Chemical Characteristics of Particulate, Colloidal, and Dissolved Organic Material in Loch Vale Watershed, Rocky Mountain National Park

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Abstract. The chemical relationships among particulate and colloidal organic material and dissolved fulvic acid were examined in an alpine and subalpine lake and two streams in Loch Vale Watershed, Rocky Mountain National Park. The alpine lake, Sky Pond, had the lowest dissolved organic carbon (DOC) (0.37 mgC/L), the highest particulate carbon (POC) (0.13 mgC/L), and high algal biomass. The watershed of Sky Pond is primarily talus slope, and DOC and POC may be autochthonous. Both Andrews Creek and Icy Brook gain DOC as they flow through wet sedge meadows. The subalpine lake, The Loch, receives additional organic material from the surrounding forest and had a higher DOC (0.66 mgC/L). Elemental analysis, stable carbon isotopic compositon, and ^{13}C-NMR characterization showed that: 1) particulate material had relatively high inorganic contents and was heterogeneous in compositon, 2) colloidal material was primarily carbohydrate material with a low inorganic content at all sites; and 3) dissolved fulvic acid varied in compositon among sites. The low concentration and carbohydrate-rich character of the colloidal material suggests that this fraction is labile to microbial degradation and may be turning over more rapidly than particulate fractions or dissolved fulvic acid. Fulvic acid from Andrews Creek had the lowest N content and aromaticity, whereas Sky Pond fulvic acid had a higher N content and lower aromaticity than fulvic acid from The Loch. The UV-visible spectra of the fulvic acids demonstrate that variation in characteristics with sources of organic carbon can explain to some extent the observed non-linear relationship between UV-B extinction coefficients and DOC concentrations in lakes.

Introduction

Particulate, colloidal and dissolved organic material can be derived from both external sources (allochthonous) and from within the aquatic ecosystem (autochthonous), with wetlands and littoral zones considered as part of the aquatic ecosystem (Wetzel 1990, Lock & Ford 1986). The production, consumption and transformation of these different forms of organic material are intimately linked to the transfer of energy within aquatic ecosystems (e.g. Hessen et al. 1990). In many aquatic ecosystems, the largest pool of organic material in the water column is dissolved organic material (DOM).

The microbial degradation of DOM can be an important process in carbon cycling, directly influencing ecosystem dynamics (Wetzel 1990). Furthermore, certain fractions of DOM can influence chemical and physical characteristics of an aquatic ecosystem and can indirectly influence ecosystem dynamics (Steinberg & Meunster 1985; McKnight 1991). For example, fulvic acids, which are defined as yellow-colored, moderate molecular weight, biologically recalcitrant organic acids, are important in light attenuation, pH buffering, and complexation of trace metals. Complexation of trace metals can regulate the bioavailibility of metals as nutrients or toxicants. Dissolved fulvic acid absorbs UV and visible radiation and may be particularly significant in the response of freshwater ecosystems to elevated UV-B radiation associated with ozone depletion.

Common practice has been to define particulate organic carbon as that which is retained by a 0.45 μm filter and dissolved organic carbon as that passing through the filter. Most bacteria are larger than 0.45 μm, and such a cutoff has a clear utility (Malcolm & Leenheer 1973). However, such an operationally-defined dissolved fraction can include colloidal particles, which are particles that are much larger than most specific biomolecules, but are small enough that their motion in water is controlled by surface effects rather than by bulk solution effects. Particulate and colloidal organic material includes detrital plant and soil material, whereas particulates can also include living aquatic organisms. Dissolved organic carbon is also heterogeneous, composed of different classes of acids, bases and neutral compounds (Leenheer & Huffman 1979). Dissolved fulvic acid is commonly the largest fraction of the DOC (40–60%) and can be isolated using chromatographic techniques based upon hydrophobic character and acidic functional group content (Malcolm 1990). Dissolved fulvic acids have a molecular weight range of 500 to 1200 daltons (Aiken & Malcolm 1987; Aiken & Gillam 1989).

Application of fractionation and ultrafiltration techniques can provide insight into specific biogeochemical processes involving detrital organic material. In a study of Mirror Lake in New Hampshire, Cole et al. (1984) found that lakewater was enriched in colloidal DOC (1.2 μm to 100,000 daltons) compared to inflowing streamwater or soilwater and concluded that this colloidal DOC was produced within the lake in association with algal growth and decomposition. In a study of streams in Quebec, Lock and Ford (1986) found that colloidal organic carbon (0.7 μm to 300,00 daltons) was dominant when streams received runoff from muskeg areas. Ranville et al. (1991) found that particulate and colloidal organic material was primarily algal-derived in a large reservoir in Colorado and that organic colloids were enriched in trace elements. In alkaline and humic lakes in the Netherlands, most of the dissolved Al was complexed by low molecular weight ligands

(De Haan et al. 1991). The chemical characteristics of dissolved fulvic acids have been studied in a range of surface waters, and consistent differences have been found between fulvic acids from algal-rich environments and those from systems dominated by organic inputs from plants and soils (McKnight et al. 1994).

Here, we examine the continuum between particulates, colloids and dissolved fulvic acids in samples collected from two lakes and two streams in an alpine/subalpine watershed. Previous studies in Loch Vale Watershed have focused on seasonal variations in DOC and fulvic acid (generally 25–65% of the DOC) and showed that during snowmelt flushing through organic rich sediments was a possible DOC source in the alpine lake while flushing of forest soils was a source for the subalpine lake (Baron et al. 1991). This study addresses two main questions: 1) Does the size continuum correspond to a continuum in chemical properties of organic material or are there clear chemical differences among fractions? and 2) How do the sources of aquatic organic material influence the chemical properties of particulate, colloidal and fulvic acid carbon? In addressing these questions, we have employed analytical methods which characterize the "bulk" properties of organic carbon, specifically elemental analysis, stable carbon isotopic composition, and ^{13}C-NMR and UV-visible spectroscopy. As a final objective, the UV-visible spectra for fulvic acids are interpreted in the context of observed relationships between UV absorbance and DOC in lakes (Scully & Lean 1994)

Site description

Loch Vale Watershed in Rocky Mountain National Park is an alpine/subalpine watershed containing three small lakes which are connected by Icy Brook (Figure 1). Table 1 presents physical and chemical characteristics of these small shallow lakes. During the late summer, fall and winter, the alpine lake, Sky Pond, has longer residence times than the other two lakes. Residence times are shorter during spring snowmelt (April-June) than during the rest of the year in all lakes. This rapid flushing in combination with the shallow depths and strong winds prevents thermal stratification during open water (Baron 1992). For this study samples of organic material were collected at four sites (Figure 1): (1) the outlet of Sky Pond (SO), (2) Icy Brook (IB), above the confluence with Andrews Creek, (3) Andrews Creek (AC), a small stream draining Andrews Glacier, above its confluence with Icy Brook, and (4) the outlet of The Loch (LO).

The watershed of Sky Pond is composed of bedrock outcrop, glaciers, and talus fields where large boulders are interspersed with tundra underlain by thin minimally developed Entisols (Baron 1992). The phytoplankton in

Table 1. Lake characteristics in Loch Vale Watershed, Rocky Mountain National Park. Physical characteristics and median water quality values are for 1983–1988 from Baron 1992; the water quality values on 8/12/93 are listed in parentheses and values on that date from Icy Brook are listed with the Glass Lake data.

	Sky Pond	Glass Lake	The Loch
Elevation (m)	3,322	3,292	3,048
Watershed area (ha)	204	257	660
Surface area (ha)	3.03	1.01	4.98
Average depth (m)	4.5	2.8	1.5
Residence time (days)			
April-June	3.9	6.9	6.1
August-March	105	18.3	16.4
Extinction coefficients[*]	0.33–0.53	–	0.34–0.49
pH	6.5	6.5	6.3
Alkalinity (μeqL^{-1})	30	38	43
Ca (μeqL^{-1})	6 (37)	65 (46)	70 (45)
Mg (μeq L^{-1})	15 (8.5)	17 (10.6)	20 (11.4)
Na (μeq L^{-1})	16 (10)	17 (13)	22 (15)
K (μeq L^{-1})	3.8	4.3	5.3
Cl (μeq L^{-1})	2.6	3.3	4.1
SO$_4$ (μeq L^{-1})	29	33	36
NH$_4$ (μeq L^{-1})	1.2	1.2	1.4
NO$_3$ (μeq L^{-1})	15	13	16
SiO$_2$ (mgL^{-1})	1.2 (1.1)	1.2 (1.1)	1.9 (1.6)
DOC (mgL^{-1})	0.8 (0.37)	0.8 (0.59)	1.4 (0.66)

