

## Sources of dissolved organic matter (DOM) in a Rocky Mountain stream using chemical fractionation and stable isotopes

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**Abstract.** Dissolved organic matter (DOM) is an important vehicle for the movement of nutrients from terrestrial to aquatic systems. To investigate how the source and composition of aquatic DOM change in both space and time, we used chemical, spectroscopic, and isotopic analyses to characterize DOM in a headwater catchment in the Colorado Front Range. Streamwater samples for DOM analyses were collected from 2 sites, a lightly vegetated alpine site and a forested, subalpine site, in the North Boulder Creek catchment during the snowmelt runoff season (May–September). Concentrations of dissolved organic carbon (DOC) peaked on the ascending limb of the snowmelt hydrograph at both the alpine (2.6 mg C l<sup>-1</sup>) and the subalpine sites (7.0 mg C l<sup>-1</sup>) and decreased sharply on the descending limb of the hydrograph. Fractionation of DOM into operationally defined humic and non-humic components showed that the fulvic acid content of DOC decreased through the season at both sites and that spectroscopic (fluorescence and ultraviolet) properties of the humic DOM fraction shifted in a manner consistent with an increase in the proportion of humic DOM derived from instream sources as compared to terrestrial catchment sources. Humic and non-humic fractions of DOM isolated near peak flow in June and during low flows in September showed a seasonal enrichment in <sup>15</sup>N and <sup>13</sup>C as well as a seasonal decrease in the ratio of aromatic to aliphatic carbon, both of which were correlated with a decrease in the C:N ratio of the DOM fractions. These results suggest that seasonal shifts in the isotopic and chemical characteristics of DOM are a result of changes in catchment sources of DOM. In particular, it appears that DOM production in alpine lakes is an important contributor to the streamwater DOM load during late season low flows, especially in the alpine reach of the catchment. Our results further suggest that stable isotopes of C and N are useful tools, particularly when combined with ancillary data such as elemental analyses and catchment discharge, for evaluating sources and transformations of DOM at the catchment scale.

### Introduction

Studies on DOM at the watershed scale in mountain catchments commonly treat nutrient components as single pools. Research has shown that concentrations and fluxes of dissolved organic carbon (DOC) vary with factors such

as hydrograph stage (Hornberger et al. 1994; Boyer et al. 1997) and annual variations in climate (Lewis and Grant 1980). Fluxes and concentrations of dissolved organic nitrogen (DON) also vary with the hydrograph and annually (Sickman et al. 2001). However, it is unclear whether the components of DOC and DON vary systematically in the same manner as bulk measurements. Treatment of DOC and DON as a single pool may be misleading from the viewpoint of understanding ecosystem processes directly related to changes in sources and biological reactivity (Kaushal and Lewis in press). Our understanding of the biological reactivity of DOM has been limited by the lack of adequate analytical tools.

In freshwaters, organic acid fractions comprise the majority of DOM. Fulvic acids are typically the most abundant DOM fraction followed by low molecular weight organic acids (Thurman 1985). Fulvic acids, as well as humic and transphilic acids, can be distinguished and isolated operationally based upon properties such as hydrophobicity, pH-dependent solubility and other characteristics of chemical structure (Aiken et al. 1992). Transphilic acids are defined as the least hydrophilic of the non-humic DOM compounds that pass through XAD-8 Amberlite resin (Croue et al. 1999). Interactions of these organic acid fractions with metals and nutrients are important in controlling the chemistry of freshwaters (e.g. McKnight et al. 1992; Hruska et al. 2001) and absorbance of light by these fractions influences the physical environment in lakes and streams (e.g. Scully and Lean 1994; Morris et al. 1995). Additionally, the microbial utilization of DOM dominates the flux of energy in many freshwater aquatic ecosystems (Wetzel 1995).

Dissolved organic matter and its constituents fractions can be derived both from terrestrial sources external to the aquatic system (allochthonous) and from sources within the aquatic system (autochthonous). In headwater catchments, streamwater DOM is typically derived primarily from allochthonous sources such as organic soil horizons (Schiff et al. 1990; Brooks et al. 1999). In contrast, in lake systems autochthonous DOM typically supports a significant fraction of community respiration. However, net ecosystem production (gross primary production – community respiration) in lakes is often negative suggesting that allochthonous DOM from the surrounding catchment is also an important source of C for respiration in lake systems (Cole et al. 2000).

In freshwaters where DOM is derived primarily from autochthonous algal/microbial precursor material, fulvic acids generally account for a lower proportion of the DOM compared to freshwaters where DOM is derived from plants and soil organic matter (McKnight et al. 1994). The relative balance of allochthonous and autochthonous material also has important implications for the chemistry of the DOM fractions. For example, elemental analysis and  $^{13}\text{C}$ -NMR characterization of fulvic acids have demonstrated that fulvic acids of algal/microbial origin have a lower C:N ratio and less aromatic carbon than fulvic acids leached from terrestrial material (McKnight et al. 1991; Aiken 1992). Further, fulvic acids derived from algal material can be expected to have

a relatively high N content because microbial cells have a greater N content than the lignaceous material in higher plants (McKnight et al. 1994). Recent work has shown that fluorescence spectroscopy is a useful tool for distinguishing autochthonous vs. allochthonous sources of fulvic acid (McKnight et al. 2001). This technique has been used in a wide variety of studies to determine sources of DOM and its constituent chemical classes in both surface waters and soils (Klapper et al. 2002; Wolfe et al. 2002; Hood et al. 2003a).

In the Colorado Rocky Mountains, techniques for the chemical and spectroscopic characterization of DOM have been applied in several catchment scale studies on DOM cycling (Baron et al. 1991; McKnight et al. 1997, 2001; Hood et al. 2003a). Taken together, the results from these studies suggest that in headwater streams without lakes DOM is derived primarily from humified terrestrial material that is flushed into the stream at high concentrations during spring snowmelt. However in catchments that contain lakes, a substantial portion of the aquatic DOM is derived from algal production in the lakes, especially during summer. The information that these studies have provided about spatial and temporal changes in sources of DOM in headwater catchments in the Rocky Mountains has important implications for the coupling of the carbon and nitrogen cycles in these watersheds.

