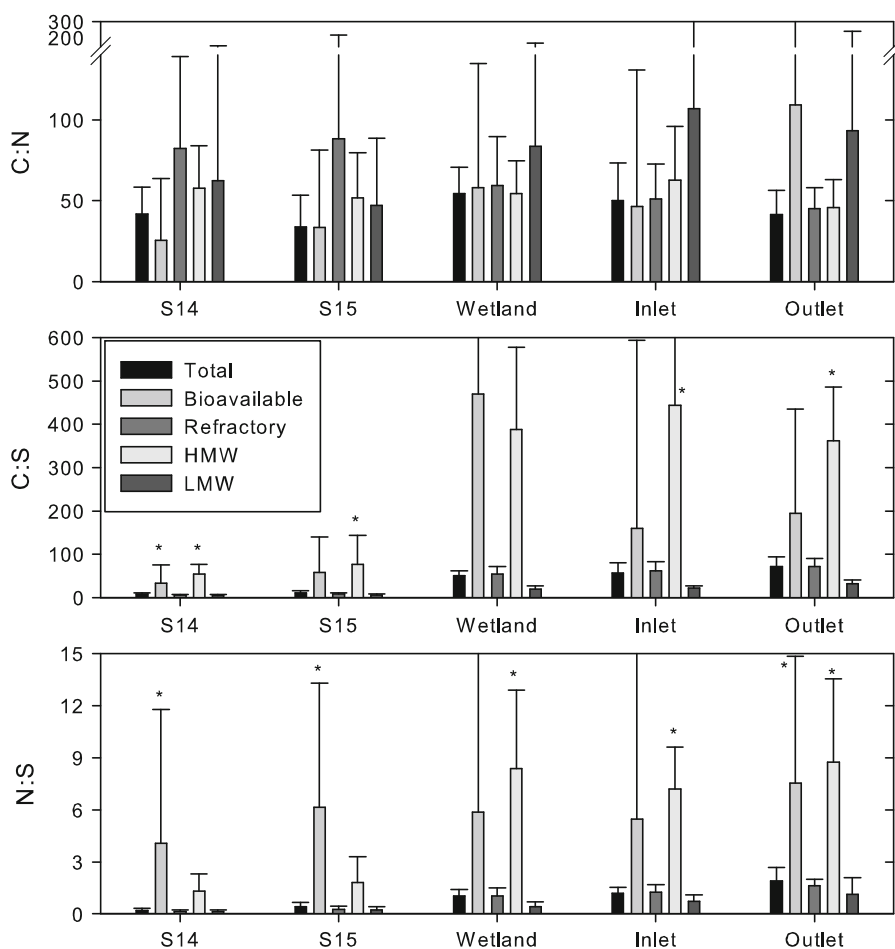


Fig. 5 Averages (SD) of molar ratios among DOC, DON, and DOS for total, bioavailable, refractory, high-molecular-weight (HMW), and low-molecular-weight (LMW) forms in the S14, S15, wetland, inlet, and outlet from May 2009 to June 2010.

One-way ANOVA was performed to test differences among forms of each site using Dunnett's comparisons with a control (total form) of each site indicated by asterisk (*) above bars ($\alpha = 0.05$ level)

SUVA values, and therefore, we found a significant relationship between the BDOC (X : $\mu\text{mol L}^{-1}$) and the SUVA value of BDOC (Y : refractory SUVA–total SUVA) ($Y = 0.009X - 0.036$, $r^2 = 0.84$, $n = 44$, $p < 0.0001$).

Stable carbon isotope ratios

Average $\delta^{13}\text{C}$ values of TDOC in surface waters had a narrow range from -27.8 to -27.1 ‰ (Table 3) and there was no clear seasonal pattern among or within sites. The $\delta^{13}\text{C}$ values of TDOC in the wetland were intermediate to those at S14 and S15, whereas between the inlet and outlet, the $\delta^{13}\text{C}$ values of TDOC in the lake outlet showed significantly more negative values than that in the inlet (one-way ANOVA with Tukey's pairwise comparison: $F_{1,18} = 5.54$, $p < 0.05$). In HMWDOC, we observed more depletion in ^{13}C in HMWDOC from the wetland site compared to upland streams (one-way ANOVA with Tukey's pairwise comparison: $F_{4,43} = 6.69$, $p < 0.001$). For LMWDOC, S14 and S15 showed lower $\delta^{13}\text{C}$ values than for HMWDOC (Table 3) and there was no significant difference ($F_{4,39} = 1.08$, $p = 0.38$) of LMWDOC $\delta^{13}\text{C}$ values among sites.