Sky Pond is more abundant than in the other lakes, and is dominated by a diatom, *Asterionella formosa*, in the spring and a filamentous cyanobacteria, *Oscillatoria limnetica*, during August and September (McKnight et al. 1988, 1990). Lake sediments are composed of rocks, boulders and organic gyttja resulting from deposition of algal material, and interstitial DOC concentration was 5.3 mgC/L. The inlet to Sky Pond drains only 20% of the watershed, and has low DOC concentrations of 0.1 to 0.4 mg C/L (Baron et al. 1991). The DOC in Sky Pond reaches peak concentrations of about 1.6 mg C/L during snowmelt, possibly a result of flushing of snowmelt through organic-rich sediments (Baron et al. 1991). Glass Lake, located at the top of a hanging valley in the alpine portion of the watershed has cliffs and boulder fields along most of the shore and has sediments composed of gyttja and rocks. The transition between the alpine and subalpine occurs at Timberline Falls below the outlet of Icy Brook from Glass Lake. The reach of Icy Brook between the falls and the confluence with Andrews Creek contains extensive wet sedge meadows.
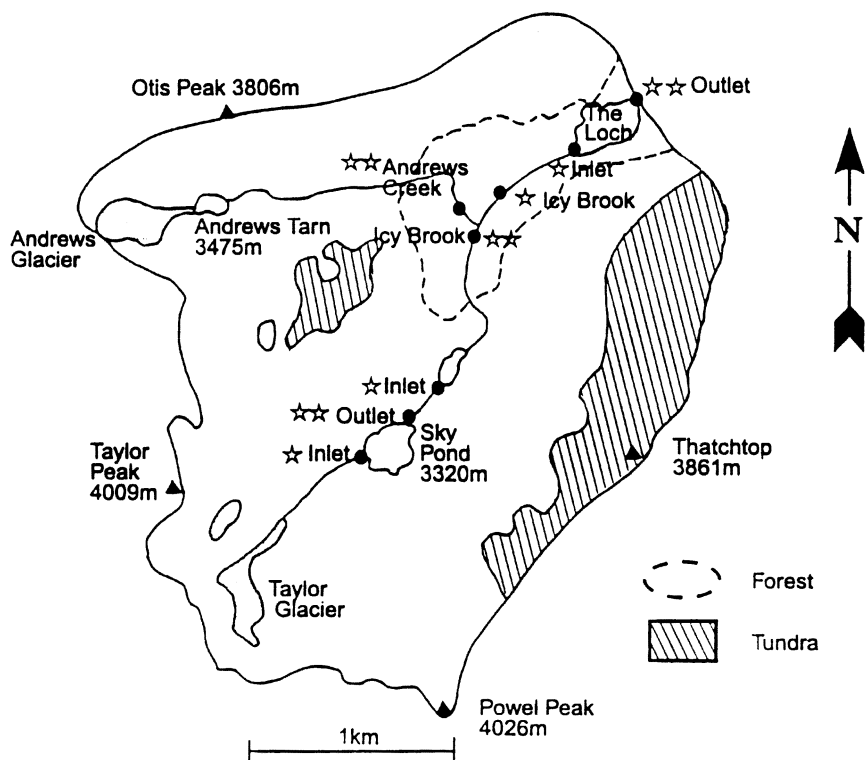
Figure 1. Map of Loch Vale Watershed showing sampling sites.

Andrews Creek originates from Andrews Glacier flows through a large wet sedge meadow before joining Icy Brook as its largest tributary. The inflow to The Loch is carried by Icy Brook as well as by several intermittent streams which flow during snowmelt. The subalpine forest surrounding The Loch is composed of old-growth Englemann spruce and subalpine fir, ranging from 200–500 years in age (Arthur & Fahey 1992). The forest soils are well-developed and DOC concentrations measured in soil lysimeters within the forest floor and root zone are about 20–30 mgC/L during the spring (Baron et al. 1991). These forest soils are a source of DOC to Icy Brook and The Loch, particularly during spring snowmelt when DOC concentrations peak at 4.5 mgC/L (Baron et al. 1991). The sediments of The Loch are composed of gyttja and sand. The phytoplankton in The Loch has a similar species composition but lower abundance than in Sky Pond (McKnight et al. 1990).

Table 2. Spectral regions for different functional group carbon atoms in ^{13}C NMR spectra of plant and microorganisms tissue and natural organic substances. The chemical shifts are given in ppm (parts per million) from tetramethylsilane (TMS) resonance at 0 ppm.

Designation	Spectral Regions	Functional Group Carbons
AL-I	0–62 ppm	Aliphatic carbons
AL-II	62–90 ppm	Alcoholic carbons in carbohydrates and other aliphatic alcohols and ethers
AL-III	90–110 ppm	Anomeric carbons in carbohydrates and some aromatic and olefinic carbons
AR	110–160 ppm	Aromatic and olefinic carbons
C-I	160–190 ppm	Carboxylate and amide carbons
C-II	190–230 ppm	Carbonyl carbons in aldehydes and ketones

Sampling and analytical methods

Samples were collected at the four sites during July and August and additional surface waters in Loch Vale were sampled on August 11, 1993. DOC samples were filtered through precombusted GFC filters, having a nominal pore size of 1.2 μm. The next day, large volume water samples (505–665 L) were collected in order to obtain preparative quantities of organic material for characterization. Table 3 lists the quantity of water processed and material obtained for the four sites. Water was collected in plastic buckets, transferred to 5-gallon cubitainers, and transported to the USGS laboratory in Boulder, CO within 7 hours of collection. Samples were transferred to 55 gallon polycarbonate barrels and stored on ice during processing. The organic carbon concentration in the GFC filtered samples collected on August 11 were similar to the concentrations of the 5.0 μm-filtered samples from August 12, and consistently greater than the concentrations in the 0.45 μm-filtered samples (Table 4).

We separated particulate and colloidal fractions using tangential flow ultrafiltration which alleviates problems of aggregation and clogging of pores that may occur in standard filtration. The details of the procedures are as follows: 1) Spiral flow filtration through a 5.0 μm polycarbonate filter; 2) tangential-flow ultrafiltration through a 0.45 μm Millipore filter membrane (HVMP000 01) using a Millipore Pelicon filtration unit; 3) tangential-flow ultrafiltration through a 100,000 Dalton Millipore filter membrane (PIHK000 01) as in step 2 and 4) isolation of fulvic acid from the filtrate obtained in step 3 using preparative scale column chromatography with XAD-8 resin following the method of Thurman & Malcolm (1981). The isolation procedure of Thurman & Malcolm (1981) produces comparable fulvic acid samples that are operationally defined relative to a set of chemical properties controlling

affinity of organic solutes for the XAD-8 and cation exchange resins and was also used in the study by Baron et al. (1991). The cation exchange resin used to hydrogen saturate the fulvic acid may selectively remove fulvic acid molecules with N-containing basic moieties. The Millipore filters were pre-cleaned with 0.1N NaOH, distilled water, 0.1N HCl and distilled water in sequence for about 30 minutes. Processing of each sample took about 36 hours. Particulate and colloidal material in the retentates obtained in steps 1–3 were freeze-dried in a small volume (250–1200 mL) of sample water. Aliquots of particulate and colloidal suspensions were retained for analysis. Fulvic acid samples were reconcentrated, desalted and freeze-dried. Fulvic acid concentration was calculated based upon a 90% efficiency of recovery of material and the carbon contents of fulvic acids. Some loss of material probably occurs during the cation exchange step and removal of the sample from the flask after freeze-drying.

Water samples were analyzed for standard water quality parameters. DOC concentrations of samples and filtrates were determined at the US Geological Survey laboratory in Boulder by UV-persulfate oxidation using an Oceano-graphic International instrument or a Dohrman high temperature combustion instrument for the samples collected in July 1993. Between 4–8 replicate DOC analyses were done for the synoptic and ultrafiltration studies and the standard deviation for these analyses was typically 0.02 mgC/L, with a range of 0.01 to 0.04 mgC/L. The samples collected at the 4 sites during July and August were also analyzed by another laboratory using a high tempera-ture combustion method, but the reported DOC concentrations were a factor of two lower than our results and were too low to account for the amount of colloidal material and fulvic acid recovered from the large volume samples. Cation concentrations were determined by inductively coupled plasma atomic emission spectroscopy and anion concentrations were determined by ion chromatography.

Lyophilized particulate, colloidal and fulvic acid samples were analyzed for elemental content by Huffman Laboratories (Huffman & Stuber 1985). For determination of ^{13}C, dried organic matter was combusted to CO_2 at $550\,^{\circ}C$ in vacuum sealed pyrex glass tubes with CuO and Ag wire. Ratios of $^{13}C/^{12}C$ were measured on a VG Prism mass spectrometer and reported in δ‰ units relative to PDB. Analytical errors are less than +/– 0.2‰.

Samples were analyzed by quantitative natural abundance ^{13}C-NMR spectroscopy with a solid-state Chemagnetics CMX spectrometer. Cross polarization-magic angle spinning (CP/MAS) ^{13}C spectra were measured at 50.3 MHz with a 7.5 mm-diameter probe. The spinning rate was 5000 Hz, with a contact time of 2 ms and a pulse delay of 1 s. A line broadening of 100 Hz was applied in fourier transformation of the free induction decay data.