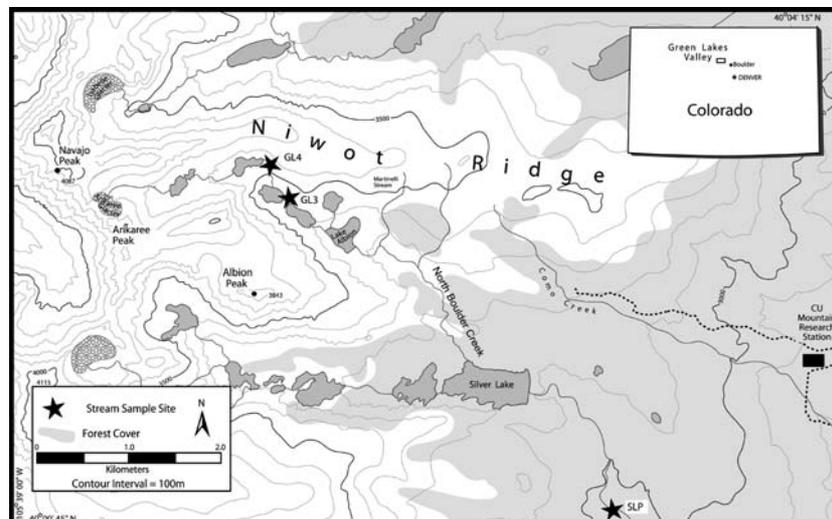
In high-elevation catchments, dissolved organic nitrogen (DON) can be a significant component of the streamwater N flux. For example, in Green Lakes Valley in Colorado, DON accounts for 10–15% of the N flux in alpine streams and nearly 50% of the N flux in streams below treeline (Williams et al. 2001; Hood et al. 2003b). Some of this DON is in humic fractions. An important consideration in studying the humic DON is that N is a trace constituent, i.e. not every fulvic acid molecule will contain a nitrogen atom (McKnight et al. 2003). Depending upon the molecular weight and the N content, the frequency of occurrence of a nitrogen-containing functional group can range from one out of every 3 to 6 fulvic acid molecules. One implication of this is that N-containing fulvic acid molecules may have different sources and reactivities than the fulvic acid fraction as a whole. The isotopic signature of  $^{15}\text{N}$  in fulvic acid has previously been used to track the sorption and desorption of N-containing fulvic acids in an alpine stream (McKnight et al. 2002). These results suggest that stable isotopes of humic fractions have the potential to provide information about the source and fate of DOM at the catchment scale. A better understanding of the coupling of C and N cycling in high-elevation watersheds is particularly important given that the terrestrial and aquatic DOM cycles in these catchments are being altered by changes in climate and precipitation chemistry (Baron et al. 1994; Williams et al. 1996a; Wolfe et al. 2001; Waters in press).

The purpose of this study is to link spatial and temporal changes in the sources of the primary constituent fractions of aquatic DOM, fulvic and transphilic acids, with changes in their N content and  $^{13}\text{C}$  and  $^{15}\text{N}$  signatures. Streamwater samples were collected from both the lightly vegetated alpine and the forested, subalpine reaches of the catchment throughout the snowmelt

runoff season (May–September). Small volume samples were collected weekly to bi-weekly and analyzed for fulvic acid content as well as UV and fluorescence spectroscopic properties. In addition, preparative quantities of fulvic and transphilic acids were collected at two points on the hydrograph in the alpine and subalpine reaches of the catchment. These samples were analyzed for elemental content, stable isotopes of C and N, and the structural group distribution of carbon. Used in concert, these analyses provide an initial assessment of the chemical and isotopic character of streamwater DOM across an alpine–subalpine ecotone. This information, in turn, provides insight into the relative balance of allochthonous and autochthonous DOM in this system and how that balance changes both seasonally and spatially within the catchment.

### Site description

The North Boulder Creek watershed is situated in the Green Lakes Valley in the Front Range of the Rocky Mountains approximately 40 km north-east of Boulder, Colorado. The catchment is 2720 ha in area and extends from below Silver Lake at 2963 m to the crest of the Continental Divide at 4000 m (Figure 1). The watershed is underlain by granodiorites and Precambrian



*Figure 1.* The North Boulder Creek catchment in the Green Lakes Valley, Colorado Front Range, USA. In 1999 and 2000, surface water grab samples were collected throughout snowmelt runoff at an alpine site above treeline (GL4) and a forested site (SLP). In 2000, large volume (100 l) water samples were collected at peak snowmelt (8 June) and during low flow in the fall (14 September) to isolate preparative quantities of fulvic and transphilic acids at an alpine site (GL3) and forested site (SLP). GL3 was sampled in place of GL4 because of the logistical difficulties of transporting large volumes of water from GL4.

metasediments. Above Lake Albion at 3250 m, the catchment is lightly vegetated and alpine in nature, dominated by steep rock walls and talus fields. Below Lake Albion, the catchment is dominated by mixed conifer forest composed of Englemann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*), with some regrowth lodgepole pine (*Pinus contorta*) lower in the catchment. This portion of the catchment is typified by developed soils on glacial till and moraine deposits. Climate is characterized by long, cool winters and a short growing season (1–3 months). At Niwot Ridge, the northern interfluvium of the catchment, mean annual temperature is  $-3.8$  °C and mean annual precipitation is 1000 mm, about 80% of which falls as snow (Williams et al. 1996b).

North Boulder Creek is typical of high-elevation headwater streams, characterized by low specific conductance (typically  $< 25$   $\mu\text{S cm}^{-1}$ ) and relatively low concentrations of dissolved nutrients (C, N, and P) (Caine 1995; Hood et al. 2002). During summer, weekly monitoring of phytoplankton populations and chlorophyll *a* concentrations in Green Lake 4 has shown that algal productivity increases in late July as the residence time and temperature in the lake increase (Gardner 2003). The chemical character of dissolved organic material (DOM) shifts dramatically during the water year from primarily fulvic acids during spring and summer snowmelt to a higher content of transphilic acids and low molecular weight material during low flows in the fall and winter. Previous measurements of the fluorescence properties of fulvic acids suggest that, particularly in the alpine reach of the catchment, this shift in character is associated with a change in the provenance of DOM from terrestrial precursor material during snowmelt to more aquatic precursor material during late summer and fall (Hood et al. 2003a).

In 2000, stream samples on the main stem of North Boulder Creek were collected at two alpine sites: Green Lake 4 (GL4) and Green Lake 3 (GL3), as well as a forested, subalpine site 5 km below treeline (SLP) (Figure 1). Elevations of the two alpine sites ranged from 3400 (GL3) to 3535 m (GL4), while the subalpine SLP site was at 2963 m. During the summer of 2000, phytoplankton monitoring in Green Lake 4 showed that chlorophyll *a* concentrations at the surface and 3 m depths increased from less than  $1$   $\mu\text{g l}^{-1}$  on July 6 to  $3$ – $4$   $\mu\text{g l}^{-1}$  on July 27 (Gardner 2003). Water level at Green Lake 4 outflow was measured continuously with a pressure transducer. Water levels were recorded hourly and converted to volumetric discharges by empirical ratings (Caine 1995). Discharge was not measured at the SLP site.

## Methods

### *Sample collection and handling*

Weekly to bi-weekly water samples were collected during the 2000 snowmelt runoff season (May–October) at GL4 and SLP. Samples were collected as 1 l

grab samples in pre-combusted amber glass bottles with teflon-lined caps and filtered through pre-combusted Gelman GF/F filters with a nominal pore size of 0.7  $\mu\text{m}$ . Large volume (100 l) samples for isolating preparative quantities of fulvic acids and transphilic acids were collected at the GL3 and SLP sites on 8 June and 14 September of 2000. The GL3 site was sampled as the closest alternative to the regularly sampled alpine site (GL4) on the two sampling dates because it was not feasible to transport 100 l of water from GL4. However, previous surface water sampling and discharge measurements have shown that the GL3 site is very similar in terms of discharge and water chemistry to the GL4 site (N. Caine, personal communication). Large volume samples were filtered through a Balston glass fiber filter type AAH (0.3  $\mu\text{m}$ ) and acidified to pH 2. The majority of samples were filtered in the field, and all samples were filtered within a day of collection. Samples were stored at 4 °C until analysis, which occurred within 2 weeks of sample collection. DOC concentrations for all samples were determined by high-temperature catalytic oxidation using a Shimadzu Total Organic Carbon Analyzer 5050A at the Institute of Arctic and Alpine Research (INSTAAR) in Boulder, CO. Three replicate analyses were done for each sample. Standard deviation was typically 0.06  $\text{mg C l}^{-1}$  with a range of 0.01–0.22  $\text{mg C l}^{-1}$ .