Correlation of SUVA with other measurements of DOM quality

SUVA values for estimating DOM quality in our study provided insight for evaluating the change of bioavailability and TDOC concentration as well as isotopic changes across surface waters (Fig. 6). The increase of TDOC from upland streams through wetland to inlet was significantly related to accumulating aromatic compounds in DOM as indexed by SUVA (Fig. 6a). The increase of aromatic C in DOM was associated with decreasing C bioavailability (Fig. 6b). Through our incubation (bioavailability) experiment, we observed that most of the DOC in surface waters with large contributing catchment areas (wetland, inlet, and outlet) compared to the upland streams consisted of higher proportions of refractory forms along with high aromatic contents (Table 1), and the weak ($r^2 = 0.27$) positive relationship between $\delta^{13}\text{C}$ values and SUVA values was shown (Fig. 6c). For all surface waters in the Arbutus Watershed stable C isotopic values in LMWDOC

and HMWDOC did not show a consistent relationship with SUVA (Fig. 6d).

Correlation among bioavailability and molecular size among DOC, DON and DOS constituents

In general, the LMW and refractory forms in DOS concentration showed similar relationships among the surface waters (Category of “General” 1 to 3 in Table 2). Upland streams showed a strong correlation between TDOC and BDOC (Category of “Upland” 1: S14; $r = 0.89$, S15; $r = 0.97$ in Table 2) as shown in Fig. 4, but each upland stream showed different correlations, indicating different internal DOS and DOC generation patterns. We observed that relationships with respect to bioavailable and LMW DOM at the lake outlet were totally different than from those of wetland-affected waters (wetland and inlet) with respect to refractory and HMW DOM.

The relative level of refractory DOM versus total DOM varied among DOC, DON, and DOS especially as a function of molecular size (Fig. 7). For DOC, there were significant positive relationships between the refractory and the HMWDOC or LMWDOC accounting for 72 and 24 % of the refractory DOC, respectively (Fig. 7a), and the regression slopes were significantly different for HMW and LMW in DOC ($F_{1,89} = 5.25$, $p < 0.05$ by ANCOVA). For DON, 51 and 48 % of the RDON were represented by HMWDON and LMWDON, respectively (Fig. 7b), and there was no significant difference of slopes between HMW and LMW ($F_{1,89} = 0.58$, $p = 0.45$ by ANCOVA). DOS concentrations showed a strong positive relationship only between RDOS and LMWDOS (Fig. 7c). The LMW DOS accounted for a large proportion of the entire refractory DOS with 88 % of the variability. Based on these results between the % BDOM versus the % HMWDOM, we developed a “bioavailability-molecular size model” (Fig. 7d) suggesting that the bioavailability of DOM varies among the size-fractions and also differs among DOC, DON and DOS.

Discussion

DOM in upland streams

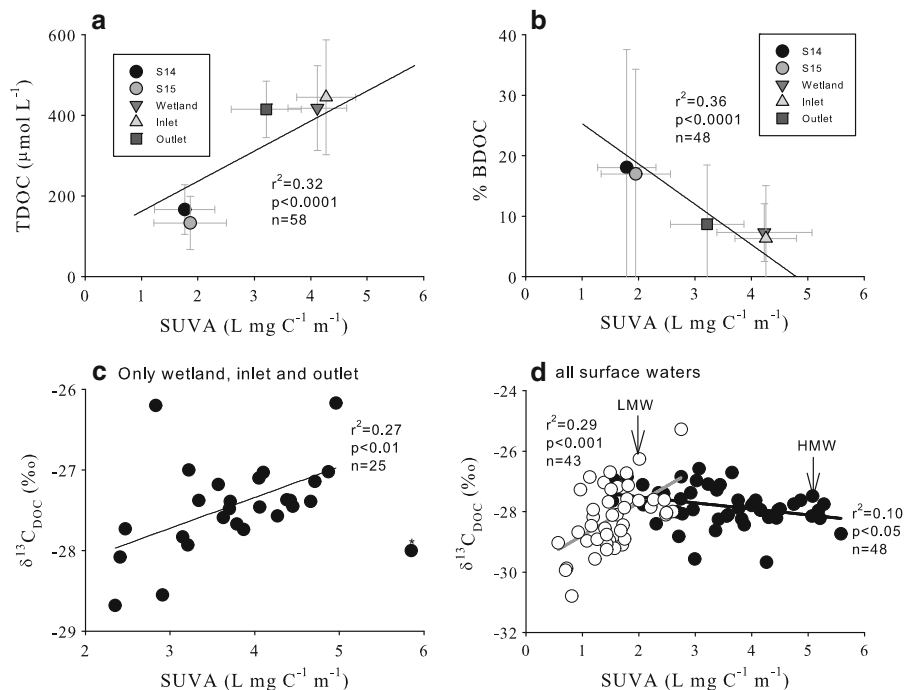
The bioavailable C fractions based upon our incubation experiments constituted 17–18 % of DOM in the