Table 3. Total mass recovery of particulates and colloidal material from lake and stream sites in Loch Vale Watershed. The value in parentheses indicates the correction factor applied to account for the removal of an aliquot of the retentate prior to freeze-drying.

Site	Volume	Large Particulates		Small Particulates		Colloids	
	L	Total Mass mg	Conc. mg/L	Total Mass mg	Conc. mg/L	Total Mass mg	Conc. mg/L
Sky Pond	627	122 (1.1)	0.21	151 (1.08)	0.26	66 (1.08)	0.11
Icy Brook	665	175 (1.08)	0.28	82 (1.09)	0.13	13 (1.05)	0.02
Andrews Creek	505	42 (1.11)	0.09	18 (1.04)	0.04	53 (1.11)	0.12
The Loch	665	64 (1.1)	0.11	140 (1.09)	0.23	13 (1.14)	0.02

The chemical shifts were calibrated with hexamethlybenzene (methyl carbon at 17.35 ppm). The major bands that have been reported in the literature for humic substances from many environments are listed in Table 2 (Wershaw 1992). The consistency in the positions of these bands is due to the general similarity of the constituent functional groups in detrital organic material. These functional groups are derived from the partial degradation of structural components of plants and microorganisms (Wershaw 1992). Degradation of vascular plants and microorganisms gives rise to carbohydrates, lignin, lipids and proteins. Proteins and lipids are generally more abundant in microorganisms than in vascular plant tissue. The variations in relative intensities of the spectral bands arise from differences in functional group distribution and may be related to differences in sources of organic material. Dominance of woody debris and other lignin-derived material would contribute to a substantial AR peak, reflecting the aromatic rings of lignin. For fulvic acids, carboxylic acids are the major acidic functional groups, and this is reflected in a prominent C-I peak. Therefore, ^{13}C-NMR spectra provide a means of assessing the origin, fate and reactivity of aquatic organic material.

For light absorbance measurements, fulvic acids were dissolved in distilled water at a concentration between 25 and 45 mg C/L with a pH between 3.85 and 4.15. Light absorbance was determined using a Pye Unicam PU 8800 UV/Vis spectrophotometer with a 1-cm cell and absorbance measurements were adjusted to a per milligram DOC per L per centimeter.

Results and discussion

Organic material concentrations and composition

In Loch Vale Watershed, DOC concentrations of surface waters typically peak during spring snowmelt (May-June) and decrease through July and August

Table 4. Organic carbon concentrations (mgC/L) in Loch Vale Watershed.

| Site | Unfiltered | Filtrates | | | |
		5 μm	1.2 μm 8/11	0.45 μm 8/12	10^6 Dalton
Sky Pond					
inlet	–	–	0.38	–	–
outlet	0.51	0.53	0.51	0.4	0.37
Glass Lake					
inlet	–	–	0.53	–	–
outlet	–	–	0.52	–	–
Icy Brook*	0.66	0.64	0.66	0.59	0.51
Andrews Creek	0.47	0.47	0.47	0.42	0.39
Icy Brook*	–	–	0.59	–	–
The Loch					
inlet	–	–	0.59	–	–
outlet	0.72	0.71	0.72	0.66	0.57

* Sites in Icy Brook are located above and below the confluence with Andrews Creek.

(Figure 2) (Baron et al. 1991). In this study, DOC concentrations at all sites were less than 1 mg C/L by late July. These values are typical for Loch Vale, and are at the lower range for temperate surface waters (Baron et al. 1991; Thurman 1985).

The recovery of total mass, including both organic and inorganic material, in the different size fractions is shown in Table 3. Particulate and colloid mass recovery was greater than 0.09 mg/L except for three low samples (Table 3). For all sites, concentrations of large particulates (>5 μm) ranged from 0.09 to 0.28 mg/L. These fractions may have contained the filamentous cyanobacteria *Oscillatoria limnetica* which is commonly abundant in August. Smaller algal cells and bacteria should have occurred as small particulates. The large and small particulates from The Loch and the streams were more likely than those from Sky Pond to contain significant quantities of plant and soil derived material.

Both temporal (Figure 2) and synoptic sampling results (Table 4) show the pattern of DOC increasing below Sky Pond, as vegetation becomes more abundant in subalpine areas. At all sampling sites, the concentration of organic carbon decreased progressively with filtration through membranes with smaller pore sizes. These reductions in organic carbon concentration were used to calculate by difference the concentration of organic carbon in particulate or colloidal size fractions (Table 5). The calculation by difference
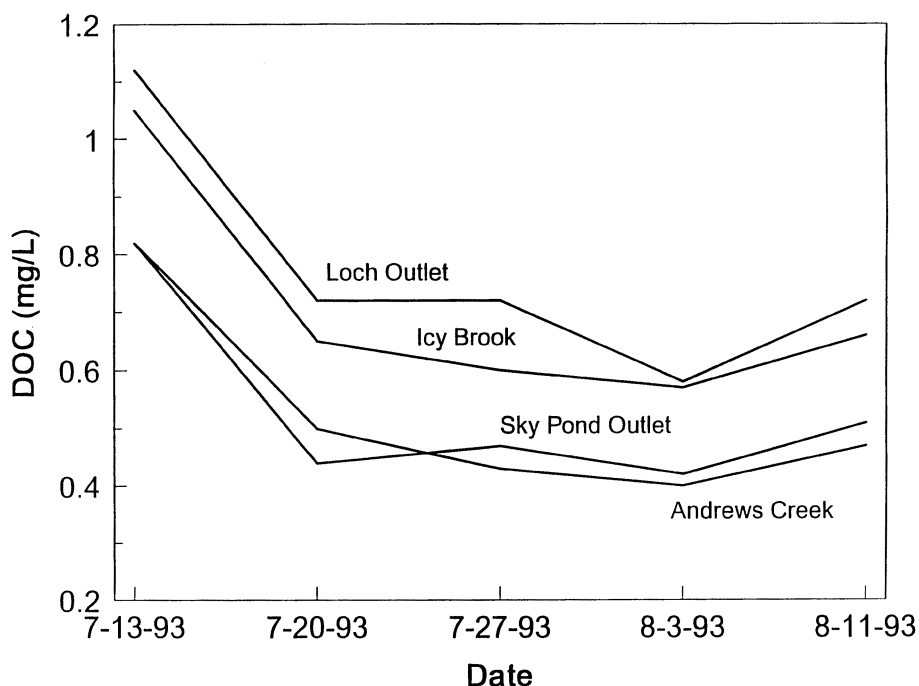
Figure 2. DOC concentrations at the four sampling sites during July and August, 1993.

resulted in low or undetectable concentrations of organic carbon greater than 5 μm.

The elemental contents and $\delta^{13}C$ of the particulates, colloids and fulvic acids differed between sites (Table 6). The ash content of large and small particulates was substantial, accounting for about 41–54% by mass. The ash-free contents for C, N, S and P should be considered with caution for the particulates, because significant concentrations of these elements may have been associated with carbonates or other mineral phases, and the ash-free contents of H and O are not presented because of probable influence of oxides. The ash contents of the colloids (about 15 to 26%) were lower than those of the particulates, indicating a lesser influence of inorganic mineral phases. Ash-free elemental contents for colloidal material were constrained within narrow ranges, e.g. C: 39–44%, H: 6.5–7.3%, O: 44–51%, N: 4.1–5.4%. Ash contents of fulvic acids were about 5–14%, and elemental contents were generally similar to those of other aquatic fulvic acids (McKnight et al. 1994).

We used the carbon contents in Table 6 and the recovery data in Table 3 to calculate the concentration of organic carbon in different size fractions (Table 5). The sums of the recovery estimates of large and small particulates were 0.13 mgC/L for Sky Pond and 0.09 mgC/L for The Loch. These con-

Table 5. Comparison of organic carbon concentration in different size fractions as calculated by difference from measurements of filtrates and by recovery of material during ultrafiltration and carbon analysis of solid material. Large particulates are greater than 5 μm in diameter; small particulates are between 5 μm and 0.45 μm in diameter; colloids are between 0.45 μm and 100,000 daltons in diameter; and fulvic acids are less than 100,000 daltons, isolated following Thurman and Malcolm (1981); – indicates insufficient sample for analysis.

	Carbon concentration (mgC/L)	
	by difference	by recovery
Large Particulates		
Sky Pond	–	0.05
Icy Brook	0.02	0.06
Andrews Creek	–	0.02
The Loch	0.01	0.03
Small Particulates		
Sky Pond	0.14	0.08
Icy Brook	0.05	0.03
Andrews Creek	0.05	0.007
The Loch	0.05	0.06
Colloids		
Sky Pond	0.03	0.035
Icy Brook	0.08	0.006
Andrews Creek	0.03	0.036
The Loch	0.09	0.009

centrations are in the same range (0.15 to 0.8 mgC/L) as POC concentrations (retained on 1.2 μm GFC filter) in Sky Pond and The Loch during July and August reported by Baron et al. (1991). They are also consistent with the higher POC concentration in Sky Pond caused by the greater phytoplankton abundance. Carbon concentrations based on recovery gave higher concentrations of organic carbon in large particulates than calculations based on difference. This discrepancy may result from incomplete oxidation or settling of large particulates during analysis of unfiltered samples. In contrast, recovery estimates were much lower than those based on difference for small particulates from Andrews Creek and colloids from Icy Brook and The Loch. These recovery estimates may have been biased by mechanical losses of material during processing. The range of colloidal carbon concentrations of 0.03 to 0.09 mgC/L indicated by the difference calculations were the more credible results.