#### *Isolation procedures*

For the weekly samples, a 200 ml subsample of every 1 l sample was fractionated into operationally defined humic and non-humic fractions using XAD-8 Amberlite resins according to the methods of Thurman and Malcolm (1981) as modified by Hood et al. (2003a). The benefit of this chemical fractionation technique is that it reproducibly isolates compositionally distinct fractions of DOM from the molecular continuum observed in natural waters (Aiken et al. 1992). In headwater streams, the humic fraction is typically composed primarily (>90%) of fulvic acids (Thurman 1985), therefore we refer to this fraction as fulvic acids following the terminology of Baron et al. (1991). The non-humic fraction refers to a heterogeneous class of substances including transphilic acids and other low molecular weight compounds that pass through the XAD-8 resin. Mass balance analyses show that DOC recovery was almost complete, with the sum of the DOC measured in the fulvic and non-humic fractions between 94 and 103% of DOC in the original samples.

Fulvic and transphilic acids were isolated from large volume (~100 l) water samples collected in May and June following the methods of Aiken et al. (1992). This isolation scheme is valid for natural water samples with DOC concentrations within the range in which sorption of organic material to the XAD resins is solubility dependent, approximately 0–25  $\text{mg C l}^{-1}$  (Aiken et al. 1992). Two-liter columns of XAD-8 and XAD-4 resin were connected in series by Teflon tubing, and 100 l of sample were passed through the XAD-8/XAD-4

column pair. After rinsing with deionized water, the XAD-8 column was back-eluted with 4 l of 0.1 NaOH to isolate the fulvic acid fraction and the XAD-4 column was back-eluted with 4 l of 0.1 NaOH to isolate the transphilic acid fraction. The non-humic DOM fraction isolated on XAD-4 resin at pH 2 has previously been referred to as the hydrophilic acid fraction (e.g. Thurman 1985; Aiken et al. 1992). Here we use the term transphilic for this non-humic fraction following the terminology of Croue et al. (1999). The eluents from both columns were immediately hydrogen saturated using a cation exchange resin and then freeze-dried within 2 days. The total mass recovery for fulvic acids was 30–274 mg and the fulvic acid composition of DOC calculated by the analytical (200 ml) and preparative (~100 l) scale techniques agreed within 4% on the June and September sample dates (Table 1).

The ash content of the fulvic and transphilic isolates was relatively large (10–45%). Analysis of several isolates indicated that the major constituents in ash were sodium and silicon. The sodium content is a result of incomplete ion exchange during the hydrogen saturation step. Silicon results from interactions between silicic acid in the water sample and both organic material and the macroporous resins (Aiken et al. 1992). It is unlikely that ash-free contents of C, N, S, and P were substantially affected by the presence of mineral phases of sodium and silicon in the isolates, however ash-free contents of H and O are not presented because of the possible influence of oxides. It is also possible that the extraction conditions could affect the N and P contents of the DOM fractions. However, previous research has shown that precursor sources of organic material and biogeochemical processes such as sorption by iron oxides control the amino acid composition of isolated fulvic acids. This suggests that the isolation method does not significantly alter the amino acid composition (McKnight et al. 1991, 1992).

#### *Fluorescence spectroscopy*

The fluorescence properties of fulvic acids isolated from weekly surface water samples were measured using a Fluoromax-2 multi-wavelength fluorescence

*Table 1.* Total mass recovery of fulvic acids from streamwater samples.

Sample	Volume (l)	Fulvic acids	DOC	Fulvic acid %	Fulvic acid %
		mass (mg)	mass (mg)	Preparative	Analytical
GL2 June	100	54	87	62	63
SLP June	100	268	438	61	58
GL2 Sept	100	30	61	49	45
SLP Sept	100	55	165	33	31

The mass of bulk DOC was calculated as the DOC concentration times the volume of sample used for fractionation. The fulvic acid composition of DOC calculated by the preparative scale technique compared well (within 4%) with the calculations using the analytical scale (200 ml sample volume) technique.

spectrophotometer with a xenon lamp. Emission intensities were measured at 450 and 500 nm using an excitation of 370 nm in order to calculate the fluorescence index (FI) following the procedure of McKnight et al. (2001). The intensity values for both the 450 and 500 nm scans were adjusted by subtracting the intensity of deionized water, which was used as a blank. The standard deviation of samples analyzed in triplicate was typically less than 0.01.

Fluorescence properties of fulvic acids are related to the presence of quinone functional groups (Klapper et al. 2002). The FI can serve as a simple tool to distinguish whether fulvic acids are derived from aquatic microbial material, which has high FI values ( $\sim 1.7$ ), or terrestrial material such as lignin, which has relatively low values ( $\sim 1.2$ ; McKnight et al. 2001). The fluorescence properties of aquatic fulvic acids from North Boulder Creek were compared to the fluorescence properties of two reference fulvic acids derived predominantly from either aquatic or terrestrial precursor material. The aquatic reference fulvic acids were isolated from Lake Fryxell in the McMurdo Dry Valleys of Antarctica and the terrestrial reference fulvic acids were isolated from the Suwannee River in the Okefenokee swamp in Georgia. Comparison to the FI of the reference fulvic acids provides a semi-quantitative evaluation of the precursor material (terrestrial vs. aquatic) for fulvic acids in North Boulder Creek (Hood et al. 2003).

#### *UV spectroscopy*

Fulvic acid isolates were analyzed for UV-visible absorbance following the procedure of Weishaar et al. (2003). UV measurements were made on a Hewlett–Packard photodiode array spectrophotometer using distilled water as a blank. A quartz cell with a 1.0 cm path length was used. Refrigerated samples were warmed to room temperature before analysis and duplicate analyses were performed approximately on every 10 samples to ensure instrument stability. Specific UV absorbance (SUVA) was calculated by dividing the UV absorbance at 254 nm by the DOC concentration and is reported in units of  $l/mg\ C * m$ .

#### *<sup>13</sup>C-NMR analysis*

Solid-state CPMAS <sup>13</sup>C-NMR spectroscopy analyses were performed on all lyophilized fulvic and transphilic acid samples on the same day ( $\sim 25$  mg) using a Bruker DPX 300 MHz NMR spectrometer. A contact time of 2 ms, recycle delay time of 1 s, a sweep width of 277 kHz, and a line broadening of 100 Hz were used. The <sup>13</sup>C NMR data were integrated in six major bands characteristic of humic substances (Wershaw 1985): 0–60 ppm, aliphatic carbon; 60–90 ppm, heteroaliphatic carbons; 90–110 ppm, acetal carbons;

110–160 ppm, aromatic carbons; 160–190 ppm, carboxyl, ester and amide carbons; and 190–230 ppm, ketonic and aldehydic carbons.

#### *Elemental and isotopic analyses*

All samples for elemental and isotopic analyses were run on the same day to ensure comparability between samples. Lyophilized fulvic acid and transphilic acid samples were analyzed for elemental content (C, N, O, H, and S) using individual elemental analyzers. Phosphorus analysis was done by ICP after decomposition by acid. Analyses were done by Huffman Laboratories in Golden, Colorado following the procedures of Huffman and Stuber (1985).

