Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley

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Received 27 February 2009; revised 30 September 2009; accepted 26 October 2009; published 23 March 2010.

The spectral characteristics of whole water dissolved organic matter (DOM) and fulvic acid were studied in samples collected from an alpine lake, a subalpine lake, and a subalpine stream during snowmelt and the summer growing season. Excitation-emission matrices of whole water DOM and fulvic acid were analyzed by parallel factor analysis (PARAFAC). Allochthonous inputs of terrestrial fulvic acid DOM were dominant during snowmelt at the alpine lake, and during both snowmelt and summer at the subalpine sites. At the alpine lake, autochthonous inputs of DOM dominated during the summer phytoplankton bloom, and the spectral characteristics of the whole water DOM diverged from those of the fulvic acid. For example, the quinone-like fluorophores in whole water DOM at the alpine lake were more oxidized and microbially derived than the fulvic acid fraction during the summer. At the subalpine sites, the seasonal changes in the source and redox state of the quinone-like fluorophores of the whole water DOM tracked those of the fulvic acid pool. However, at both lake sites there was a greater contribution of amino acid-like fluorophores in the whole water DOM than the fulvic acid fraction. This trend was not observed at the subalpine stream site. Principal components analysis (PCA) of the PARAFAC components suggests that during snowmelt, the chemical quality of the DOM at the alpine lake was similar to that of the subalpine stream; whereas the alpine site was more similar to the subalpine lake during the summer. Spectral characterization and PCA of the PARAFAC components suggest that nonhumic quinone-like and amino acid-like fluorophores were produced in the alpine lake during the summer phytoplankton bloom. Our results show that different types of water bodies produce different seasonal patterns in whole water DOM and fulvic acid quantity and quality.


1. Introduction

Biogeochemical processes taking place in aquatic ecosystems and the surrounding terrestrial environment influence the chemical quality of dissolved organic matter (DOM). In turn, these biogeochemical processes are influenced by seasonal changes in climate as well as alteration of landscape characteristics and hydrologic flow paths [Hood et al., 2003; Larson et al., 2007; Jaffe et al., 2008]. The chemical quality of DOM can influence its reactivity such as in metal binding [Yamashita and Jaffe, 2008] and in electron transfer [e.g., Scott et al., 1998; Ratusuk and Nanny, 2007]. Monteith et al. [2007] demonstrated that increasing dissolved organic carbon (DOC) concentrations in north temperate surface waters were associated with recovery from acidification. In light of these observed increases in DOC, greater understanding of the connections between DOM quality and reactivity would be useful in evaluating the underlying processes driving these trends (e.g., recovery from acidification), and evaluating the potential impacts of these increases on ecosystem processes and drinking water supplies.

[1] The spectral characteristics of whole water dissolved organic matter (DOM) and fulvic acid were studied in samples collected from an alpine lake, a subalpine lake, and a subalpine stream during snowmelt and the summer growing season. Excitation-emission matrices of whole water DOM and fulvic acid were analyzed by parallel factor analysis (PARAFAC). Allochthonous inputs of terrestrial derived fulvic acid DOM were dominant during snowmelt at the alpine lake, and during both snowmelt and summer at the subalpine sites. At the alpine lake, autochthonous inputs of DOM dominated during the summer phytoplankton bloom, and the spectral characteristics of the whole water DOM diverged from those of the fulvic acid. For example, the quinone-like fluorophores in whole water DOM at the alpine lake were more oxidized and microbially derived than the fulvic acid fraction during the summer. At the subalpine sites, the seasonal changes in the source and redox state of the quinone-like fluorophores of the whole water DOM tracked those of the fulvic acid pool. However, at both lake sites there was a greater contribution of amino acid-like fluorophores in the whole water DOM than the fulvic acid fraction. This trend was not observed at the subalpine stream site. Principal components analysis (PCA) of the PARAFAC components suggests that during snowmelt, the chemical quality of the DOM at the alpine lake was similar to that of the subalpine stream; whereas the alpine site was more similar to the subalpine lake during the summer. Spectral characterization and PCA of the PARAFAC components suggest that nonhumic quinone-like and amino acid-like fluorophores were produced in the alpine lake during the summer phytoplankton bloom. Our results show that different types of water bodies produce different seasonal patterns in whole water DOM and fulvic acid quantity and quality.

used fractionation and spectroscopic approaches for characterizing natural organic material to show that the source of the fulvic acid fraction shifted from terrestrial to microbial during the spring to summer transition. Hood et al. [2005] also found that the C/N ratio and aromaticity of a nonhumic fraction, the transphilic acids [Aiken et al., 1992], decreased from snowmelt into the summer season. Thus, the results of these studies suggest that there may be concomitant changes in the quality of the nonhumic DOM as snowmelt proceeds and autochthonous production of organic matter increases with algal growth in summer.