Table 6. Elemental analysis of particulate, colloidal and dissolved fulvic acid from Loch Vale Watershed. Ash-free values are presented parenthetically for colloids and fulvic acids. Large particulates are greater than 5 μm in diameter; small particulates are between 5 μm and 0.45 μm in diameter; colloids are between 0.45 μm and 100,000 daltons in diameter; and fulvic acids are less than 100,000 daltons, isolated following Thurman and Malcolm (1981); – indicates insufficient sample for analysis.

	Elemental Content (% by weight)							C:N ratio	$\delta^{13}C$ (‰)
	C	H	O	N	S	P	Ash		
Large Particulates									
Sky Pond	21.6 (45.5)	3.7	22.1	2.4 (5.1)	0.26 (0.55)	0.25 (0.53)	52.5	10.5	–22.1
Icy Brook	20.4 (43.9)	3.5	21.8	2.0 (4.3)	0.24 (0.52)	0.23 (0.49)	53.5	11.9	–23.7
Andrews Creek	23.4 (48.5)	3.8	19.8	1.5 (3.1)	–	–	51.8	18.2	–25.1
The Loch	29.0 (53.3)	4.7	18.6	1.9 (3.5)	–	–	45.6	17.8	–25.1
Small Particulates									
Sky Pond	29.0 (49.8)	4.8	19.7	3.4 (5.8)	0.61 (1.0)	0.60 (1.0)	41.8	9.9	–23.4
Icy Brook	24.3 (43.8)	4.1	24.8	3.6 (6.5)	–	–	44.5	7.9	–22.2
Andrews Creek	16.6 (36.2)	3.1	22.1	–	–	–	54.2	–	–
The Loch	25.2 (43.0)	4.3	26.2	3.1 (5.3)	0.85 (1.4)	0.76 (1.2)	41.4	9.5	–22.9
Colloids									
Sky Pond	35.9 (44.4)	5.9 (7.3)	36.3 (44.9)	3.7 (4.6)	–	–	19.2	11.3	–22.5
Icy Brook	30.6 (39.2)	5.1 (6.5)	39.9 (51.1)	4.2 (5.4)	–	–	22.0	8.5	–23.3
Andrews Creek	30.1 (40.6)	4.8 (6.5)	–	–	–	–	25.8	–	–
The Loch	35.0 (41.3)	5.7 (6.7)	37.7 (44.5)	3.5 (4.1)	–	–	15.2	11.7	–
Fulvic Acids									
Sky Pond	47.3 (51.0)	4.8 (5.2)	37.3 (40.2)	1.95 (2.1)	–	–	7.16	28.3	–25.3
Icy Brook	48.3 (50.9)	4.5 (4.7)	38.7 (40.8)	1.75 (1.8)	0.55 (0.58)	0.033 (0.035)	5.16	32.2	–25.8
Andrews Creek	46.3 (50.3)	4.5 (4.9)	38.4 (41.7)	1.46 (1.6)	0.73 (0.79)	0.028 (0.030)	8.0	37.0	–26.5
The Loch	44.3 (51.7)	4.3 (5.0)	33.9 (39.6)	1.5 (1.75)	0.54 (0.63)	0.040 (0.047)	14.35	34.5	–25.8

Table 7. Fulvic acid concentrations in surface waters in Loch Vale Watershed. DOC refers to organic carbon concentration of 100,000 dalton filtrate.

Site	Volume of filtrate (L)	Fulvic Acid[*]			
		Recovered quantity (mg)	Concentration (mg C/L)	Percent of DOC	AR/AL-I[**]
Sky Pond	575	65.4	0.06	16	0.49
Icy Brook	660	185.7	0.15	30	0.51
Andrews Creek	525	104.3	0.10	26	0.40
The Loch	480	217.2	0.22	39	0.67

[*] Concentration was calculated based upon the recovered quantity of fulvic acid divided by the filtrate volume, multiplied by the organic carbon content (Table 6), and divided by 0.9 to account for losses of material during processing.
[**] From ^{13}C-NMR spectra in Figure 8.

Although N, S and P were minor constituents of the organic materials, these elements can significantly influence biogeochemical processes in aquatic systems. In large and small particulates, these elements could have been incorporated in living organisms (e.g. algal cells) as well as in detrital material. Small particulates had higher concentrations of N and lower C:N ratios than large particulates (Table 6). The limited data available for S and P also indicated higher concentrations in small particulates than in large particulates. Lignaecous material from higher plants does not contain much N, S or P; a greater abundance of woody detritus relative to microorganisms in large particulates could explain these results. The N contents and C:N ratios of colloids were comparable to those of small particulates.

Fulvic acid concentrations increased and fulvic acid became a more significant percentage of the DOC in the transition from Sky Pond to subalpine surface waters (Table 7), as observed by Baron et al. (1991). For The Loch, the fulvic acid percentage was similar in this and the previous study; however, for Sky Pond the percentage in this study was about half of the average percentage in the previous study (16% vs. 34%). The higher fulvic acid percentages were associated with peak snowmelt DOC concentrations in Sky Pond (Baron et al. 1991), whereas the lower percentages in August may be more representative for periods of reduced water renewal. In studies of other algal-dominated lakes, fulvic acid has been found to account for about 20–25% of the DOC (McKnight et al. 1994).

The ash-free nitrogen content of the fulvic acid was greatest in Sky Pond (2.1%) and least in Andrews Creek (1.6%) (Table 6), representing the range associated with different organic sources in Loch Vale, specifically algal material in the water column and sediments of Sky Pond and wet sedge meadow draining into Andrews Creek. A higher N content is expected for
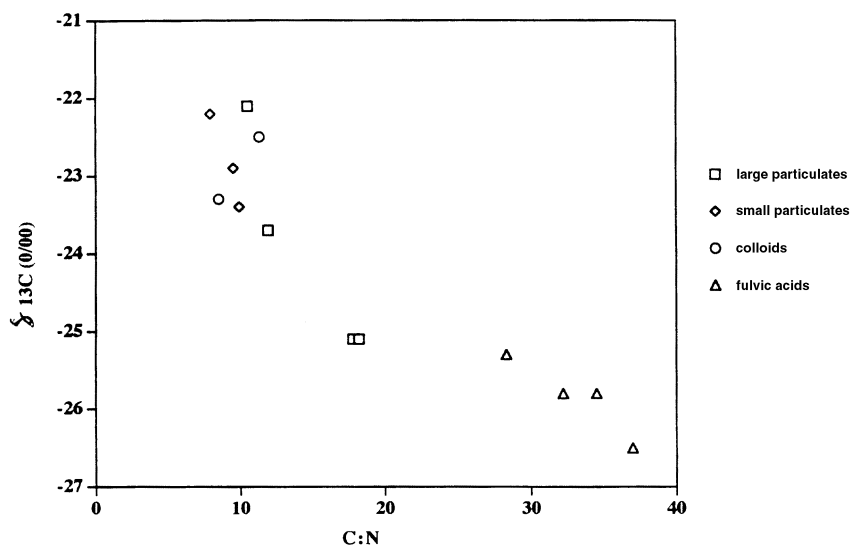
Figure 3. Diagram of the relationship between C:N ratio and δ13C for the Loch Vale samples.

algal-derived fulvic acids because algal cells contain significant N while the lignaeceous material in higher plants does not (McKnight et al. 1994). The fulvic acid concentration increased from 0.06 mgC/L to 0.15 mgC/L between the Sky Pond outlet and the Icy Brook sampling site (Table 7). The most probable source for this additional fulvic acid was the wet sedge meadow. The decrease in the N content of fulvic acid from Sky Pond outlet to Icy Brook could be accounted for by the addition of fulvic acid with the same N content as the Andrews Creek acid. The fulvic acid concentration in The Loch was greater than in Icy Brook and Andrews Creek and yet the N content remained at an intermediate value of 1.8%. Therefore, fulvic acids from the subalpine forest must have had N contents of at least 1.8%. This value is somewhat higher than N contents of other forest-derived fulvic acids (Malcolm 1990; McKnight et al. 1992), and may reflect the sustained high deposition of N in Loch Vale Watershed (Baron et al. 1994).