$\delta^{13}\text{C}$  of lyophilized fulvic and transphilic acid samples was measured on a CE Elantech 1110 CHN analyzer coupled with a Micromass Isoprime continuous flow isotope ratio mass spectrometer. Isotopic ratios are reported relative to the international Pee Dee Belemnite (PDB) standard. The standard deviation for standards in the same run was 0.11‰ for  $\delta^{13}\text{C}$ .  $\delta^{15}\text{N}$  of the DOM was measured on a PDZ Europa 20-20 continuous flow isotope ratio mass spectrometer. Isotopic ratios are reported relative to  $\text{N}_2$  of air. Instrument precision was  $\pm 0.2\text{‰}$  for  $\delta^{15}\text{N}$ . Field duplicates for isotopic analyses were not collected in this study due to logistical constraints, however previous studies using the same methods have shown that two co-located field duplicates for large volume isolates varied by  $< 0.2\text{‰}$  for  $\delta^{13}\text{C}$  (Baron et al. 1991). This variability is consistent with other isotopic studies on DOM that report analytical errors of  $\pm 0.2\text{‰}$   $\delta^{13}\text{C}$  between independently field sampled replicates (Schiff et al. 1990, 1997) and standard deviations of  $< 0.25\text{‰}$   $\delta^{13}\text{C}$  and  $< 0.16\text{‰}$   $\delta^{15}\text{N}$  between field replicates for three separate DOM samples (Elder et al. 2000). In addition, McKnight et al. (2002) found that three coincident samples taken along a 150 m reach of stream varied by 0.5‰ for  $\delta^{13}\text{C}$ . Taken together these studies suggest that precision for  $\delta^{13}\text{C}$  of DOM can reasonably be estimated at  $\pm 0.5\text{‰}$ . Precision for  $\delta^{15}\text{N}$  is likely lower due to the lower levels of N present in DOM but can be reasonably be estimated at  $< \pm 1\text{‰}$  based on the results of Elder et al. (2000).

## **Results**

### *Seasonal trends – DOC, fulvic acid content, fluorescence, and SUVA*

The seasonal peak in discharge from snowmelt runoff at GL4 in 2000 occurred in early June (Figure 2a). Concentrations of DOC peaked on the ascending limb of the hydrograph at both sites and then declined on the descending limb of the hydrograph (Figure 2a). Concentrations of DOC were always 2–3 times greater at the downstream SLP site compared to the alpine GL4 site.

The fulvic acid content of DOC at both GL4 and SLP was generally highest on the ascending limb of the hydrograph and then declined on the recession

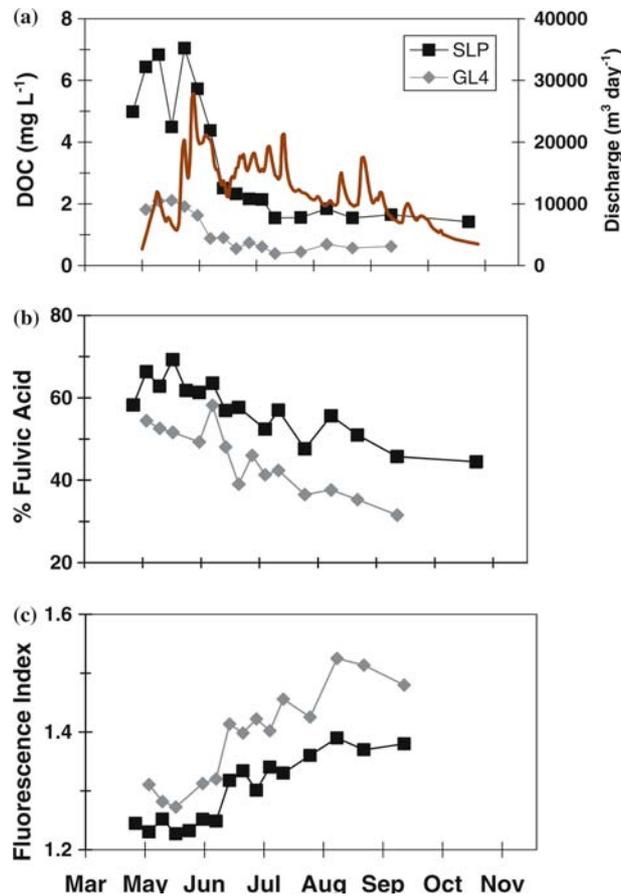


Figure 2. Time series of DOC concentration (a), the fulvic acid content of DOC (b), and the fluorescence index (c) at GL4 and SLP during the 2000 snowmelt runoff season. Daily stream discharge at GL4 is shown in (a) with DOC concentrations.

limb (Figure 2b). The fulvic acid content of DOC in the alpine (GL4), which ranged from 35 to 60%, was consistently lower than the fulvic acid content of DOC at the subalpine SLP site. Fluorescence index values for fulvic acids at both sites showed minima on the ascending limb of the hydrograph with values close to that of the Suwannee River terrestrial endmember (1.23). On the descending limb of the hydrograph, FI values increased at both sites although they did not reach the value of the Lake Fryxell aquatic endmember (1.70). (Figure 2c). Over the season, the FI of fulvic acids from GL4 was always greater than at the downstream SLP site.

The specific UV absorbance for fulvic acids at both sites peaked in the early summer and decreased to lower values in the late season (Figure 3). Seasonally, SUVA values for fulvic acids were consistently lower in the alpine (GL4)

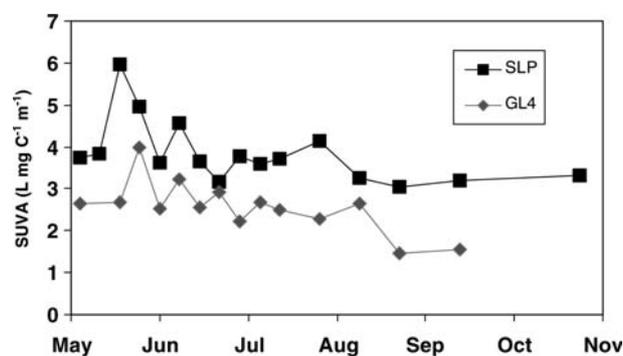


Figure 3. Time series of SUVA at 254 nm (expressed as l/mg C \* m) for fulvic acids collected at the alpine GL4 site and subalpine SLP site in the North Boulder Creek watershed.

compared to the subalpine (SLP), consistent with the physical properties of the fulvic fraction changing downgradient in the catchment. For all samples, SUVA values of fulvic acids showed a significant inverse correlation with the fluorescence index of fulvic acids at SLP ( $R^2 = 0.50$ ,  $p = 0.002$ ,  $n = 16$ ), however the correlation between these parameters was not significant at GL4 ( $R^2 = 0.17$ ,  $p = 0.16$ ,  $n = 13$ ).

#### Composition of fulvic and transphilic acids

##### Elemental analyses

There was a consistent difference in elemental composition between the fulvic and transphilic acid isolates (Table 2). The fulvic acid isolates typically had a

Table 2. Ash-free elemental content (as percent) and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for fulvic and transphilic acids collected at an alpine site (GL3) and a forested, subalpine site (SLP).