Spectroscopic characterization of DOM is a useful approach for assessing DOM source and quality [e.g., Coble et al., 1990; McKnight et al., 2001; Stedmon et al., 2003; Weisshaar et al., 2003; Jaffe et al., 2008]. A significant negative linear relationship was observed between specific ultraviolet absorbance at a wavelength of 254 nm (SUVA_{254}) and fluorescence index (FI [McKnight et al., 2001]) values in a study involving a data set that included samples from a diverse set of environments [Jaffe et al., 2008]. The low SUVA_{254}/high FI values were from sites that receive substantial microbial inputs of DOM; whereas the high SUVA_{254}/low FI values were from sites receiving significant DOM inputs from the terrestrial environment.

Spectroscopic approaches have also been used to quantify autochthonous production of DOM in aquatic ecosystems [Bade et al., 2007; Gondar et al., 2008; Miller et al., 2009a]. As part of a larger study, Bade et al. [2007] compared SUVA_{254} and FI values of the whole water to the fulvic acid fractions of DOM from samples collected on a single date from two lakes; one of which had been experimentally enriched in nutrients. The SUVA_{254} was lower and the FI was higher in both the whole water and fulvic acid fractions of the nutrient enriched lake. These spectroscopic comparisons suggest that algal derived DOM was produced in the nutrient enriched lake. The difference in FI values between the whole water and fulvic acid fraction suggests that this algal derived DOM may have been composed of both nonhumic and humic quinone-like fluorophores. Miller et al. [2009a] used reactive transport modeling and spectroscopic data to demonstrate that both nonhumic DOM and microbially derived fulvic acid were produced in an alpine lake during the summer phytoplankton bloom.

Although spectroscopic methods such as SUVA_{254} and FI are useful simple methods for DOM characterization, it is important to consider potential interferences from other dissolved constituents [Chin et al., 1994; Weisshaar et al., 2003]. Specifically, for SUVA_{254}, both dissolved iron and nitrate absorb across the region of the spectrum that includes the wavelength of 254 nm, where the SUVA_{254} measurements are made. The potential for these inorganic species to interfere with SUVA_{254} measurements increases with decreasing DOC and fulvic acid concentration. This is because the same concentration of iron or nitrate can have little influence in high DOM samples but have a large influence on SUVA_{254} in low DOM samples with a low proportion of humic material. Such interferences are less likely to occur when the humic fraction is isolated from the sample by a chromatographic or ultrafiltration method prior to characterization. In their development of a fluorescence index (FI) based on the slope of the descending limb of the emission peak at an excitation wavelength of 370 nm, McKnight et al. [2001] specified that the FI would be useful for comparison for water samples in which the peak emission occurred in the range characteristic of humic material (e.g., 460–470 nm) for instrument corrected spectra [Cory and McKnight, 2005]). For some of the Antarctic lake waters examined by McKnight et al. [2001], the emission peak was at lower wavelengths, which may have reflected abundant nonhumic fluorophores.

While a temporal shift in the spectroscopic characteristics of the fulvic acid fraction of the DOM has been demonstrated to occur in the alpine lake studied by Hood et al. [2003, 2005] and Miller et al. [2009a], differences between the spectroscopic characteristics of the whole water DOM and the fulvic acid fraction of the DOM have not been investigated. Comparison of the spectral properties of these two DOM pools provides insight into how the source and quality of the fulvic acid and nonhumic fractions of the DOM change seasonally at different sites. This study focuses on evaluating the patterns in spectral characteristics of DOM in the whole water and the fulvic acid fraction in three different water bodies: an alpine lake, a subalpine lake, and a subalpine stream. We hypothesized that spectroscopic characteristics of the whole water DOM would be similar to those of the fulvic acid DOM at the subalpine sites dominated by allochthonous inputs of terrestrially derived fulvic acid DOM. On the other hand, we anticipated that the whole water and fulvic acid fractions of the DOM would deviate from one another when autochthonous production of DOM is dominant, such as during the summer phytoplankton bloom at the alpine lake. Specifically, because of increased rates of primary productivity in the summer months, we expected that nonhumic quinone-like fluorophores produced in the lake would be a dominant source of DOM during the summer growing season in the alpine lake. The biogeochemical response of the DOM at these sites provides insight into how different ecosystems may respond to future changes in climate and/ or land use.

2. Materials and Methods

2.1. Site Description

Two of the three sample sites are located in the Green Lakes Valley and the third is located in the adjacent Como Creek watershed. The Green Lakes Valley is located approximately 6 km east of the Continental Divide along the Colorado Front Range, and is part of the Niwot Ridge Long-term Ecological Research Site (NWTLTER). It is located within the Silver Lake Watershed, which provides 40% of the water supply for Boulder, Colorado, and public access is prohibited. The NWTLTER project has conducted extensive climatic, atmospheric, water quality and biological studies for the last fifty years [e.g., Bowman and Seastedt, 2001].