Table 3 Average (standard deviation) SUVA values ($\text{L mg C}^{-1} \text{ m}^{-1}$) and stable C isotope values ($\delta^{13}\text{C}$, ‰) of the total, refractory, high-molecular-weight (HMW; $>1,000$ Da) andlow-molecular-weight (LMW; $<1,000$ Da) DOM in the S14, S15, wetland, inlet and outlet

| Form | S14 | S15 | Wetland | Inlet | Outlet |
|--|--------------|--------------|-------------|--------------|-------------|
| SUVA ($\text{L mg C}^{-1} \text{ m}^{-1}$) | | | | | |
| Total | 1.8 (0.5) | 1.9 (0.6) | 4.4 (0.7) | 4.3 (0.5) | 3.2 (0.6) |
| Refractory | 2.0 (0.7) | 2.0 (0.7) | 4.5 (0.5) | 4.4 (0.4) | 3.4 (0.6) |
| HMW | 2.8 (0.5)* | 2.6 (0.7)* | 4.3 (0.7) | 4.7 (0.5) | 3.4 (0.5) |
| LMW | 1.2 (0.4)* | 1.2 (0.3)* | 1.7 (0.6)* | 1.9 (0.5)* | 1.8 (0.4)* |
| $\delta^{13}\text{C}$ (‰) | | | | | |
| Total | −27.7 (0.7) | −27.1 (0.5) | −27.4 (0.3) | −27.2 (0.4) | −27.8 (0.7) |
| HMW | −27.3 (0.4) | −27.3 (0.4) | −28.1 (0.6) | −28.2 (0.6)* | −28.1 (0.6) |
| LMW | −28.8 (1.0)* | −28.3 (1.0)* | −28.1 (1.1) | −27.8 (1.3) | −28.0 (0.9) |

Note that there was no measurement of $\delta^{13}\text{C}$ values in the refractory form. One-way ANOVA was performed to test differences among forms of each site ($\alpha = 0.05$ level) and the result of Dunnett's comparisons with a control (total form) of each site was indicated by asterisk (*) above bars (family error rate = 0.05). ANOVA was separately run for SUVA and stable C isotope

Fig. 6 Regression models depicting the relationship of SUVA with **a** TDOC concentration across sites (S14, S15, wetland, inlet, and outlet), **b** % BDOC across sites, **c** stable C isotope values of TDOC in the wetland, inlet and outlet, and **d** stable C isotope values in LMW- and HMWDOC across sites. Note that an asterisk in the (c) panel was not included for the linear regression

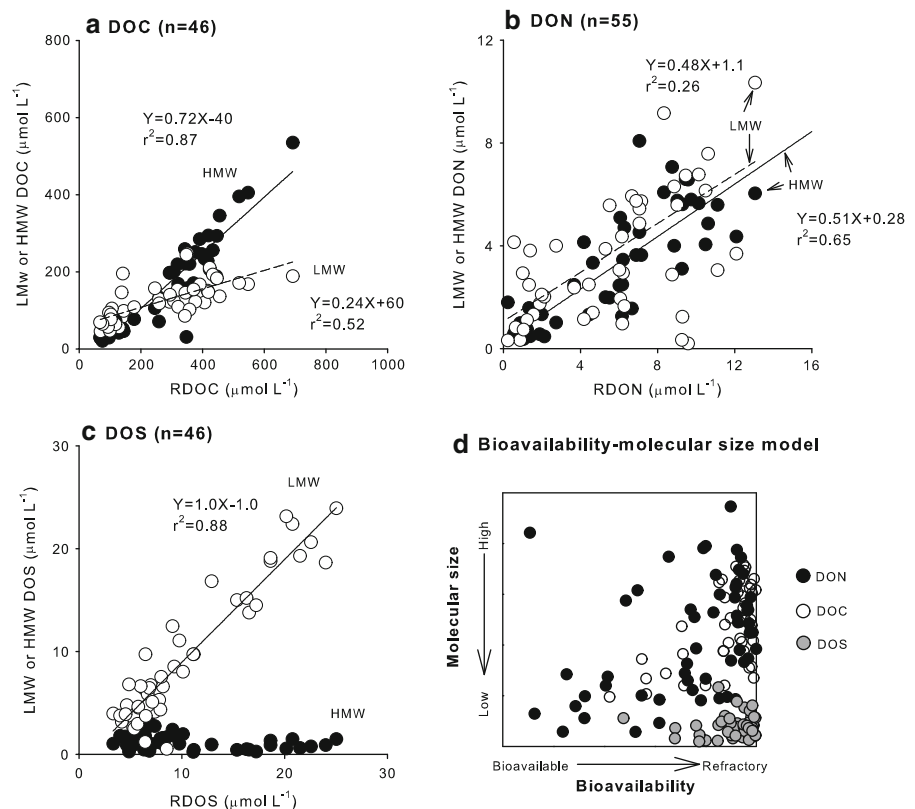


upland streams of the Arbutus watershed and were similar to the values summarized by Søndergaard and Middleboe (1995) in lakes (14 ‰) and rivers (19 ‰). These results suggest that relative proportion of bioavailable C fraction in DOM is similar among diverse lotic systems (del Giorgio and Davis 2003). The higher percentage of the bioavailable DON fraction (42–43 ‰) compared to DOC (17–18 ‰)

suggests the potential importance of DON as an N source.