	Elemental content				C:N ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
	C	N	S	P			
<i>Fulvic acids</i>							
GL4 June	53.4	2.2	0.81	–	24.2	–27.0	0.8
SLP June	55.1	1.2	0.66	0.04	45.9	–26.5	0.1
GL4 Sept	48.9	2.7	0.96	–	18.1	–26.2	1.2
SLP Sept	54	1.8	0.73	0.09	30.2	–25.6	0.2
<i>Transphilic acids</i>							
GL4 June	45.1	2.8	2.1	–	16.1	–25.9	2.3
SLP June	48.8	1.7	0.92	–	28.7	–25.2	1.0
GL4 Sept	27.7	2.3	1.35	–	12.0	–24.7	2.5
SLP Sept	46.9	2.5	1.27	–	18.8	–24.5	1.5

Samples were collected near peak snowmelt runoff in June and during a lower flow period in September. Phosphorus was only analyzed on two of the fulvic acid samples.

lower content of N and S than transphilic acids isolated from the same sample. This difference in N content was reflected in the C:N ratios of the fulvic fractions which were 50–60% higher than the C:N ratios of the corresponding transphilic acids. These findings are consistent with previous studies on the elemental content of fulvic and transphilic acids from a wide range of environments (Aiken et al. 1992, 1996; McKnight et al. 1997).

Seasonally, the N and S content of both fulvic and transphilic acids tended to increase between the June (peak flow) and September (low flow) sampling periods (Table 2). Similarly, the limited data available for P show that the phosphorus content of fulvic acids at SLP more than doubled between June and September. The temporal shift in N content was reflected in C:N ratios of the isolates collected in September, which were 30–50% lower than the C:N ratio of the June isolates. Within the catchment, the N and S content of fulvic and transphilic isolates consistently decreased moving from the alpine (GL3) to the forested, subalpine (SLP) reach of the catchment. As a result the C:N ratio of both fractions increased strongly (60–90%) in the downstream direction. These results show that the shift in elemental composition of streamwater organic material between the physically distinct upper and lower reaches of the catchment was more pronounced than the seasonal changes in the elemental composition of DOM at an individual site.

#### *Stable isotopic analyses – $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$*

The  $\delta^{13}\text{C}$  values for fulvic and transphilic acids were clustered between  $-24.5$  and  $-27\text{‰}$  while  $\delta^{15}\text{N}$  values for the isolates ranged from  $0.1$  to  $2.5\text{‰}$  (Table 2). Differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between DOM fractions collected at the same site were on the order of  $1\text{--}1.5\text{‰}$ . For all samples, the transphilic acids were significantly enriched in both  $\delta^{13}\text{C}$  ( $p = 0.02$ ) and  $\delta^{15}\text{N}$  ( $p = 0.03$ ) compared to fulvic acids.

Seasonally,  $\delta^{13}\text{C}$  for fulvic and transphilic acids at both sites increased significantly ( $p = 0.004$ ) between peak snowmelt and baseflow in the fall. Values of  $\delta^{15}\text{N}$  for the isolates did not change significantly between June and September ( $p = 0.06$ ). Between the alpine and subalpine, there was a consistent shift in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for the two isolates, with a significant increase in  $\delta^{13}\text{C}$  ( $p = 0.02$ ) and a significant decrease in  $\delta^{15}\text{N}$  ( $p = 0.004$ ) moving downstream from GL3 to SLP. It should be noted, however, that the downstream increase in  $\delta^{13}\text{C}$  for transphilic acids in September ( $0.2\text{‰}$ ) was less than the estimated precision for  $\delta^{13}\text{C}$ . Similar to the shifts in the chemical content of the fulvic and transphilic acids, changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values matched with shifts in the elemental content of the two fractions. The enriched  $\delta^{13}\text{C}$  values for both isolates matched with lower C:N ratios (Figure 4a). Similarly, an increase in  $\delta^{15}\text{N}$  values showed a strong correspondence to a decrease in C:N ratios (Figure 4b).

#### *$^{13}\text{C}$ -NMR analyses*

The broad-banded nature of the  $^{13}\text{C}$ -NMR spectra suggests that both fractions are complex organic mixtures, however there were important structural

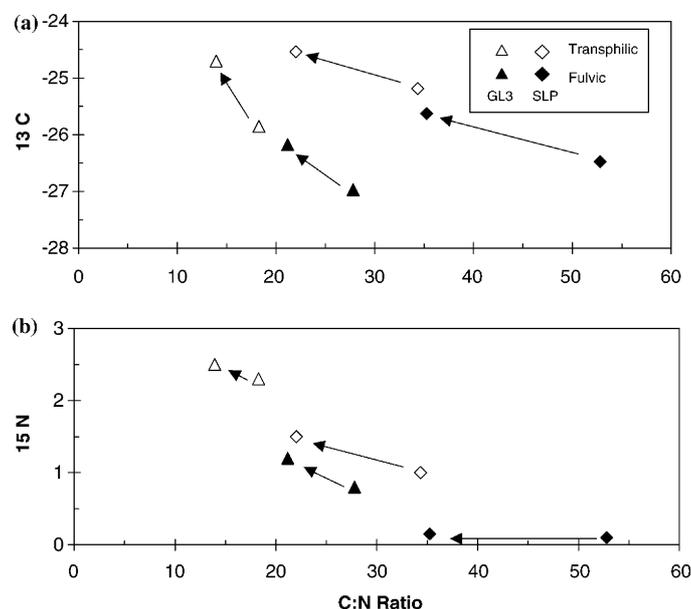


Figure 4. Diagram of the relationship between C:N ratio and  $\delta^{13}\text{C}$  (a) and  $\delta^{15}\text{N}$  (b) for fulvic and transphilic acids collected at an alpine site (GL3) and a forested subalpine site (SLP) in the North Boulder Creek catchment. Organic acid isolates collected at the alpine site are denoted by triangles, while those collected at the subalpine site are denoted by diamonds. Fulvic acids are denoted by solid symbols and transphilic acids are denoted by open symbols. Arrows indicate the change in isotopic values and C:N ratios between June (snowmelt) and August (late summer low flow).

differences between the two fractions (Table 3). The quantitative  $^{13}\text{C}$ -NMR spectra from fulvic and transphilic acids collected at SLP in September illustrate these differences, which were most apparent in the aromatic and aliphatic II regions (Figure 5). For all samples, the fulvic acids had significantly larger peaks in the aromatic region ( $p = 0.008$ ) and significantly smaller peaks in the aliphatic II region ( $p = 0.04$ ) compared to the transphilic acids. These chemical differences between the two DOM fractions are consistent with previous results from the Yakima River in Washington (Aiken et al. 1992) and Lake Fryxell, Antarctica (Aiken et al. 1996). The fulvic acids also had larger peaks in the aliphatic-I and ketone regions and smaller peaks in the carboxyl regions, though these differences were not statistically significant.

In both the alpine and subalpine, there was a decrease in the aromaticity of fulvic and transphilic acids between June and September (Table 3), although this seasonal shift was not statistically significant ( $p = 0.1$ ). Within the catchment, fulvic and transphilic isolates demonstrated a significant decrease in aliphatic II carbon ( $p = 0.007$ ) and significant increase in aromatic carbon ( $p = 0.02$ ) moving from the alpine GL3 site downstream to the forested SLP site (Table 3). These changes in the quantitative  $^{13}\text{C}$ -NMR spectra suggest that there may be a shift in the chemical structure of DOM moving across the

Table 3. Peak areas as a percentage of total spectrum area for quantitative  $^{13}\text{C}$ -NMR spectra of fulvic and transphilic acids collected at GL3 and SLP.