The Green Lakes Valley is separated into two catchments (Figure 1). The upper alpine catchment, which includes Green Lake 4 as well as the Arikaree Glacier, has an area of approximately 2 km². Green Lake 4 is an unmanaged oligotrophic alpine lake at an elevation of 3450 m. Green Lake 4 has a volume of 2.2 × 10⁵ m³ and residence times range from about 8 days during snowmelt to 30 days late in the summer [Miller, 2008]. The entire catchment is fed by the Arikaree Glacier and receives the majority of its precipitation in the form of snowfall during the winter and
spring months. A lower subalpine–alpine catchment, which includes Lake Albion, has an area of approximately 5 km$^2$. Lake Albion is a managed reservoir at the subalpine–alpine boundary (3350 m) with a well defined inlet and outlet. The lake has a volume of approximately $5.1 \times 10^5$ m$^3$ and residence times range from 20 days during snowmelt to approximately 100 days late in the summer. Como Creek is a subalpine stream in an adjacent catchment that does not contain any upstream lakes. The hydrology of the three catchments is dominated by spring snowmelt, during which time there is an annual ionic pulse that results in high nitrate and other major ion concentrations in surface waters in the upper reaches of the catchment (http://culter.colorado.edu/NWT/). Concentrations of ferric iron in surface waters in the catchment are low (<0.05 mg L$^{-1}$). Nitrate concentrations in the valley are highest at the alpine sites and peak concentrations during snowmelt at Green Lake 4 are about $2–3$ mg L$^{-1}$ (http://culter.colorado.edu/NWT/). Additional details about all three sampling sites can be found elsewhere [e.g., Hood et al., 2003; http://culter.colorado.edu/NWT/].

2.2. Field Sampling and Laboratory Protocols

Water samples were collected as grab samples on a weekly basis from 25 May to 29 September 2006 from the outlets to Green Lake 4 and Lake Albion as well as at Como Creek. Samples were transported to the lab after collection and filtered immediately. The filtrate was stored in the dark at 4°C and all analyses were preformed within one week of collection. Discharge was measured continuously with a stream gage at the outlet to Green Lake 4 and at Como Creek. Additionally, a stream gage measured discharge at a stream site approximately 1 km downstream of the outlet to Lake Albion. In Green Lake 4, samples for phytoplankton enumeration were collected, preserved, and identified as described by Gardner et al. [2008] at the surface and a depth of 3 m on 6, 13, 20, and 27 July and 10 August 2006. Samples for bacterial enumeration were collected from all three sites on most sampling dates and processed as described by Miller et al. [2009b].

For DOM and chlorophyll a analyses, 500 ml water samples were filtered through 0.7 μm, 47 mm Whatman GF/F glass–fiber filters, which were combusted at 450°C for 4–6 h prior to use, and the filtrate was used for DOM analyses. Immediately following filtration, chlorophyll a was extracted from the filters using hot ethanol extraction and analyzed following the spectrophotometric methods of Marker et al. [1980], which includes a phaeopigment correction. Briefly, the extracted ethanol solutions were stored at 0°C for 1–2 days and filtered through 0.22 μm nylon filters prior to analysis on an Agilent 8453 UV–Visible spectrophotometry system. A portion of the filtrate was acidified with dilute hydrochloric acid and the blank subtracted absorbance values at 665 nm and 750 nm for the acidified and unacidified fractions were used to calculate chlorophyll a concentrations. The detection limit for the sample volumes filtered in this study was 1 μg L$^{-1}$ and the standard deviation for samples analyzed in triplicate was ±5%.