The $\delta^{13}C$ values were clustered either between –25 to –27‰ (Table 6), the range for plant-derived carbon, or between –22 to –24‰. The large particulates from Sky Pond and all the small particulates and colloids had $\delta^{13}C$ values in the heavier range, and potentially could have originated from algal or microbial carbon. As shown in Figure 3, these heavier $\delta^{13}C$ values matched with lower C:N ratios, which are also indicative of aquatic microbial material. The large particulates from The Loch and Andrews Creek had lighter $\delta^{13}C$ values, well within the range of terrestrially-derived organic material, and greater C:N ratios.

The fulvic acids from all the sites had lighter δ^{13}C values in the range of terrestrially-derived organic material. Baron et al. (1991) found that in The Loch δ^{13}C values remained fairly constant in the spring and summer, whereas in Sky Pond values became heavier following snowmelt, shifting from –33.1‰ in April to –24.5‰ in July. The constant values for The Loch fulvic acid suggest that the abundant soils and vegetation around The Loch are the dominant and continuous source of fulvic acid for the lake. Whereas, there are several possible interpretations for the changing δ^{13}C values in Sky Pond. There may be seasonal changes in the δ^{13}C of the dissolved inorganic carbon (DIC), e.g. lighter in winter from respiration under icecover, that cause changes in the δ^{13}C of the source organic material for the fulvic acid. Autochthonous precursor organic material may be microbial biomass in the water column or algal-derived organic material in the lake sediments, which are flushed during spring snowmelt. This interpretation is consistent with the higher C:N ratio of the Sky Pond fulvic acid and the spectroscopic results presented below. An alternative explanation is that the sparse vegetation in the Sky Pond watershed becomes a significant source of fulvic acid during the summer.

Structural group distributions for organic carbon

Carbon bonding in particulates, colloids and fulvic acids was characterized by ^{13}C-NMR spectroscopy to determine distributions of structural groups. The ^{13}C-NMR spectra for the organic fractions were separated into three groups based on the relative intensities of the major spectral bands: large and small particulates (Figures 4, 5), colloidal material (Figure 6), and fulvic acids (Figure 7).

The grouping of large and small particulates together (Figures 4, 5) is consistent with the similarities in elemental and ash content of these fractions. The particulate spectra consisted of bands with maxima in regions attributable to aliphatic components: 25–30 ppm (AL-I), 70–75 ppm (AL-II), and 175–178 ppm (C-I). In addition, a weaker band near 100 ppm (AL-III) is present in many of the spectra. The AL-I band probably represents CH_2 groups and some CH_3 groups. The AL-II band represents -HCOH- groups mainly in carbohydrate structures as evidenced by the anomeric carbon band (AL-III). The ratio of AL-II to AL-III is 5 in hexoses. Therefore the deviation of this ratio from 5 can indicate the presence of noncarbohydrate alcohols in a sample. In all spectra of large particulates and in the Sky Pond and Icy Brook small-particulate spectra, the AL-II/AL-III ratio is approximately 5. The ratio is significantly less than 5 in the spectra for small particulates from Andrews Creek and The Loch, probably indicating the presence of
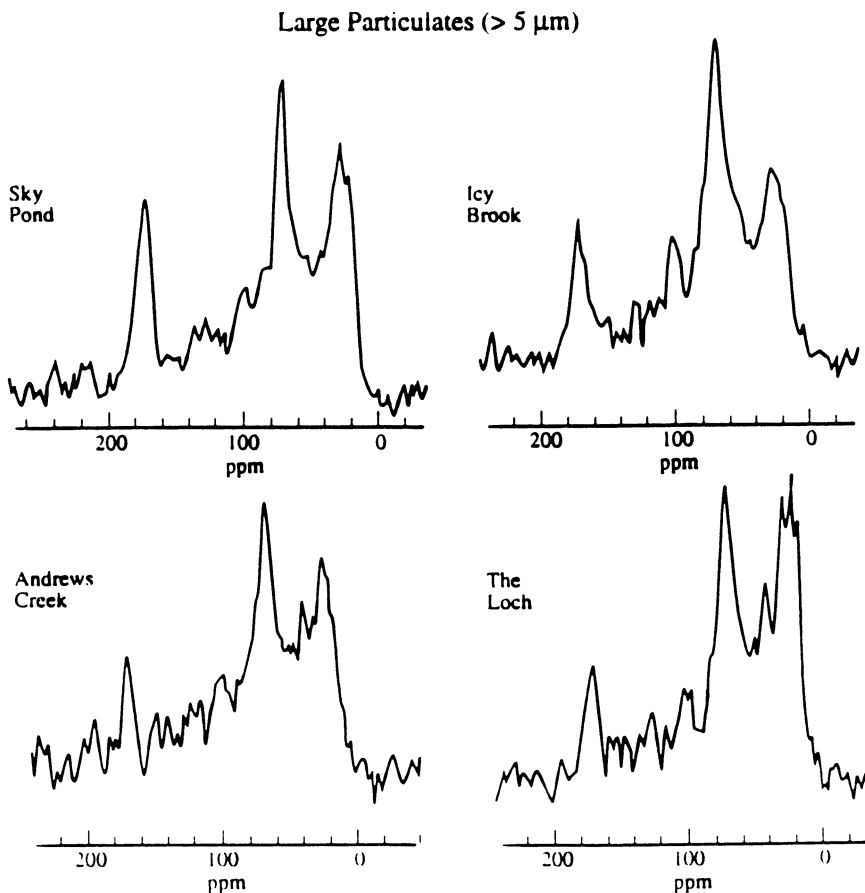
Large Particulates (> 5 μm)

Figure 4. ^{13}C-NMR of large particulates (>5 μm).

sugar acids. All particulate samples contained a C-I band. This band could correspond to carboxylic acid groups associated with soil-derived particulate humic and fulvic acids sorbed to mineral particles or could reflect oxidation of particulate organic material. Alternatively, amide groups in proteins could contribute to this band (Leenheer et al. 1995), because the N content of the particulates (3.6 to 1.5%) is comparable to the carbon represented by this band (5–10% of the organic carbon, which comprised 17–29% of the particulate mass).

The colloid spectra (Figure 6) were similar to each other and differed from particulate spectra in that the AL-II bands were much more pronounced. The AL-II/AL-III ratios were approximately 5 in the colloid spectra, indicating that most carbohydrates were not oxidized to acids. The C-I bands in these

Small Particulates
(between 5 μm & 0.45 μm)
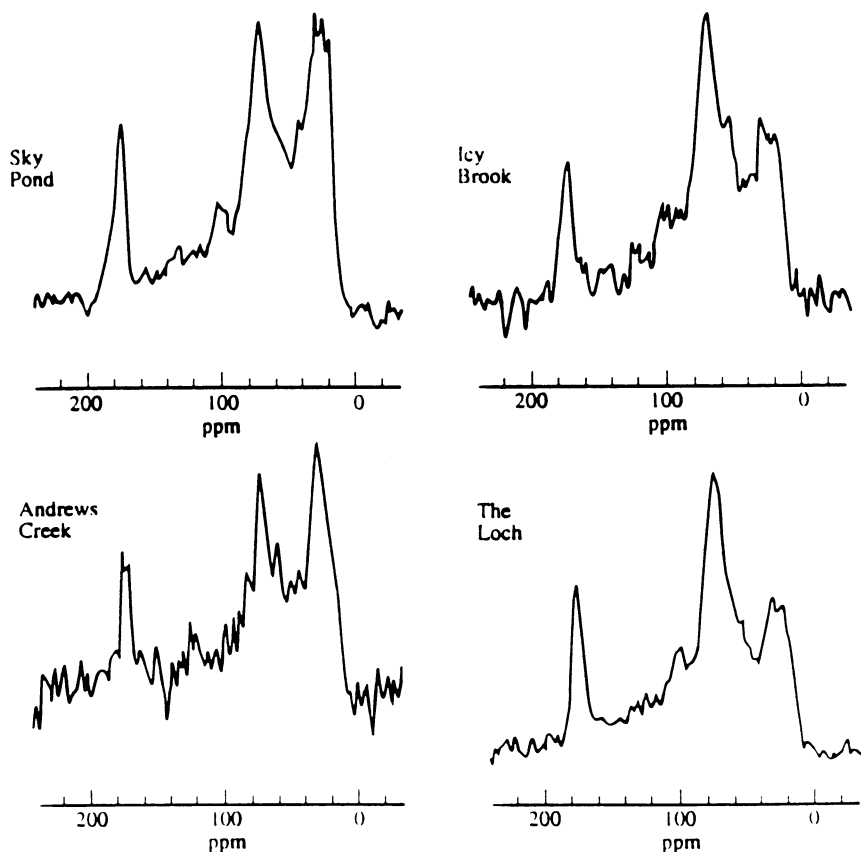
Figure 5. ^{13}C-NMR of small particulates (between 5 μm and 0.45 μm).

spectra probably represent nonsugar aliphatic acids (e.g. lipids); however, a small fraction of sugar acids may be present. In general, two maxima in the AL-I band were observed at about 20 and 30 ppm . The maximum at 20 ppm may indicate terminal methyl groups (Wershaw 1992).