	Region								Ratio AR:AL-I
	Aliphatic-I 0–60 ppm	Aliphatic II 60–90 ppm	Acetal 90–110 ppm	Aromatic 110–160 ppm	Carboxyl 160–190 ppm	Ketone 190–230 ppm			
<i>Fulvic acids</i>									
GL3 June	35.8	23.3	7.3	15.7	13.9	4.1			0.44
SLP June	33.4	15.0	6.8	22.3	16.5	6.1			0.67
GL3 Sept	38.8	22.9	6.8	13.9	14.4	3.3			0.36
SLP Sept	39.9	17.0	5.5	16.6	15.6	5.4			0.42
<i>Transphilic acids</i>									
GL3 June	31.9	33.3	9.3	7.0	14.8	3.8			0.22
SLP June	33.1	21.8	8.4	11.9	19.4	5.4			0.36
GL3 Sept	35.1	32.7	8.7	6.6	14.1	2.9			0.19
SLP Sept	36.5	25.8	8.0	9.0	17.1	3.5			0.25

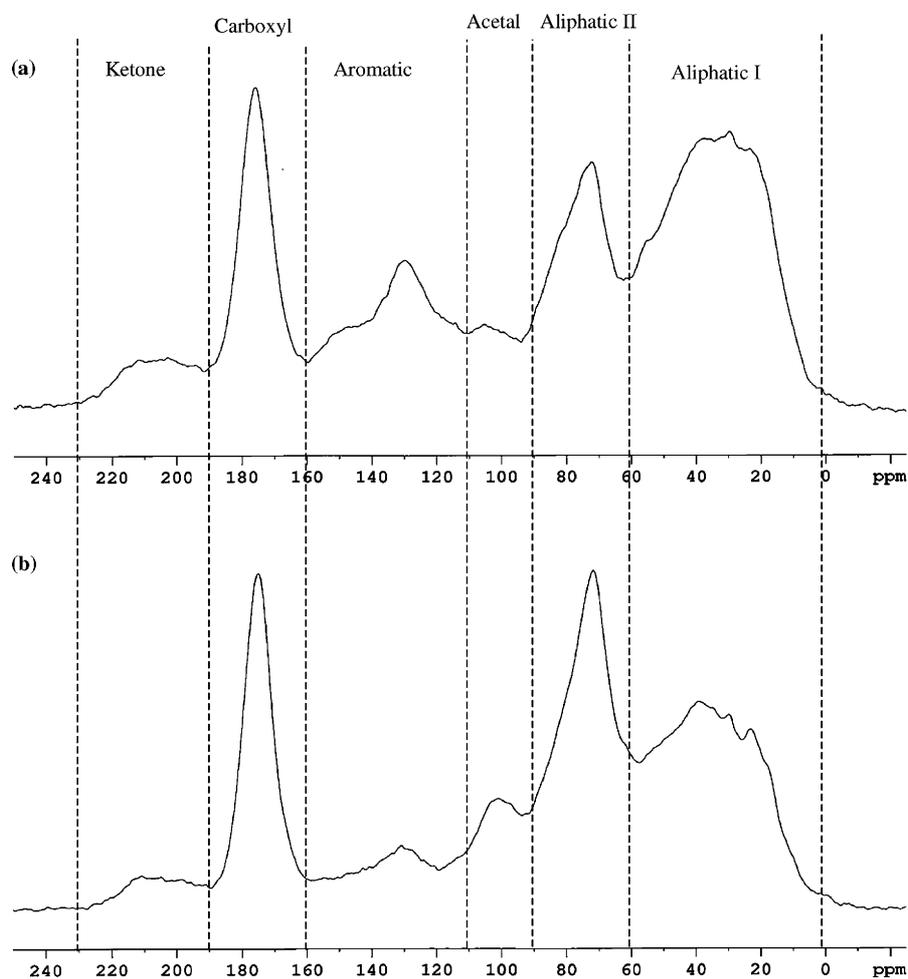


Figure 5. Quantitative  $^{13}\text{C}$ -NMR spectra for fulvic and transphilic acids collected at the subalpine SLP site in September.

alpine–subalpine ecotone. A comparison of fulvic and transphilic acids based on their C:N ratio and aromaticity (measured as the ratio of AR:AL-I; McKnight et al. 1994) showed that changes in chemical structure occurred in concert with changes in elemental content. For all samples, decreasing aromaticity in fulvic and transphilic isolates was strongly correlated ( $R^2 = 0.87$ ,  $p = 0.002$ ,  $n = 8$ ) with an increase in C:N ratio (Figure 6a). Shifts in the chemical structure of fulvic and transphilic acids also affected attenuation of UV radiation as evidenced by the strong positive correlation between specific UV absorbance at 254 nm and aromatic carbon content in the two isolates (Figure 6b).

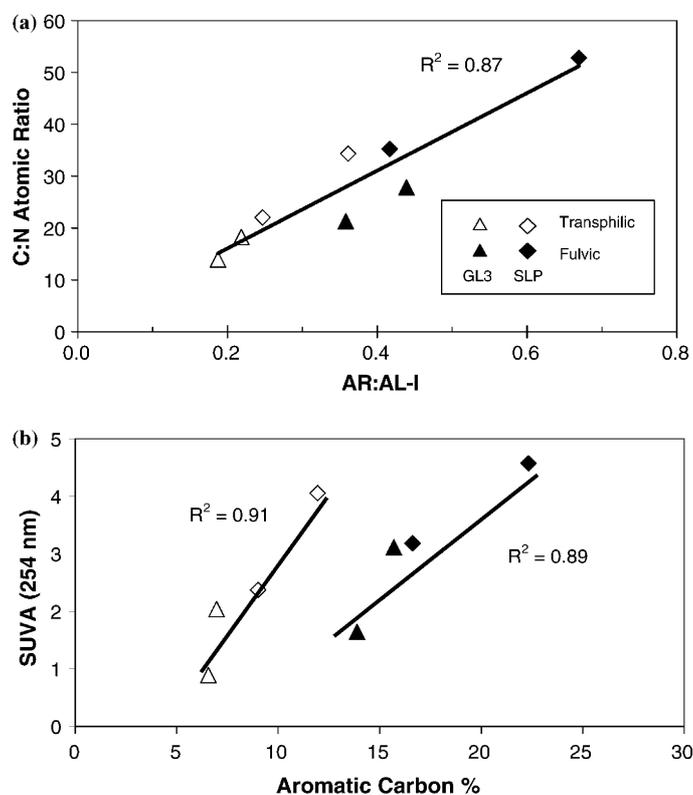


Figure 6. Diagram of (a) C:N ratio vs. the ratio of Aromatic to Aliphatic-I carbons (Ar:ALI) and in fulvic and transphilic acids and (b) SUVA at 254 nm (expressed as  $l/mg\ C * m$ ) vs. aromatic carbon content of fulvic and transphilic acids collected at an alpine site (GL3) and a forested subalpine site (SLP) in the North Boulder Creek watershed. Organic acid isolates collected at the alpine site are denoted by triangles, while those collected at the subalpine site are denoted by diamonds. Fulvic acids are denoted by solid symbols and transphilic acids are denoted by open symbols.