Leaf fall in the autumn and the addition of organic matter to the Arbutus watershed surface waters resulted in increasing TDOC (Fig. 3, inlet and wetland) and %BDOC (Fig. 3, S14 and S15) as has been found in other studies (i.e., Aitkenhead-Peterson et al. 2003). Most studies on DOM and BDOM have

Fig. 7 Relationship between the refractory form and high (>1 kDa; HMW)- or low (<1 kDa; LMW)-molecular-weight form of **a** DOC **b** DON and **c** DOS of all surface waters (*closed*: HMW, *open*: LMW). All regression results are significant ($p < 0.001$). A bioavailability-molecular size model **d** developed through modifying those relationships indicates the different characteristics among DOC, DON, and DOS in surface waters in the Arbutus Lake watershed



been confined to the growing-season (i.e., Fellman et al. 2008). The sporadic increase of % BDOC(N) in December might suggest the export of the accumulated labile DOM in a dormant season that might be associated with melt events. Kurian et al. (2012) found that for the S14 and S15 streams that melt events resulted in elevated concentrations of DOC and other solutes.

Isotopic values of DOC in surface and soil waters are affected by sources (i.e., C3 vs. C4 plants; O'Leary, 1988), vegetation cover (Amiotte-suchet et al. 2007), metabolic processes in terrestrial production (e.g., Blair et al. 1985) and transformations including microbial decomposition (Nadelhoffer and Fry 1988), adsorption (Qualls and Haines 1992; Kaiser and Zech 1998; Kaiser et al. 2001). We observed the weak temporal variation of the $\delta^{13}\text{C}$ values through surface waters since in terrestrial and aquatic ecosystems, most of the C isotopic values from OM precursor, terrestrial sources and algae overlap (Schiff et al. 1990), therefore, resulting in weak temporal patterns, i.e., in streams in Canada (80 % of data falling within 0.5 ‰ of -27.0 ‰; Schiff et al. 1997).

Our size-fractionated DOM concentration revealed that the concentration of % HMWDOC(N) (approximately 30–35 %) in upland streams was low compared to the wetland (Table 1). The small proportion of HMWDOC(N) might be linked to the deep and shallow groundwater which was a major source of DON that entered surface waters (Christopher et al. 2008). Clearly internal soil processes played an important role in changing the form of DOM and the biogeochemically-affected DOM in soil water was exported through discharges into streams. For example, the decrease of HMWDOC and increase of LMWDOC from precipitation, throughfall, soil horizon to stream waters was observed previously at Hubbard Brook, New Hampshire, suggesting the importance of soil microbial processing of DOM (Aitkenhead-Peterson et al. 2003; Cole et al. 1984; McDowell 1982). And the decrease of MW of DOC in pore waters was also found via hyporheic zone and runoff in a temperate forest of northern Wisconsin (Schindler and Krabbenhoft 1998), suggesting that the change of DOM flux was dependent on soil solution flow path, and soil characteristics (Aitkenhead-

Peterson et al., 2003). For microbial processing, significantly higher $\delta^{13}\text{C}$ values in HMWDOC than LMWDOC (Table 3) supports the microbial decomposition of the HMW form in DOC because microbes use preferentially lighter C (^{12}C) isotopes during DOC decomposition (Schiff et al. 1997). Along with biotic process, chemical adsorption is widely recognized as an important mechanism that removes DOC by mineral soil and the amount of adsorption varies with hydrological flow pathways (Aitkenhead-Peterson et al. 2003). We also observed the relatively low contribution of the HMW SUVA fraction of the total SUVA in spite of the relatively high aromaticity of HMWDOC. The enriched ^{13}C in HMW forms in streams might also be attributed to preferential sorption of the ^{13}C -depleted hydrophobic fractions in the soil (Dai et al. 1996; Jardin et al. 1989; Kaiser and Zech 1998; Kaiser et al. 2001; Qualls 2000). Inamdar et al. (2011) also showed that aromatic contents were lower from deeper soil sources (i.e., deep groundwater) than from surficial sources (throughfall, litter, and soil water) in a forested mid-Atlantic watershed, supporting the decrease of HMWDOM through as solutes move through the soil profile.