Figure 1. The Green Lakes Valley and adjacent Como Creek Catchment. Stars represent the sampling locations; including the outlet to Green Lake 4 (GL4), an alpine lake, the outlet to Lake Albion (ALB), a subalpine lake, and Como Creek (CC), a subalpine stream site.
A 200 ml subsample of each 500 ml filtrate was fractionated into nonhumic and humic fractions using columns with a 10 ml bed volume packed with XAD-8 resin following the methods presented by Thurman and Malcolm [1981]. The effluent from the columns contains the DOM that does not sorb to the XAD-8 resin at low pH and corresponds to the nonhumic fraction. This fraction includes low molecular weight compounds such as carbohydrates, amino acids, and carboxylic acids [Thurman, 1985]. The humic fraction sorbs to the XAD-8 resin and was obtained by back eluting the XAD-8 resin with 20 ml of 0.1 N NaOH. The humic fraction was immediately acidified to pH 2 with concentrated HCl. The percent of the DOM that was humic was determined using a mass balance approach similar to that described by Hood et al. [2003]. Deionized (DI) water blanks were regularly loaded onto the columns and eluted with 0.1 N NaOH to check for bleed from the resin. The mass of DOC in the DI blank eluates was compared with the measured mass of DOC of the humic fraction. DOC concentrations were measured with a Shimadzu TOC-5000A Total Organic Carbon Analyzer, with a coefficient of variance of 0.2 mg L⁻¹. The mass of DOC was determined by multiplying the DOC concentration by the sample volume. The average mass of DOC in the DI blank eluted associated with the 0.1 N NaOH elution step was 0.025 ± 0.005 mgC (n = 16; corresponding to a concentration of 1.2 ± 0.2 mg L⁻¹ in the 20 ml volume of eluate). Therefore, the measured DOC mass of the humic fraction was not used to calculate percent fulvic acid. Rather, the mass of humic DOC, and subsequently percent fulvic acid, was calculated as the difference between the mass of the whole water DOC and the mass of the nonhumic DOC. Thurman [1985] reported that in surface waters the majority of the humic DOM is fulvic acid. Therefore, the percent humic DOM is referred to as the percent fulvic acid following the terminology of Baron et al. [1991] and Hood et al. [2003].

Absorbance and three-dimensional fluorescence scans were collected on both the filtered whole water and fulvic acid fractions of the samples. Isolates were diluted for highly absorbing samples prior to collecting fluorescence scans, such that the absorbance at 254 nm was less than 0.2. Absorbance scans were collected on an Agilent 8453 UV-Visible spectroscopy system and absorbance was measured every nm from 190 to 1100 nm using a 3 ml quartz cuvette with a 1 cm path length. Whole water SUVA₂₅₄ values were calculated as the decadic absorption coefficient at 254 nm normalized to the DOC concentration of the whole water sample. Fulvic acid SUVA₂₅₄ values were calculated using the decadic absorption coefficient of the fulvic acid fraction measured at 254 nm. To account for the DOC contribution from the bleed of the XAD-8 resin, the fulvic acid decadic absorption coefficient was normalized to a corrected DOC value. This correction was done by subtracting the average mass of DOC in the DI blank eluate (0.025 ± 0.005 mgC) from the measured mass of DOC in the fulvic acid fraction and then normalizing to the 20 ml sample volume collected during the back elution. Additionally, SUVA₂₅₄ values were calculated for the nonhumic fraction of five samples collected during snowmelt from each site. The standard deviation of the absorbance at 254 nm for samples analyzed in triplicate was ±5%.

Three-dimensional fluorescence scans were collected on a Fluoromax-3 fluorometer (Horiba Jobin-Yvon) and were corrected following the methods of Cory [2005]. Scans were collected in ratio (S/R) mode, a Milli-Q water blank was subtracted from each scan, and scans were corrected for excitation and emission in Matlab [Cory, 2005] using the instrument specific excitation and emission correction files. Samples were also corrected for the inner-filter effect [McKnight et al., 2001]. Additionally, the area underneath the water raman peak was calculated and used to normalize all sample intensities. Therefore, sample intensities are reported in Raman units. The effectiveness of the manufacturer supplied emission correction file to remove instrument bias was evaluated by regular collection and correction of an emission scan of quinine sulfate. The corrected scan was nearly identical to the National Institute of Standards and Technology (NIST) reference spectra for quinine sulfate.

Fluorescence index (FI) values were calculated as the ratio of the intensity of emission at 470 nm to 520 nm at an excitation wavelength of 370 nm [McKnight et al., 2001; Cory, 2005]. The standard deviation of samples analyzed in triplicate analysis was 0.01. The fluorescence scans were fit to the 13 component PARAFAC model described by Cory and McKnight [2005]. The advantages of fitting our data to the Cory and McKnight [2005] model as opposed to building our own PARAFAC model are that a large sample size is not required, and a greater amount of variation in the source and quality of the fluorescent fraction of the DOM is likely to be identified. The redox state of the quinone-like moieties of the fluorescent fraction of the DOM was calculated using the redox index (RI) derived from the PARAFAC analysis [Miller et al., 2006]. The percent contribution of amino acid-like fluorophores was represented as the sum of the Fmax values, as percent, of components 8 and 13 from the Cory and McKnight [2005] PARAFAC model.