The result that carbohydrate moieties were more prominent in the colloid spectra than in the spectra for the particulates or fulvic acids leads to an explanation of the chemical uniformity of the colloids. This material could be more highly labile and more rapidly consumed by microbial degradation than larger more aromatic particulate material. At the same time, carbo-hydrate rich colloids could be produced as mucilage of algae, released by herbivorous zooplankton as they graze on phytoplankton cells, or released by

Colloids (0.45 μm to 10^6 Daltons)
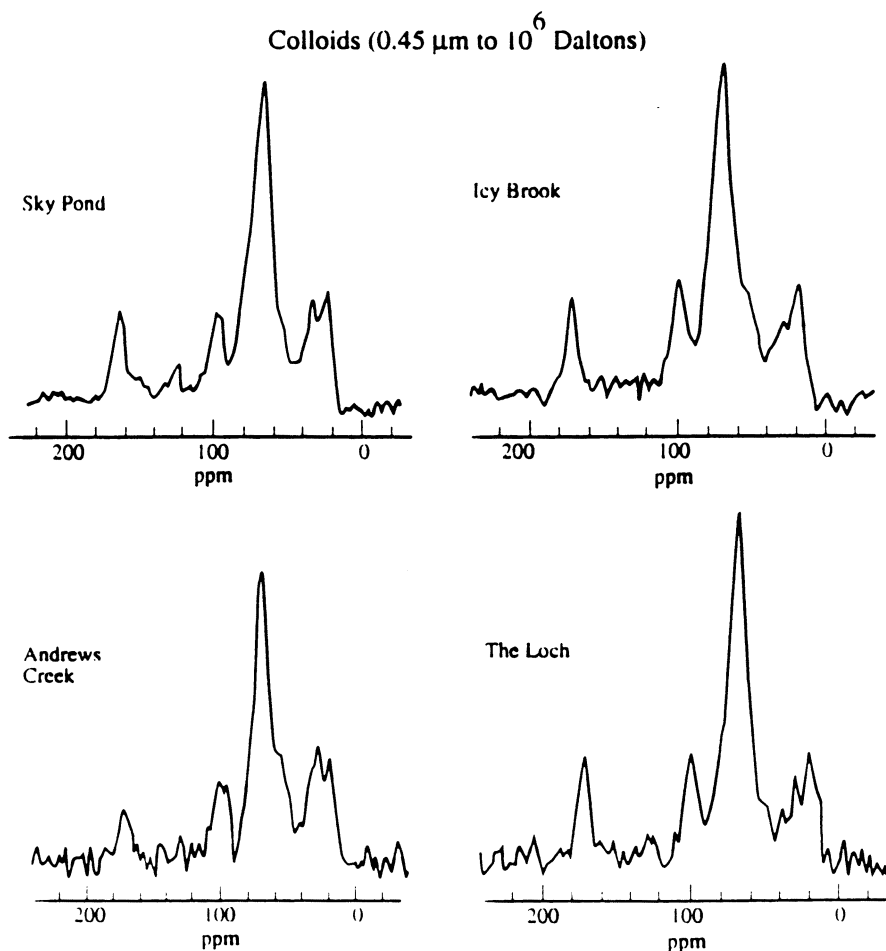
Figure 6. ^{13}C-NMR of colloids (between 0.45 μm and 10^6 Daltons).

benthic invertebrates grazing on periphyton. The low concentration of colloidal carbon and the potential high turnover rates within aquatic ecosystems would result in autochthonous processes and carbon sources determining the chemical properties of colloids.

Spectra for the fulvic acids were typical of aquatic fulvic acids (Wershaw 1992), showing prominent Al-I bands with maxima between 35 and 40 ppm and well-defined C-I bands. For fulvic acids the C-I bands represent carboxylic acid groups, which can account for about half of the cation excess in these surface waters (Table 1). The AL-II bands were relatively weak and well-defined AL-III bands were absent. It is possible that the AL-III bands were obscured by the overlap of aromatic (AR) bands. However, it is also
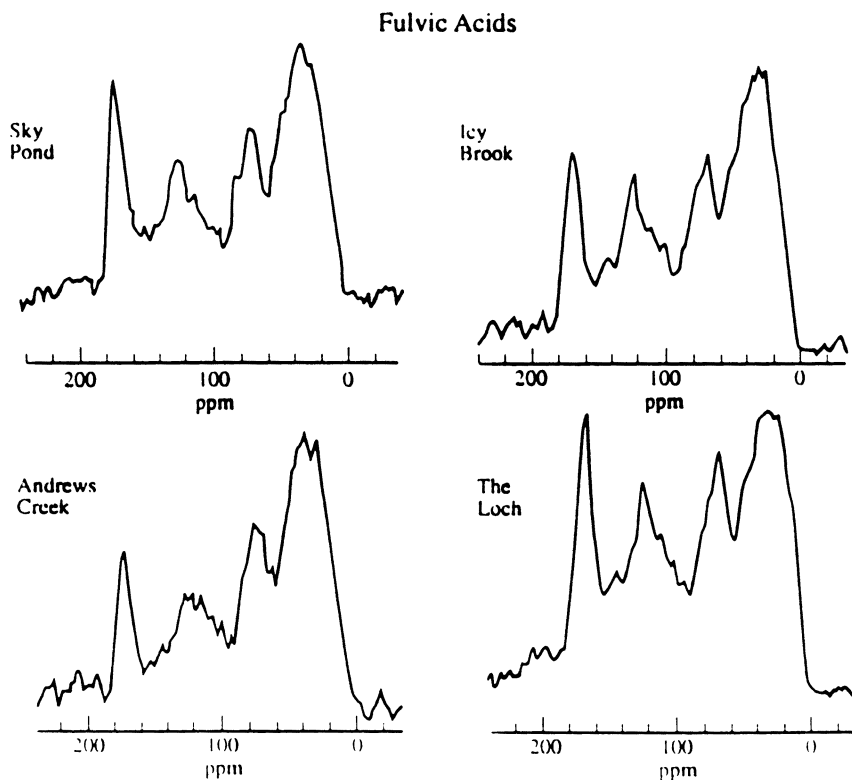
Fulvic Acids

Figure 7. ^{13}C-NMR of dissolved fulvic acids.

possible that most of the anomeric carbon atoms of carbohydrates have been oxidized to carboxylate groups.

Variations in fulvic acid characteristics

The relative abundance of aromatic and aliphatic carbons can indicate the importance of terrestrial lignin-containing sources to fulvic acid (McKnight et al. 1994). Comparison of AR/AL-I ratios (Table 7) shows that The Loch fulvic acid is the most aromatic, Andrews Creek fulvic acid is the least aromatic, and Sky Pond and Icy Brook fulvic acids are similar. This pattern of variation appears unrelated to chemical characteristics of particulates and colloids at the sites. One explanation for this lack of relationship is that characteristics of particulates and colloids are more strongly influenced by processes occurring within the lake or stream, e.g. production, consumption, aggregation, and deposition. In contrast, fulvic acids are produced as solutes within a source environment, are less susceptible to decomposition, and are transported to

and within aquatic environments retaining discernible characteristics of their origin.

The aromaticity of The Loch fulvic acid reflects the contribution from the sub-alpine forest, and the AR/AL-I ratio of 0.67 is similar to the value of 0.72 for fulvic acid from Deer Creek, a stream in another forested watershed in the Colorado Rocky Mountains (McKnight et al. 1992). The increase in fulvic acid concentration between the Icy Brook site and the Andrews Creek confluence was about 0.10 mgC/L. To account for the associated increase in the AR/Al-I ratio from 0.50 to 0.67 requires the fulvic acid entering The Loch to have an even higher ratio of about 1.0.

The low aromaticity of the Andrews Creek fulvic acid was not anticipated. Although the sedges and other meadow vegetation would contain some lignin, lignin does not appear to be as important in the formation of dissolved fulvic acid in the meadow as in the sub-alpine forest. The intermediate AR/AL-I ratio for the Sky Pond sample is similar to ratios for algal-rich ponds (McKnight et al. 1994). In contrast to the N content, the AR/AL-I ratio of Icy Brook fulvic acid was too high to be explained as a mixture of Sky Pond fulvic acid with meadow-derived fulvic acid similar to that in Andrews Creek.

Fulvic acids from different environments can be compared based upon the ratios of C:N and AR:AL-I (McKnight et al. 1994). Algal-derived fulvic acids have lower ratios than fulvic acids derived from soil and higher plants. Loch Vale samples fall within the general range of samples from other environments (Figure 8). Sky Pond fulvic acid had lower ratios than The Loch fulvic acid, as would be expected. However, the Andrews Creek and Icy Brook samples did not fall in between these two samples, indicating that the fulvic acid gained as the stream flows through the wet sedge meadow has different derivation pathways than either the algal-lake sediment source in Sky Pond or the soil-forest vegetation source for The Loch.