DOM in wetland-affected streams

The seasonal variation of both TDOC and TDON concentrations suggested the importance of biotic control such as the accumulation during the growing season as found in other studies (Devito et al. 1989; Fellman et al. 2008; Fraser et al. 2001; Wiegner and Seitzinger 2004). The increase of the refractory DOC and DON concentrations and proportion in the wetland-affected streams (wetland and inlet) from upland streams supported the previous study of Piatek et al. (2009) in the Arbutus watershed showing the importance of wetlands in generating high concentrations of DOC (Table 1). In addition, we found that the generation of DOM in wetlands was also important in the production of refractory and HMW forms (Table 1, 2). The quality of exported DOM from wetlands compared to upland streams resulted in increased C-richness and this increase of aromaticity in HMW. Possibly, the higher C content might be related to the accumulation of high refractory and aromaticity in the DOC and hence higher relative amounts of ^{12}C -depleted HMWDOC (Table 3) due to microbial decomposition (Schiff et al. 1997).

The C bioavailability fraction (6–7 %) was substantially smaller in our study than in other studies of this DOC component (e.g., in bogs in the Pine Barrens region of New Jersey (22 %; Wiegner and Seitzinger 2004), in wetland soil in coastal temperate watershed in Alaska (23–42 %; Fellman et al. 2008), in Japanese mountain bog pools (40 %, Satoh and Abe 1987), in freshwater marshes (45 %; Mann and Wetzel 1995), and in sewage treatment plants and industrial effluent (66 %; Markosova 1991). A synthesis by Wiegner and Seitzinger (2004) on DOC derived from soil organic matter concluded that wetlands soils/sediments were the most important sources of refractory of DOC compared to other sources.

DON bioavailability was also affected by the soil process in the wetland areas where refractory DON increased from upland streams as shown in DOC bioavailability. Our wetland-area DON bioavailability (12–13 % of DOM) within palustrine peatland (Greenwood Mucky peats) was higher than Swedish freshwater wetland (1 %) in spruce forests with sandy soils and flooded meadows with peats showed (Stepanaukas et al. 1999), supporting that wetland soil process may change the DON bioavailability (Wiegner and Seitzinger 2004). The DON bioavailability in wetlands in forest has been shown to vary with vegetation and land use (Wiegner and Seitzinger 2004). Several studies showed similar values of DON bioavailability, e.g., 30 % in forests with shallow muck soils (Wiegner and Seitzinger 2004), and 24 % in forest export and 29 % in agricultural export (Seitzinger et al. 2002; Wiegner and Seitzinger 2001). Urban/suburban runoff showed higher DON availability (56 %) (Seitzinger et al. 2002).

The difference in the bioavailability of C and N likely affects microbial processes. For example, due to the greater lability of DON this would result in more rapid utilization of DON resulting in higher relative concentrations of C versus N in DOM. Gregorich et al. (2003) showed higher biodegradation of DON than DOC in hot- and cold-water extractable organic matter in agricultural soils. Additionally, high N turnover was consistent with many studies that have shown higher turnover rates of dissolved free amino acid versus glucose in various environments, i.e., lakes, estuaries, and ocean (Kirchman 2003). Our data supported the idea that the bioavailability of DOC and DON changed as water passed through the Arbutus watershed with wetlands showing a marked effect on the amount of forms of DOM.

DOM in Arbutus Lake (outlet)

In lake ecosystems, there are two major sources of DOM: allochthonous DOM from terrestrial sources (Aitkenhead-Peterson et al. 2003) and autochthonous DOC from production by phytoplankton, benthic algae and macrophytes (Bertilsson and Jones 2003). In spite of numerous studies, the separation between two sources in lake ecosystems has been known to be difficult (i.e., Cole et al. 1984). In our study, we not only observed changes in DOM concentrations, i.e., a small decrease of TDOC following an increase of BDOC as well as all of the DON fractions (Table 1), but also found the shift of major fractions from more refractory and HMW forms in the inlet to more bioavailable and LMW fraction in the outlet (Table 2). Our results also suggest that Arbutus Lake served both as a net sink for DOM, but also affected the form of DOM that could have been affected by both autotrophic and heterotrophic processes including algal production (Owen et al. 1999).

Evidence of the terrestrial DOM decomposition (DOM sink) was supported by the decrease of allochthonous RDOC and HMW concentrations in the Lake (Table 1) and the decrease of aromatic content in HMW and refractory fractions (Table 3). But the decrease of allochthonous DOM was not substantial since, for example, considering the change of HMW-DOC with approximately $35 \mu\text{mol L}^{-1}$ (Inlet–Outlet) this content accounted for approximately 8 % of the total outlet DOC. This small decrease may indicate the slow degradation of allochthonous DOM dominated by refractory forms (Wetzel 2001).