Statistical Analyses

To assess the difference in spectral characteristics between the whole water and fulvic acid DOM, the difference between the whole water FI and RI values and the fulvic acid FI and RI values was calculated for each sample. Using these data the average difference in FI and RI (ΔFI and ΔRI, respectively) was calculated for each season at each site. A two sample t test for the two tailed hypothesis was used to test for significant differences between ΔFI and ΔRI values between sites and over time. The snowmelt period was differentiated from the summer period based on the timing of large changes in flow and phytoplankton biomass, using data from the hydrographs and chlorophyll a values. Linear regression analyses were used to assess the relationships between FI and chlorophyll a concentrations as well as FI and SUVA₂₅₄ for the whole water samples collected at all three sites.

Additionally, two principal components analyses (PCA) including the Fmax values of the thirteen components from the Cory and McKnight [2005] PARAFAC model were performed. One PCA was run on the PARAFAC component distributions from the whole water samples only and another from the fulvic acid fractions only. The Fmax values were fourth root transformed prior to analysis to account for large differences in Fmax values and improve the distribution of the data [Clarke and Warwick, 1998]. The PCA was run using
the Matlab code provided by Legendre and Gallagher [2001] and chord distance was used as a metric to examine the relationship between samples.

3. Results

3.1. Hydrologic, Chemical, and Biologic Time Series

[18] The maximum discharge during snowmelt in early June was 2.5 × 10^4 m^3 d^-1 at Green Lake 4 (Figure 2a), 6.0 × 10^4 m^3 d^-1 at the Albion site (Figure 2b), and 1.7 × 10^4 m^3 d^-1 at Como Creek (Figure 2c). Green Lake 4 became ice-free at the end of June and Lake Albion became ice-free at the beginning of June. Following snowmelt, discharge decreased at all three sites until the beginning of July when there was an unusual 3 day rain event. The rain event increased discharge to peak values about 2.5 fold greater than the snowmelt discharge values at Green Lake 4 and the Albion site, but did not increase discharge above the snowmelt maximum at Como Creek. Discharge decreased to base flow conditions at all three sites following the rain event.

[19] DOC concentrations were consistently low at Green Lake 4 (<2 mg L^-1) and peaked during snowmelt (Figure 2d). At the outlet to Lake Albion, DOC concentrations were low early in the season (~1 mg L^-1) and steadily increased to a peak value of 3.7 mg L^-1 in late September (Figure 2e). At Como Creek DOC concentrations were highest during snowmelt at 10.1 mg L^-1 and decreased to base flow values of 3–4 mg L^-1 (Figure 2f).

[20] During snowmelt, the percent fulvic acid was high (60–80%) at all three sites (Figures 2d–2f). Following snowmelt, the percent fulvic acid decreased into the summer months at the outlets to Green Lake 4 and Lake Albion. The lowest percent fulvic acid measured at Green Lake 4 was 8% in late September and 23% at Lake Albion in September. In contrast, the percent fulvic acid remained high during the entire study period at Como Creek, with the lowest percent fulvic acid value measured at Como Creek being 59%. The percent fulvic acid values calculated by taking the difference between the mass of the whole water and nonhumic DOC were within 5% of the values calculated by correcting the mass of the humic fraction DOC with the average mass of the DI blank eluates. The mass of DOC in the DI blank eluates was on average <5% of the mass of DOC in the eluates of samples with DOC concentrations >2 mg L^-1 (i.e., subalpine lake and stream samples). For the Green Lake 4 samples, which all had DOC concentrations <2 mg L^-1, the mass of DOC in the DI blank eluate was on average 10% ± 3.2% of the mass of DOC in the sample eluates.

[21] During snowmelt chlorophyll a concentrations were below the detection limit of 1 µg L^-1 at Green Lake 4 and were about 3 µg L^-1 at Lake Albion (Figures 2g and 2h). Concentrations of chlorophyll a then increased by approximately a factor of 4. Following the rain event, chlorophyll a concentrations decreased abruptly to values less than the detection limit at Green Lake 4. During that time, the phytoplankton analysis showed that several of the dominant phytoplankton species were flushed from Green Lake 4 (http://culter.colorado.edu/NWT/). The peak chlorophyll a concentration, 5.8 µg L^-1, at Green Lake 4 was measured in mid-August. During the summer, the phytoplankton species with the greatest biovolumes were the chlorophytes Chlamydomonas sp., and Chlorococcum sp. as well as the chrysophyte Chrysococcus sp. [Miller et al., 2009b]. A peak chlorophyll a value of 15.7 µg L^-1 was measured at Lake Albion in late September. The chlorophyll a concentrations at Como Creek were low (less than the detection limit) during the entire study period, with the exception of early May when the chlorophyll a concentration peaked at 1.5 µg L^-1 (Figure 2i). At all sites, bacterial cells were most abundant during snowmelt (~1.5 × 10^7 cells mL^-1) and then decreased in abundance and showed no consistent trend through the remainder of the sampling season (Figures 2g–2i). Based on the hydrographs and chlorophyll a values, the snowmelt period (high flow, low chlorophyll a) was defined as 25 May through 13 July and the summer period (low flow, high chlorophyll a) was defined as 20 July through 29 September.