## Discussion

### *Seasonal trends in DOC and DOM sources*

The seasonal trends in DOC concentrations in North Boulder Creek are consistent with previous results from other montane environments in Colorado (Baron et al. 1991; McKnight et al. 1992; Hornberger et al. 1994; Boyer et al. 1997; Hood et al. 2003a, b). During the winter and spring, soluble organic material accumulates beneath the snowpack as a result of heterotrophic microbial activity (Brooks et al. 1999). The peak in DOC concentration on the ascending limb of the hydrograph is caused by snowmelt flushing of this soluble organic carbon from organic soil horizons and is similar to the increase of DOC

concentration during storm events in forested catchments (e.g. Buffam et al. 2001). The smaller peak in DOC at GL4 relative to SLP is due to the sparse vegetation and organic soil horizons in the upper reaches of the catchment.

Hood et al. (2003a, b) interpreted the seasonal trends in the fulvic acid content and fluorescence properties of fulvic acid in North Boulder Creek as showing that on the descending limb of the hydrograph a higher proportion of streamwater DOM is derived from aquatic precursor material in alpine lakes. The results from Green Lake 4 monitoring showing an increase in phytoplankton during late summer as residence time and temperature increase support this interpretation (Gardner 2003). Thus, the trends observed in this study agree with the results from previous years and the phytoplankton and SUVA results provide further support for the initial interpretation. DOM from algal precursor material has a low fulvic acid content compared to DOM from terrestrial sources (McKnight et al. 1994), therefore the observed late season decrease in the fulvic acid content is consistent with increased algal production, particularly in the alpine reach of the catchment. Because high FI values are significantly correlated with autochthonous precursor material (McKnight et al. 2001), the late season increase in the fluorescence index of fulvic acids, which is most evident at GL4, also suggests a relative increase in the proportion of autochthonous streamwater DOM. This finding is also consistent with the idea that allochthonous DOC from the terrestrial portion of the catchment is a more important influence in subalpine reach of the catchment.

The SUVA of fulvic acids has been shown to be a useful surrogate for aromatic carbon content because of the strong linear relationship between SUVA and aromaticity Chin et al. 1994; McKnight et al. 1997; Weishaar et al. 2003). Results from the fulvic and transphilic acid samples collected in North Boulder Creek demonstrated a strong correlation between increasing absorptivity (254 nm) and elevated aromatic carbon content. The strength of this relationship for both transphilic and fulvic acids was similar to that reported previously by Weishaar et al. (2003);  $R^2 = 0.94$ . The high SUVA values near peak snowmelt runoff suggest that the aromaticity of DOM peaks at this time and decreases on the descending limb of the hydrograph. This finding is consistent with snowmelt flushing of aromatic terrestrial DOM. The seasonal decrease in SUVA is consistent with a decrease in the proportion of DOM from terrestrial sources. Similarly, the increase in SUVA moving downstream between GL4 and SLP is consistent with increased terrestrial contributions of DOM lower in the catchment. The relationship between SUVA and the fluorescence index at both sites suggests that seasonal changes in the aromaticity of fulvic acids are to some extent linked to changes in humic precursor material.

The UV absorbance of fulvic acids can also be influenced by biogeochemical processing in lakes and streams. For example, photobleaching causes a decrease in SUVA suggesting that DOM is less aromatic after photobleaching. In contrast, photobleaching causes the FI to decrease slightly, which is indicative of an increase in the proportion of quinone-containing DOM from aromatic terrestrial sources (Mladenov 2004). The weaker relationship between

SUVA and FI at the alpine GL4 suggests that this processing may have different effects on the fluorescence and UV-absorbance properties of DOM. Overall, these results suggest that FI and SUVA provide complementary but distinct information about the factors controlling the chemical properties of humic DOM fractions. The FI provides an indication of the nature of DOM precursor material (aquatic vs. terrestrial), while SUVA provides information about DOM aromaticity.

#### *Relationship between DOM source and N content*

The range for the ash-free N content of fulvic acids in North Boulder Creek (1.2–2.7%) falls between the N-content of fulvic acids from other forested catchments (Malcolm 1990) and the N content of fulvic isolated from streams in Antarctica (3.1–4.9%; Aiken et al. 1996), where DOC is derived exclusively from autochthonous algal material. The range of N contents determined in this study is also broader than the range reported for these fractions in other high-elevation systems in Colorado (1.6–2.2%; McKnight et al. 1992, 1997). Because the carbon content does not vary much among fulvic acids, the C:N ratios for the four fulvic acids isolated in the study span much of the range in C:N ratio reported for fulvic acids isolated from diverse environments (McKnight et al. 1997). The N content of the transphilic acids is greater than the N content of the corresponding fulvic acids, as initially reported by Aiken et al. (1992), and follows the same pattern of change with source as the fulvic acids.

The relatively high N content of fulvic acids from the North Boulder Creek catchment, particularly at the alpine GL3 site, is consistent with the other changes in fulvic acid content and chemistry which suggest that streamwater DOM in the upper reaches of the watershed is at least partially derived from algal material in catchment lakes. The increase in N content and coincident decrease in the C:N ratio of both organic acid isolates between peak streamflow in June and low streamflow in September is consistent with the FI changes observed in this study and reflects the greater contribution of algal-derived DOC during the low-flow summer period, when the water residence time and the phytoplankton populations in the alpine lakes are greater.

This shift in C:N ratio may also reflect seasonal changes in hydrologic flowpaths characteristic of high-elevation catchments. On the descending limb of the hydrograph, soluble carbon stocks have been flushed from near-surface soils (Boyer et al. 1997) and the majority of water entering the stream in these systems is routed through deeper soil horizons (Caine et al. 1989; Sueker et al. 2000). Research in forested catchments in Europe has shown that the C:N ratio of soil solution DOM decreases with depth in the soil profile (Mulder and Clarke 2000). Three-component hydrograph mixing models using end-member mixing analysis at GL4 show that discharge was dominated by snowmelt runoff on the rising limb of the hydrograph in June and that subsurface flow dominated the hydrograph in September (Liu et al. 2004 (in press)). Thus, the

decrease in the C:N ratio of streamwater organic acids could be due in part to the leaching of deeper soils horizons containing DOM that is relatively enriched in N.

Fulvic and transphilic acids isolated in both June and September showed a downstream change in elemental stoichiometry with higher C:N ratios in the subalpine reach compared to the alpine. There are two possible mechanisms for this shift in DOM quality, which likely operate in concert. First, the downstream increase in the concentration of organic acids indicates that there was a source of DOM between the two sample sites. Intensive sampling along an elevational transect on North Boulder Creek has shown that concentrations of DOM are relatively constant in the alpine but increase sharply below treeline at Lake Albion (Hood et al. 2003a, b). Therefore the most likely source of DOM between GL2 and SLP is terrestrial material in the subalpine reach of the catchment which has a relatively low N content and would therefore lower the overall N content of organic acids at the SLP site. Second, the N content of DOM could be altered by selective abiotic or microbial removal (Moran and Hodson 1990; Cleveland et al. 2004). In particular, there is a significant positive relationship between the C:N ratio of fulvic acids and their bioavailability to natural bacterial assemblages (Hunt et al. 2000).

Like nitrogen, sulfur and phosphorus are minor constituents of DOM but play an important role in the biogeochemical processes of aquatic systems. The S content of fulvic and transphilic acids in North Boulder Creek was somewhat higher than values reported for forested sites in Colorado (McKnight et al. 1992), but lower than the S content of DOM derived from microbial material in Antarctic streams (Aiken et al. 1996). Interestingly, the P content of fulvic acids at the SLP site increased more sharply than either N or S between the June and September sampling dates. The reason for this sharp increase in P content is unclear.