UV-visible spectra of fulvic acids

The chromophores in fulvic acid are associated to a large extent with aromatic moieties. The aromaticity of fulvic acids is therefore significant in an ecological context because light absorbance by fulvic acids can control photic zone depth and attenuation of ultraviolet light. The UV-visible spectra of Loch Vale fulvic acids are shown in Figure 9. The Sky Pond and Icy Brook samples had lower absorptivities than the more aromatic sample from The Loch. However, the Andrews Creek fulvic acid had the lowest AR:AL-I ratio (0.4) and the greatest absorptivity.

Chin et al. (1994) documented a strong linear relationship ($r^2 = 0.9$) between molar absorptivity at 280 nm and aromatic carbon content for seven aquatic fulvic acids. The relationships between absorptivity at 300 nm and
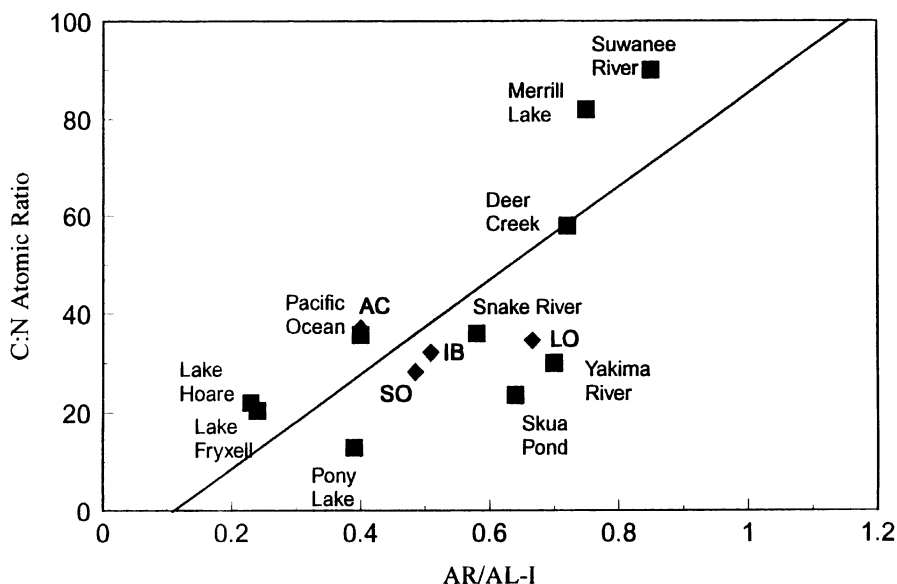
Figure 8. Diagram of C:N vs AR:AL-I, showing position of Loch Vale fulvic acids relative to other aquatic fulvic acids; SO = Sky Pond Outlet, AC = Andrews Creek, IB = Icy Brook, and LO = Loch Outlet. Other fulvic acids are described in McKnight et al. 1992 and 1994.

450 nm and aromatic carbon content for the Loch Vale samples and four other fulvic acids also show a trend of increasing absorptivity with aromatic carbon content (Figure 10), but the strength of the linear relationship (r^2 = 0.76 and 0.43 at 300 and 450 nm, respectively) is less than that found by Chin et al. (1994). The reason for the poorer fit could be that at higher wavelengths aromatic moieties would contribute less to total absorbance than at 280 nm.

These results can help to explain the relationship between UV-B extinction coefficients in lakes and DOC concentration observed by Scully and Lean (1994), which was best represented as a power function. Scully and Lean (1994) hypothesized that the nonlinearity in the relationship is caused by a change in the character of UV-absorbing DOC as DOC concentration increases. In their data, UV-B extinction coefficients increased more rapidly above DOC concentrations of about 2 mgC/L. This transition may correspond to a transition in the dominant source and therefore chemical characteristics of fulvic acid. This analysis supports the results of Scully & Lean (1994), by showing that UV absorbance does vary with the chemical characteristics of fulvic acid. Our results further serve as a basis for a more detailed hypothesis that lakes with "paler" (less aromatic), algal-derived fulvic acid are clustered in the low DOC range and lakes with "darker" (more aromatic) terrestrially-derived fulvic acids have higher DOC concentrations.
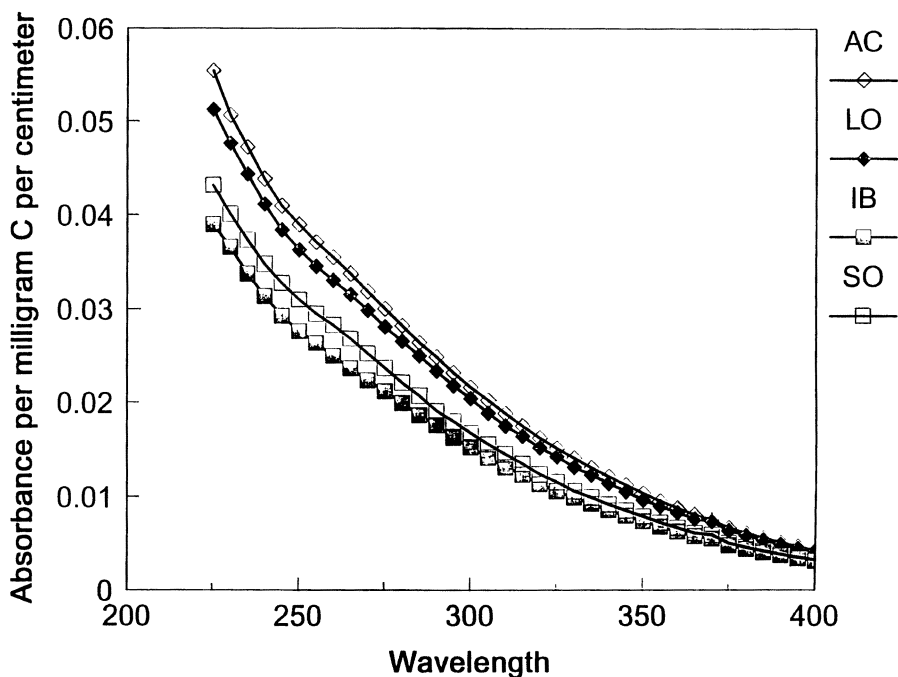
Figure 9. Absorbance of Loch Vale fulvic acids, SO = Sky Pond Outlet, AC = Andrews Creek, IB = Icy Brook, and LO = Loch Outlet.

In addition to changes in chemical characteristics of fulvic acid as the proportion of allochthonous to autochthonous DOC changes, the percentage of DOC that is fulvic acid would be expected to change. Our studies of Loch Vale and Antarctic lakes have shown that fulvic acid accounts for a lower percentage of the DOC in aquatic systems where algal-sources dominate. Therefore, in the transition to higher DOC concentrations a greater percentage of the DOC would absorb UV radiation, further contributing to a nonlinear relationship and amplifying the rise in slope.

The outlier sample, Andrews Creek, is a reminder that variations in fulvic acid sources contribute to scatter in the relationship between UV-B extinction coefficients and DOC. Other factors contributing to scatter could be decreases in aromaticity by photo-bleaching associated with long water residence times (e.g. McKnight et al. 1988) and removal of more aromatic fulvic acid molecules from solution by sorption on metal oxides or other sorptive surfaces (e.g. McKnight et al. 1992).
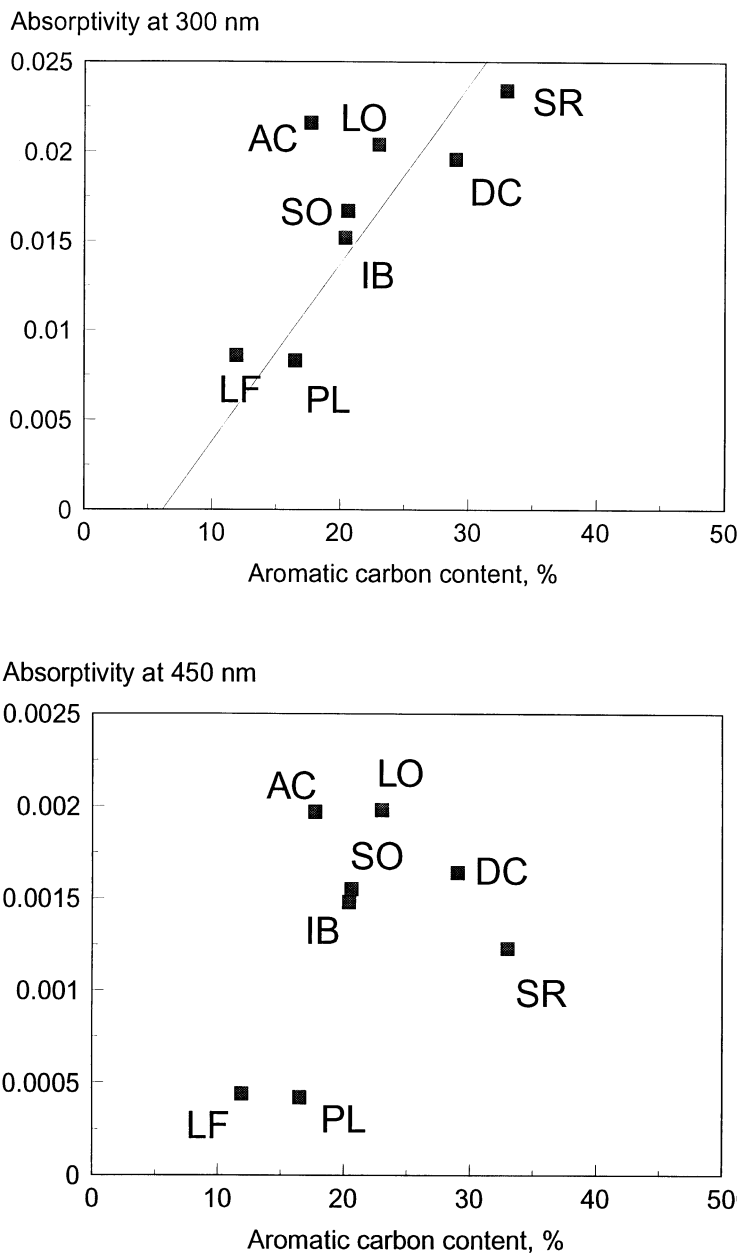
Absorptivity at 300 nm