3.2. Times Series of Spectroscopic Characteristics of Whole Water and Fulvic Acid DOM

[22] The SUVA254 values of the whole water DOM and fulvic acid fraction were generally lower at the two lake sites (<4 L mgC^-1 m^-1) as compared with Como Creek (>4 L mgC^-1 m^-1), indicating variation in the aromaticity of the DOM between stream and lake sites (Figures 3a–3c). At all three sites the SUVA254 values of the whole water DOM and fulvic acid were greatest during snowmelt and decreased into the summer. The largest seasonal decline in whole water SUVA254 occurred at Green Lake 4, corresponding with the lowest percent fulvic acid in summer. The SUVA254 values of the nonhumic DOM, which were only measured during snowmelt, were consistently less than the whole water and fulvic acid SUVA254 values.

[23] During snowmelt at all three sites, the FI of both the whole water DOM and fulvic acid fractions of the DOM were low (1.2–1.3), indicating a terrestrial source of DOM (Figures 3d–3f). The FI values of the whole water increased to varying degrees during the summer months, with the greatest increase in Green Lake 4 and the least in Como Creek. In Green Lake 4 and Lake Albion the FI of the fulvic acid increased during the summer, although the change in the Lake Albion was less than the difference in FI of ±0.1 that was initially proposed as indicative of a change in source of the DOM [McKnight et al., 2001] (Figures 3d and 3e). In Como Creek, the changes in FI of fulvic acid were also slight, with a decrease during snowmelt and a return to initial values of 1.2–1.3 during the summer (Figure 3f).

[24] During snowmelt at all three sites, and during the summer at Lake Albion and Como Creek, the RI values of both the whole water and fulvic acid DOM were consistently high (0.42–0.56), indicating that the quinone-like fluorophores of the DOM were in a reduced state (Figures 3g–3i). The redox state of DOM in aquatic environments plays an important role in many ecosystem processes including electron transfer, metal binding, and nutrient availability [e.g., Davidson et al., 2003; Ratasuk and Nanny, 2007]. The highest RI value (0.56) was measured at Como Creek. During the summer at Green Lake 4 the quinone-like fluorophores of the whole water DOM became more oxidized (RI = 0.27) than the quinone-like fluorophores of the fulvic acid fraction.

[25] The percent contribution of amino acid-like fluorophores in the whole water and fulvic acid fraction was greater at the lake sites (>6%) as compared to the Como
Figure 2. Temporal variations at Green Lake 4 (GL4), Lake Albion (ALB), and Como Creek (CC) in (a–c) discharge (Q), (d–f) dissolved organic carbon (DOC, dotted lines) concentrations and percent fulvic acid (%FA, solid lines), and (g–i) chlorophyll a concentrations (dotted lines) and bacterial counts (solid lines). Shapes of the symbols, which are site-specific, differ in Figures 2d–2f and 2g–2i. Also note the difference in scale on the y axes in Figures 2a–2c. All chlorophyll a values that were below the detection limit (1 μg L⁻¹) are reported as half of that value (0.5 μg L⁻¹).
Figure 3. Temporal variations at Green Lake 4 (GL4), Lake Albion (ALB), and Como Creek (CC) in whole water (black solid lines) and fulvic acid (black dotted lines) (a–c) specific ultraviolet absorbance (SUVA$_{254}$), (d–f) fluorescence index (FI), (g–i) redox index (RI), and (j–l) percent contribution of amino acid-like fluorophores (percent amino acid). Shapes of the symbols, which are site-specific, differ in Figures 3a–3c, 3d–3f, 3g–3i, and 3j–3l. The SUVA$_{254}$ values for early season nonhumic DOM (gray solid lines) are shown in Figures 3a–3c, the FI values of the Suwannee River (SRFA) and Lake Fryxell (LFFA) fulvic acid end-members are shown in Figures 3d–3f, and the average RI values for stream and wetland samples [Miller et al., 2006] collected in the catchment are shown in Figures 3g–3i.
The percent contribution of amino acid-like fluorophores was consistently greater for the whole water DOM as compared with the fulvic acid fraction at Green Lake 4 and Lake Albion. However, the percent contribution of amino acid-like fluorophores in the whole water and fulvic acid fraction were similar throughout snowmelt and the summer at Como Creek.