The strong correlation among LMW and bioavailable DOC and DON in Arbutus Lake suggests the change of DOM characteristics is due to within-lake processes (Mitchell et al. 2001). Generally, extracellular DOC release from algae in lakes have been found to be highly bioavailable and labile (Kirchman et al. 1991), and the increase of the slope between TDOC and % BDOC between inlet and Arbutus Lake (Fig. 4) supports the importance of algal production. In addition, the highly significant correlation between BDOC and BDON (Table 2) suggests a linkage between lake N and C autochthonous production. The significant correlations between TDOC(N) and LMWDOC(N) and the increase of LMW forms may be attributed to algae-excreted LMWDOC (i.e., Lignell 1990).

Distinct bioavailability and molecular size of DOS

The markedly high DOS concentrations in terms of refractory and LMW forms in upland streams unlike DOC and DON patterns differed from the study of Goller et al. (2006) who showed a parallel pattern among DOC, DON and DOS in soil waters at a montane forest in Ecuador. Prior to discussing the mechanisms for DOS formation, the proportion of DOS to total dissolved S needs to be considered. The contribution of TDOS to TDS ranged from 12 % (all site except S14) to 20 % (only S14), which was within the values of the boreal streams of Giesler et al. (2009) where TDDOS ranged from 10 to 50 % of TDS in northern Sweden (Giesler et al. 2009). These values are much lower than those found (10–80 %) in pore waters from a highly polluted peatland in the UK (Bottrell et al. 2010) and in montane forest soil water in Ecuador where values ranged from 30 to 80 % (Goller et al. 2006).

Most studies have previously focused on the effect of DOS transport and adsorption through the soil resulting in surface water DOS concentrations being similar to those of the deep mineral horizons (David et al. 1987; Houle et al. 2001; Mitchell et al. 1989). But the markedly high LMW DOS concentrations and the low N:S ratios in upland streams differ from these previous studies suggesting a different mechanism influencing DOS formation in some of the upland streams of the Arbutus watershed. Bottrell et al. (2010) showed for pore waters in a peatland that DOS was formed by the incorporation the SH^- , derived from bacterial dissimilatory sulfate reduction (BSR). Our study found that this DOS was found in the LMW fraction.

BSR under anaerobic conditions has been known to participate in the biochemical formation of DOS since SH^- as a product of sulfate reduction can be incorporated into DOM (Giesler et al. 2009; Werne et al. 2004). The existence of BSR in the inlet (Archer Creek) catchment had been suggested previously, i.e., the reoxidation of SH^- derived from BSR to sulfate might control low $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ ratios (Campbell et al. 2006; Mitchell et al. 2006, 2008). These isotopic results are consistent with products of BSR becoming incorporated into a larger component of LMWDOS versus HMWDOS.

Kaiser and Guggenberger (2005) studied DOS in soil solutions under beech and pine tree species in

Germany and showed that ester sulfate S, derived from microbial activity that utilized C-bonded S (Mitchell et al. 1998), was found in the hydrophilic fractions. Kaiser and Guggenberger (2005) also showed the higher mobility of DOS than DOC in soil solutions, indicating that neutral hydrophilic non-charged carbohydrates might be associated with DOS (Guggenberger and Zech 1994). Further research on DOS should include determinations of the relative contributions of carbon-bonded S and ester sulfates as well as other potential organic S constituents including those associated with BSR.

Stoichiometry as an indicator for DOM change

The C:N ratios in the surface waters in our study were similar to the range of values of other studies [e.g., in Catskills Mountains (mean: 21; Lovett et al. 2000), Adirondack lakes (mean: 31, range: 13–58; Ito et al. 2005), streams at the Coweeta Hydrologic Laboratory, North Carolina (mean: 36; Qualls and Haines 1991), White Mountain National Forest streams, New Hampshire (range: 20–62; Goodale et al. 2000), and in remote sites in Chile (range: 45–57; Hedin et al. 1995). Across sites both within our study and within other regions, surface waters showed large variation in bioavailable and refractory C:N ratios. Regarding the DOM quality and the use of C:N ratios as an index of the bioavailability in our study, more C was lost from DOM than N during incubation. This same phenomenon was observed in other studies (Fellman et al. 2008; Wiegner and Seitzinger 2004) that suggested the decrease in DOC was the combined effect of respiration and incorporation into microbial biomass. It has also been suggested that the decomposition of DOM might be affected by nutrient limitation of N and P (Fellman et al. 2008).