Absorptivity at 450 nm

Figure 10. Relationship between absorptivity at 300 nm (A) and 450 nm (B) and aromatic carbon content of fulvic acids from Loch Vale and other sites; SO = Sky Pond Outlet, AC = Andrews Creek, IB = Icy Brook, LO = Loch Outlet, LF = Lake Fryxell, PL = Pony Lake, SR = Suwannee River, and DC = Deer Creek. Other fulvic acids are described in McKnight et al. 1992 and 1994. The line in A (300 nm) is a linear fit to the equation Abs = Abs_o + bAR, where Abs=absorbance, AR = percent aromaticity, Abs_o = –0.007 and b = 1.1 × 10^{-3}.

Summary

Our results demonstrate that consistent chemical differences exist between particulates, colloids and fulvic acids in Loch Vale. The chemical composition of the small and large particulates was somewhat more heterogeneous. The isotopic composition and C:N ratios indicated that the large particulates in The Loch and Andrews Creek were soil or plant derived, while the large particulates at the other sites and the small particulates were comprised of algal or aquatic microbial carbon. Colloidal material had a pronounced carbohydrate character and varied least among sites. Colloids may be a more labile fraction, possibly being turned over rapidly by in situ heterotrophic organisms and therefore more disconnected from watershed sources. Dissolved fulvic acids changes in composition from the alpine lake to the subalpine lake as material derived from different precursor material enters the stream/lake system. Our results on the relationship between sources of organic material and chemical characteristics within this watershed provide more details to augment previously developed relationships. A general conclusion of this study is that the size continuum does not correspond to a simple continuum in chemical properties. Relationships between source and chemical composition based on the bulk properties measured in this study could be augmented or tested using trace biomarker compounds, such as degradation products of pigments or lignin.

References

Aiken GR & Malcolm RL (1987) Molecular weight of aquatic fulvic acids by vapor pressure osmometry. Geochem. Cosmochim. Acta 51: 2177

Aiken GR & Gillam AH (1989) Determination of molecular weights of humic substances by ultracentrifugation. In: Hayes MB, MacCarthy P & Malcolm (Eds), Humic Substances II In Search of Structure (pp 515–544). John Wiley and Sons, New York

Arthur MA & Fahey TJ (1992) Biomass and nutrients in an Englemann spruce-subalpine fir forest in north central Colorado: pools, annual production, and internal cycling. Can. J. For. Res. 22: 315–325

Baron, JS (1992) Biogeochemistry of a Subalpine Ecosystem. Springer-Verlag, NY

Baron J, McKnight DM & Denning AS (1991) Sources of dissolved and particulate organic material in Loch Vale Watershed, Rocky Mountain National Park, Colorado, USA. Biogeochemistry 15: 89–110

Baron JS, Ojima DS, Holland EA & Parton WJ (1994) Analysis of nitrogen saturation potential for Rocky Mountain tundra and forest: implications for aquatic systems. Biogeochemistry 27: 61–82

Chin YP, Aiken GR, O'Loughlin E (1994) Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ, Sci. Technol. 28: 1853–1858

Cole JJ, McDowell WH & Likens GE (1984) Sources and molecular weight of dissolved organic carbon in an oligotrophic lake. Oikos 42:1–9

De Haan H, De Boer T & Voerman J (1991) Size distribution of dissolved (<200nm) organic carbon and aluminum in alkaline and humic lakes. Hydrobiol. Bull. 24: 145–151

Hessen DO, Andersen T & Lyche A (1990) Carbon metabolism in a humic lake: Pool sizes and cycling through zooplankton. Limnol. Oceanogr. 35: 84–99

Huffman EWD Jr & Stuber HA (1985) Analytical methodology for elemental analysis of humic substances. In: Aiken GR, McKnight DM, Wershaw RL & MacCarthy P (Eds) Humic Substances in Soil, Sediment and Water (pp 433–456). John Wiley and Sons, New York

Leenheer JA, Wershaw RL & Reddy MM (1995) Strong-acid, carboxyl-group structures in fulvic acid from the Suwannee River, Georgia. 1. Minor structures. Environ. Sci. Technol. 29: 393–398

Leenheer JA & Huffman EW Jr (1979) Analytical method for dissolved organic carbon fractionation. US Geological Survey Water Resources Investigations 78–4

Lock MA & Ford TE (1986) Colloidal and dissolved organic carbon dynamics in undisturbed boreal forest catchments: a seasonal study of apparent molecular weight spectra. Freshwater Biol. 16: 187–195

Malcolm RL (1990) The uniqueness of humic substances in each of soil, stream and marine environments. Analytica Chimica Acta 232: 19–30

Malcolm RL & Leenheer JA (1973) The usefulness of organic carbon parameters in water quality investigations. In: Proceedings of the Institute of Environmenal Sciences 1973 Annual Meeting (pp 336–340). Anaheim

McKnight DM (1991) Feedback mechanisms involving humic substances in aquatic ecosystems. In: Schneider SH & Boston PJ (Eds) Scienstists on Gaia (pp 330–338). MIT Press, Cambridge, MA

McKnight DM, Andrews ED, Spaulding SA & Aiken GR (1994) Aquatic fulvic acids in algal-rich antarctic ponds. Limnol. Oceanogr. 39(8): 1972–1979

McKnight DM, Bencala KE, Zellweger GW, Aiken GR, Feder GL & Thorn KA (1992) Sorption of dissolved organic carbon by hydrous aluminum and iron oxides occurring at the confluence of Deer Creek with the Snake River, Summit County, Colorado. Environ. Sci. Technol. 26: 1388–1396

McKnight DM, Miller C, Smith RL, Baron J & Spaulding S (1988) Phytoplankton populations in lakes in Loch Vale, Rocky Mountain National Park, Colorado: Sensitivity to acidic conditions and nitrate enrichment. US Geological Survey Water-Resources Investigations Report 88–4115

McKnight DM, Smith RL, Bradbury JP, Baron JS & Spaulding S (1990) Phytoplankton dynamics in three Rocky Mountain lakes, Colorado, USA. Arctic & Alpine Research 22(3): 264–274

McKnight DM, Thorn KA, Wershaw RL, Bracewell JM & Robertson GW (1988) Rapid changes in dissolved humic substances in Spirit Lake and South Fork Castle Lake, Washington. Limnol. Oceangr. 33: 1527–1541

Nobili MD, Gjessing E & Sequi P (1989) Sizes and shapes of humic substances by gel chromatography. In: Hayes MB, MacCarthy P & Malcolm (Eds), Humic Substances II In Search of Structure (pp 561–593). John Wiley and Sons, New York

Ranville JF, Harnish RA & McKnight DM (1991) Particulate and colloidal organic material in Pueblo Reservoir, Colorado: Influence of autochthonous source on chemical composition. In Baker RA (Ed) Organic Substances and Sediment in Water Volume 1 (pp 47–73). Lewis Publishers, Chelsea, MI

Scully NM & Lean DRS (1994) The attenuation of ultraviolet light in temperate lakes. Arch. Hydrobiol. 43: 135–144

Steinberg C & Meunster U (1985) Geochemistry and ecological role of humic substances in lakewater. In: Aiken GR, McKnight DM, Wershaw RL & MacCarthy P (Eds) Humic Substances in Soil, Sediment and Water: Geochemistry, Isolation, and Characterization (pp 105–146). John Wiley and Sons, New York

Thurman EM (1985) Organic geochemistry of natural waters. Nijhoff/Junk Publishers, Boston

Thurman EM & Malcolm RL (1981) Preparative isolation of aquatic humic substances. Environ. Sci. Technol. 15: 463–466

Wershaw, RL (1992) Membrane-micelle model for humus in soils and sediments and its relation to humification. U.S. Geological Survey Open-File Report 91–513

Wetzel RG (1990). Land-water interfaces: Metabolic and limnological regulators. Verh. Internat. Verein. Limnol. 24: 6–24