3.3. Comparison of Seasonal Changes in Whole Water DOM and Fulvic Acid Among Sites

In general, the SUVA$_{254}$ values of the fulvic acid fraction of the DOM were greater than the SUVA$_{254}$ values of the whole water DOM at all sites (Figure 4a), indicating that the fulvic acid was the more chromophoric fraction of the DOM. At Green Lake 4, the greatest difference occurred during the summer. During snowmelt the fulvic acid and whole water SUVA$_{254}$ values were actually similar, with the SUVA$_{254}$ value of the fulvic acid being less than that of the whole water during late snowmelt on 29 June 2006. For Lake Albion there was not a seasonal trend in the difference between whole water and fulvic acid SUVA$_{254}$ values. Whereas, at Como Creek, the greatest difference between fulvic acid and whole water SUVA$_{254}$ values occurred during snowmelt.

At all sites, the whole water FI values were generally slightly higher than the FI values of the fulvic acid (Figure 4b). The exception to this trend occurred at Green Lake 4 during the summer months, when the difference between the average whole water FI value and the average fulvic acid FI value ($\Delta$FI) was 0.17; indicating a nonhumic source of microbially derived DOM (Table 1). In particular, in mid-August at Green Lake 4 the whole water FI value peaked at 1.71 and the fulvic acid FI value peaked at 1.43, a difference of 0.28 (Figure 3d). Further, at Green Lake 4 the summer $\Delta$FI was significantly greater than the snowmelt $\Delta$FI value ($p < 0.05$). As with the large decline in whole water SUVA in summer at this lake, the large $\Delta$FI corresponds with the low percent fulvic acid. At all other sites during both the snowmelt and summer periods the $\Delta$FI was less than the 0.1 difference indicative of a change in source [McKnight et al., 2001]. Additionally, the summer $\Delta$FI at Green Lake 4 was not...
Table 1. Average Differences ±1 Standard Deviation Between the Whole Water and Fulvic Acid FI and RI at Each of the Three Sampling Sites During the Snowmelt and Summer Periods

<table>
<thead>
<tr>
<th>Site</th>
<th>Green Lake</th>
<th>Lake Albion</th>
<th>Como Creek</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Snowmelt</td>
<td>Summer</td>
<td>Snowmelt</td>
</tr>
<tr>
<td>ΔFI</td>
<td>0.08 ± 0.03</td>
<td>0.17 ± 0.01</td>
<td>0.04 ± 0.02</td>
</tr>
<tr>
<td>ΔRI</td>
<td>0.01 ± 0.01</td>
<td>−0.09 ± 0.06</td>
<td>0.01 ± 0.01</td>
</tr>
</tbody>
</table>

*aΔFI, average difference between the whole water and fulvic acid FI; ΔRI, average difference between the whole water and fulvic acid RI.

significantly greater than the summer ΔFI values at Lake Albion and Como Creek \( p < 0.05 \).

The only consistent deviation from the 1:1 line for whole water versus fulvic acid RI values occurred during the summer months at Green Lake 4 (Figure 4c). The difference between the average whole water RI and the average fulvic acid RI (ΔRI) at Green Lake 4 during the summer was −0.09, indicating a nonhumic source of oxidized quinone-like DOM (Table 1). The ΔRI at all other sites regardless of season was close to zero \( (−0.01–0.03) \). The summer ΔRI at Green Lake 4 was significantly less than the snowmelt ΔRI value at Green Lake 4 as well as the summer ΔRI values at Lake Albion and Como Creek \( p < 0.05 \).

The percent contribution of amino acids to the fluorescent fraction of the whole water and fulvic acid DOM was lowest at Como Creek (Figure 4d). Moreover, there was little difference between the whole water and fulvic acid percent contribution of amino acids at this site. At the outlets to Green Lake 4 and Lake Albion, the percentages of the PARAFAC components that represent amino acids were greater by 1–6% in the whole water DOM as compared to the fulvic acid fraction during snowmelt and the summer growing season.

3.4. Relationships Between DOM Source, DOM Quality, and Biological Activity

There was a statistically significant positive linear relationship between whole water DOM source (FI) and chlorophyll \( a \) concentrations \( p < 0.01; \) Figure 5a). The points that deviated most from the line of best fit were summer samples collected at the outlet to Green Lake 4. These samples had FI values on the upper end of the measured range \( (∼1.5–1.7) \) and had intermediate chlorophyll \( a \) concentrations \( (∼2–6 \mu g L^{-1}) \).

There was a statistically significant negative relationship between whole water FI and SUVA\( _{254} \) values \( p < 0.001; \) Figure 5b). The high SUVA\( _{254} \)-low FI end of the spectrum showed the least deviation from the trend line and was dominated by Como Creek samples and Green Lake 4 snowmelt samples. On the other hand, the greatest deviation from the trend line occurred on the low SUVA\( _{254} \)-high FI end of the spectrum, where summer samples from Lake Albion and Como Creek and Green Lake 4 were grouped.