The C:S ratios in our study were generally lower compared to the limited studies in other forested ecosystems. For example, Kaiser and Guggenberger (2005) investigated at pine and beech sites in Germany showing C:S ratios of DOM in forest floor leachates and at 90 cm depth ranged from 208 to 445 and from 98 to 613, respectively. In addition, in the study of fulvic and humic acids across various humification stages of aquatic samples (groundwater, bog lake, podsol seepage water) of DOM, molar C:S ratios (calculated from mass percent data) ranged from 93 to 474, and an decrease of C:S ratios was attributed to the

increase of the stage of humification indicating preferential loss of C compared to S (Abbt-Braun and Jahnel 2001). In 1,238 lakes of Québec regions, the C:S ratios ranged from 44 to 434 with the average of 122 (Houle et al. 1995). In addition, the C:S ratios in an oligotrophic Adirondack lake (David and Mitchell 1985) and streams of the Rocky Mountains (Mitchell et al. 1986) ranged from 4 to 11, and from 22 to 42, respectively, indicating that C:S ratios might vary according to the characteristics of the sources of DOM. In our study, the increases of bioavailable DOS and HMW forms of DOS versus total DOS (Table 1, Fig. 4) might be a result from the greater rate of loss of DOC than DOS loss, the latter of which had higher proportion of refractory and LMW forms associated with DOS formation from BSR (Abbt-Braun and Jahnel 2001; Bottrell et al. 2010).

There have been few studies that have compared the relative contributions of DON and DOS in surface waters. In the study of Abbt-Braun and Jahnel (2001), N:S ratios in fulvic and humic acids of DOM ranged from 1.6 to 6.3 (calculated from mass percent data). A forested site adjacent to the Arbutus Lake watershed contained 1,152 and 7,985 kg organic N ha⁻¹ in the forest floor and mineral soil, respectively, and 70 and 1,133 kg organic S ha⁻¹ within the forest floor and mineral soil, respectively, that corresponds molar N:S ratios of 38 and 16 (Mitchell et al. 1992). The N:S ratios in microbial biomass from native aquatic and cultured bacteria range from 6.5 to 45.7 (Fagerbakke et al. 1996). The N:S ratio has been known to be referred to as an indicator of protein-derived S compounds, i.e., in biogenic material derived from proteins (N:S = approximately 11) (Abbt-Braun and Jahnel 2001; Schlegel 1985) and as a criterion in soil organic matter for assessing S status for wheat production (N:S = approximately 17) (Stewart and Whitfield, 1965). Clearly our N:S ratios in surface water across sites were low compared to ratios found in the forest floor and microbial biomass, but would be consistent with the high S content in DOM being due to the contribution of BSR.

Indicator of overall watershed DOM change

We found a positive relationship ($p < 0.0001$) between SUVA and TDOC indicating the relevance of aromatic C content with catchment DOC increasing. Our observation was consistent with the positive

relationship between SUVA and HMW DOC in refractory organic substance found by Hesse and Frimmel (1999). In addition, in our study TDOC values were positively correlated ($p < 0.05$) with HMWDOC and RDOC (Table 2). In contrast, some studies showed no correlation between DOC and SUVA in multiple sites (streamwater, lake, and estuary) in US (Jaffé et al. 2008), in forested watershed (Inamdar et al. 2011), and in tropical streams in Venezuela (Yamashita et al. 2010) suggesting that the aromaticity as a fraction of DOC concentration varies across biomes and catchments. In addition, we observed a negative relationship ($p < 0.0001$) between %BDOC and SUVA that was consistent with other studies (Fellman et al. 2008; Kalbitz et al. 2003; Marschner and Kalbitz 2003; Saadi et al. 2006) suggesting the linkage between aromaticity and bioavailability in surface waters. Aromaticity can be linked to the extent of humification and degradation of DOM, i.e., humification index (band ratio of fluorescence intensities) (Kalbitz and Geyer 2001; Kalbitz and Geyer 2002) and the negative relationship of humic-like DOM with labile DOM (Inamdar et al. 2011; Maie et al. 2006; Qualls and Haines 1992). We also quantified the biodegraded DOC using SUVA values and showed that decomposition of non-aromatic compounds resulted in a net increase in the more refractory DOC constituents in aromatic/humic components that agrees with the findings of Fellman et al. (2008).

Kalbitz and Geyer (2002) reported that the level of humification was closely related to increasing $\delta^{13}\text{C}$ in DOC in degrading peat. Our finding in the wetland-affected streams and the lake supported the importance of DOM degradation on changing C isotopic signature. Our isotopic results suggest that allochthonous DOC was much more important than autochthonous DOC. The isotopic results (Fig 6c) showed that DOM degradation in wetlands and Arbutus Lake was linked with the preferential decomposition of light C (^{12}C) (Kalbitz and Geyer 2002; Ludwig et al. 2000; Schiff et al. 1990; 1997). In our study, there was little seasonal change in $\delta^{13}\text{C}$ values within Arbutus Lake again suggesting the dominance of allochthonous DOC. In addition, the different pattern of LMW and HMW DOC (Fig 6d) suggests that $\delta^{13}\text{C}$ values combined with size information might be useful for evaluating the source of DOM and its changes in DOM characteristics (Kaiser et al. 2004; McKnight et al. 2001).