The C:N ratio of the DOM fractions collected at both sites demonstrated an increase with increasing aromaticity. This finding provides further evidence that changes in the chemical character of the DOM fractions is linked with changes in DOM precursor material. In general, the aromaticity of fulvic acids in North Boulder Creek is on the low end for values reported for fulvic acids from a wide variety of aquatic environments (McKnight et al. 1997). This finding is consistent with an autochthonous contribution to streamwater DOM because fulvic acids derived from algal material are characterized by low aromaticities (McKnight et al. 1994). Further, the low point in aromaticity for both organic acid fractions was recorded at the alpine GL3 site in September, coincident with the late-July increase in algal biomass in the alpine lakes.

#### *Linkage between DOM source and stable isotopes of C and N*

The range of  $\delta^{13}\text{C}$  values we report for fulvic and transphilic acids in North Boulder Creek is consistent with  $\delta^{13}\text{C}$  values reported previously for surface

water fulvic acids in the montane Loch Vale catchment in Colorado ( $-24.1$  to  $-27.4\%$ ) (Baron et al. 1991; McKnight et al. 1997) and in a forested catchment in Canada ( $-27.0$  to  $-27.6\%$ ) (Schiff et al. 1990). Using DOC- $^{13}\text{C}$  measurements to separate sources of DOC at the watershed scale can be difficult because of both the overlap of  $\delta^{13}\text{C}$  values for different DOC precursor materials and the small size of isotopic fractionations affecting DOC cycling (Palmer et al. 2001; Raymond and Bauer 2001; Schiff et al. 1997). However, when viewed in the context of other information on DOM quality, such as the elemental content of the humic fractions,  $\delta^{13}\text{C}$  values do appear to be useful for elucidating potential shifts in DOM source material.

Both fulvic and transphilic acids in North Boulder Creek showed a significant seasonal and longitudinal enrichment in  $\delta^{13}\text{C}$ . Although the magnitude of these shifts in  $\delta^{13}\text{C}$  was small ( $<2\%$ ), they were relatively tightly coupled with decreases in the C:N ratio of both DOM fractions. This finding suggests that the shifts in  $\delta^{13}\text{C}$  values are due more to changing source material than to other processes such as seasonal variations in the  $\delta^{13}\text{C}$  of the dissolved inorganic carbon (DIC) (Kendall et al. 2001). The downstream enrichment of DOC- $^{13}\text{C}$  values in other systems has also been attributed to removal of DOM by heterotrophic bacteria and abiotic mechanisms (Elder et al. 2000; Raymond and Bauer 2001). We cannot assess the extent to which instream processing influences  $\delta^{13}\text{C}$  values of fulvic and transphilic acids as North Boulder Creek. However this type of selective DOM removal would be also consistent with the observed seasonal and longitudinal changes in the N content of DOM.

The range of  $\delta^{15}\text{N}$  values that we report for the organic acid isolates is consistent with values reported previously for fulvic acids (McKnight et al. 2002), whole DOM (Elder et al. 2000), and sediment organic material (Wolfe et al. 2001) in headwater streams. There are very few reports of  $\delta^{15}\text{N}$  values for transphilic acid fractions. Kendall (1998) reports that the transphilic acid fraction typically has a heavier  $\delta^{15}\text{N}$  value than the fulvic acid fraction. Our results are consistent with this observation although the reason for the difference in  $\delta^{15}\text{N}$  between the two fractions is unclear. Because DOM derived from plant material has a high fulvic acid content compared to DOM derived from algal material (McKnight et al. 1994), it is possible that the difference in  $\delta^{15}\text{N}$  values between the two fractions is a function of the fact that they are derived at least partially from different sources. This would further imply that algal material is enriched in  $^{15}\text{N}$  compared to terrestrial plant material. While we cannot verify that this is the case in North Boulder Creek, other studies have shown that planktonic material in freshwater (Kendall 1998) and saltwater (Peterson and Howarth 1987) ecosystems is typically enriched in  $^{15}\text{N}$  compared to terrestrial plant material.

Similar to  $\delta^{13}\text{C}$ , the ranges of  $\delta^{15}\text{N}$  values for sources of riverine organic material are overlapping, making it difficult to discriminate sources using  $\delta^{15}\text{N}$  values (Kendall et al. 2001). However,  $\delta^{15}\text{N}$  values for fulvic and transphilic acids on North Boulder Creek also demonstrated a strong negative correlation

to C:N ratio suggesting that shifts in  $\delta^{15}\text{N}$  values are also linked to shifts in DOM precursor material. Assuming that algal material is enriched in  $^{15}\text{N}$  relative to terrestrial material, the trends in  $\delta^{15}\text{N}$  values on North Boulder Creek would be consistent with a seasonal increase in the importance of autochthonous algal material as a source of DOM, particularly above treeline. The extent to which shifts in hydrologic flowpaths on the descending limb of the hydrograph alter  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values is unclear. Bulk soil organic matter typically becomes enriched in  $^{13}\text{C}$  at depth (Garten et al. 2000). Similarly, Kendall (1998) reports that  $\delta^{15}\text{N}$  values of DON in forest soils increase with age and depth. Fulvic and transphilic acids derived from terrestrial sources and deeper flowpaths in September would therefore be consistent with the observed seasonal enrichment in  $\delta^{13}\text{C}$  values, particularly at the subalpine SLP site. Taken together, the observed changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values on North Boulder Creek appear to be useful for elucidating spatial and temporal changes in the source and processing, both hydrologic and biogeochemical, of aquatic DOM.

### Conclusions

Coincident measurements of the fulvic acid content of DOM and the fluorescence and UV spectra of fulvic acids indicated that there were seasonal and longitudinal shifts in the source and chemical character of aquatic DOM in North Boulder Creek. These analyses can be performed on relatively small volume (200 ml) water samples and could readily be applied in other catchment-scale studies of DOM cycling. Preparative scale isolation of the two primary fractions of aquatic DOM, fulvic and transphilic acids, demonstrated that there were consistent chemical differences between DOM in the alpine and subalpine reaches of the catchment. In particular, organic acids collected in the alpine had a higher N content, were depleted in  $^{13}\text{C}$ , were enriched in  $^{15}\text{N}$ , and had a lower aromatic carbon content compared to organic acids collected in the forested subalpine. Relatively few studies have used stable isotope analyses to study catchment-scale processes that affect DOM cycling. Our results indicate that stable isotope analyses of both C and N are a useful tool, particularly when combined with ancillary data such as elemental analyses and catchment discharge, for evaluating sources and transformations of DOM at the catchment scale.

Taken as a whole, this suite of chemical, isotopic, and spectroscopic analyses indicates that DOM production in alpine lakes is an important contributor to the streamwater DOM load. However, the spatial and temporal changes in DOM properties that we observed are also consistent with known hydrologic controls on the delivery of allochthonous DOM from the terrestrial system. One implication of these findings is that in headwater catchments containing lakes, primary production in the lakes may be able to somewhat offset the decrease in DOM imported from the terrestrial system during low water years because of low flushing rates and lower water residence times in the lakes. Additionally, the relative contribution of DOM derived from autochthonous sources such as

algal biomass in the lakes may continue to rise in coming decades as a result of increased algal growth due to atmospheric deposition of inorganic N.

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