Bioavailability-molecular size model

Since the microbial decomposition of DOM is influenced by the variation of DOM quality and quantity, it is critical to understand the dynamic nature of DOM sources and transformations. Our study shows that the variable molecular-size concentrations reflect not only the complexity of the quantity of DOM entering from variable sources in the watershed, but also the diverse bioavailability of DOM indicates the importance of microbial decomposition on DOM transformations. We found clear patterns by looking at both the refractory contribution on the molecular size of DOM and the variation among DOC, DON and DOS (Fig. 7). The linkage of the HMW with the refractory in DOC and DON (Fig 7a, b) was consistent with the bioavailability model by Saunders (1976) and supports the importance of polymeric molecules in DOM (Cummins et al. 1972; Kaplan and Newbold 2003; Thurman 1985; Yu et al. 2002). But the lower slope of LMWDOC versus HMWDOC may represent the importance of smaller molecular size of DOM versus the contribution of humic substances on the microbial transformation of DOM. We observed also a higher bioavailability of DON than that of DOC (Table 1), and HMWDON showed similar bioavailability to LMWDON, compared to HMWDOC. This suggests that HMWDON forms in various sources might influence on microbial transformation, resulting in the higher bioavailability of DON versus DOC. The distinctly different DOS relationships compared to DOC and DON showed an important role of LMWDOS for the S bioavailability, and the DOS derived from a different source (e.g., BSR) compared to terrestrial production of DOC and DON might also play an important role in altering DOM bioavailability.

These different elemental relationships can be further explained in the context of the proportion of bioavailability (Fig. 7d). Our “bioavailability-molecular size model” suggested the following: (1) DON is composed of a much wider range of MW and lability (bioavailability) than DOC and especially DOS; (2) DOC showed an intermediate lability between the high reactive DON and the very low reactive DOS; (3) The sources and microbial transformations of DOC and DON were variable due to different contributions of upland streams, wetland-area and Arbutus Lake; and (4) DOS consisted of mostly refractory LMW DOS, which was the prevalent DOS form through surface waters in the Arbutus Lake watershed.

Generally, it is a widely recognized assumption for understanding bioavailable correlates of DOM that terrestrial sources of DOM have highly refractory than algal-derived DOM which is largely bioavailable (del Giorgio and Davis 2003). This assumption involves the chemical difference between allochthonous and autochthonous DOM (Wetzel 2003). Our model updates further this rationale that the sources and transformations of DOM are dependent on its elemental composition. In addition, the DOC and DON characteristics in upland streams were more bioavailable than those in wetland areas, indicating that terrestrially derived DOM was less refractory than might be expected based on other studies (e.g., Sinsabaugh and Findlay 2003). Our results suggest that the different soil types in the uplands (e.g., Becket-Mundall) versus the wetlands (e.g., Greenwood Mucky peats) produced DOM with specific chemical and physical characteristics. These different soil sources affected the spatial variation and temporal patterns in the amount and form of allochthonous DOM within the catchment. Further quantification of the factors affecting the contribution of various soil types on DOC, DON and DOS in forested catchments is needed.

Conclusion

Our study emphasizes the importance of using a range of methods to characterize the heterogeneity of DOM in terms of molecular size, lability, aromaticity, stoichiometry, and chemical isotopic values as well as differences among DOC, DON and DOS. To our knowledge, this is the first study using DOS biogeochemistry for understanding the bioavailability and size in DOM biogeochemistry combined with analyses of DOC and DON. The major findings are:

1. Aromaticity (SUVA) was a useful index for elucidating DOM characteristics among surface water locations within a forested watershed, and was related to bioavailability and humification with respect to stable C isotopic values.
2. Our approach found linkages among various DOM qualities (size and bioavailability) that varied among upland stream, wetlands, lake inlet and lake outlet sites. The water that passed

through the wetlands resulted in substantial changes in the DOM characteristics.

3. DOC and DON biogeochemical relationships showed more similarities than for DOS where dissimilatory sulfate reduction played an important role.

Future work should include further evaluations of DOS sources in the upland catchments, more detailed analyses of how the formation of DOM varies both spatially and temporally in conjunction with other factors including changing atmospheric deposition and climate. For example, the increase of sulfate exports due to previously stored soil S in spite of decreasing an atmospheric S input might affect DOS biogeochemistry including a possible increase of an export of DOS produced under changing redox conditions and as a function of soil wetness within catchments (Mitchell and Likens 2011).

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