3.5. Principal Components Analysis of PARAFAC Component Distributions

The first and second axes of the PCA for the whole water samples accounted for 70% and 18%, respectively, of the variance in PARAFAC component distribution (Figures 6a–6c). Although the PCA included all samples from all sites, the PCA scores were plotted on the first two axes separately for each site to avoid crowding of points on a single plot. The PCA scores of the Green Lake 4 samples had a wide distribution; whereas the PCA scores showed a clumped distribution at the two subalpine sites. Furthermore, the Lake Albion samples were clearly separated from the Como Creek with scores grouped in opposite quadrants. PARAFAC components that had strong positive weightings on both axes, the region where all Lake Albion points and most of the Green Lake 4 summer points were clustered, included the microbially derived components that represent the proteins tryptophan and tyrosine, as well as the oxidized quinone-like component Q3 (Table 2). The unknown microbially derived C3 component and microbially derived SQ3 component also had weaker positive weightings on both axes. The only component with a strong negative

Figure 5. Relationships between whole water FI values and (a) chlorophyll \( a \) concentrations and (b) whole water SUVA\( _{254} \) values for all sites during both seasons. Also shown are the equations for the lines of best fit, the \( R^2 \) values, and the \( p \) values corresponding to linear regression analyses. Note that all chlorophyll \( a \) values below the detection limit of 1 \( \mu g L^{-1} \) are reported as half of the detection limit \( (0.5 \mu g L^{-1}) \).
weighting on both axes, where the Green Lake 4 snowmelt points and Como Creek points were clustered, was the component representing a terrestrially derived semiquinone, SQ1. Additionally, the unidentified components C1 and C6 as well as Q2, SQ2, and Q1 had weaker negative weightings on both PCA axes.

The two Green Lake 4 summer samples that had strong positive weightings on axis 1 and strong negative weightings on axis 2 of the whole water PCA were from samples collected during the peak of the phytoplankton bloom (17 and 24 August) (Figure 6a). The FI and RI data indicated that a large fraction of the whole water quinone-like fluorophores at this time were microbially derived, nonhumic, and oxidized. Analysis of the PARAFAC distributions for these two samples indicated an increase in the relative contribution of the unidentified component 10, as well as the microbially derived oxidized quinone-like Q3 component and the component representing the protein tyrosine (data not shown). Indeed, component 10 has a positive weighting on axis 1 and a negative weighting on axis 2. Additionally, the Q3 and tyrosine-like components are the most strongly weighted components on axis 1.

The first and second axes of the PCA for the fulvic acid samples accounted for 80% and 17%, respectively, of the variance in PARAFAC component distribution (Figures 6d–6f). There was a clear distinction along axis 2 between the snowmelt and summer samples collected at Green Lake 4 (Figure 6d). In general, the Green Lake 4 snowmelt samples had positive scores on axis 2 (similar to the Como Creek samples) and the Green Lake 4

Figure 6. Principal components analysis (PCA) scores for the 13 components for all (a–c) whole water samples and (d–f) fulvic acid fractions of the samples fit to the PARAFAC model presented by Cory and McKnight [2005]. The PCAs for the two sample types (whole water and fulvic acid) were run separately, and each run included the PARAFAC component distributions from all three sites. The results of each PCA run are presented separately for each site for clarity: Green Lake 4 (GL4) (Figures 6a and 6d), Lake Albion (ALB) (Figures 6b and 6e), and Como Creek (CC) (Figures 6c and 6f).
summer samples had negative scores on axis 2 (similar to the Lake Albion samples). The average axis 2 score of the Green Lake 4 snowmelt samples (0.45) was significantly greater than the average axis 2 score of the summer samples (−1.00) \((p < 0.01)\). The Green Lake 4 samples with negative scores on axis 1 were collected either early in the season (presnowmelt and at the initiation of snowmelt) or late in the summer, during base flow conditions. The only component with a strong negative weighting on axis 1 was the tyrosine-like component. Green Lake 4 samples with positive scores on axis 1 were collected in the middle of the sampling season (late snowmelt and early summer). Components with strong positive weightings on axis 1 included the microbially derived tryptophan-like component, semiquinone-like (SQ3) component, and oxidized quinoline-like (Q3) component.

[35] Axis 2 of the fulvic acid PCA clearly separated the Lake Albion samples from the Como Creek samples (Figures 6e and 6f). The PARAFAC component representing tyrosine-like fluorescence had strong negative weightings on both axes, in the region where the majority of the Lake Albion samples were clustered (Table 2). On the other hand, the components that had negative weightings on axis 1 and strong positive weightings on axis 2, where the majority of the Como Creek points were clustered, were the reduced hydroquinone-like component (HQ) and the terrestrially derived semiquinone-like component (SQ1). Additional components with weaker PCA weightings in this region of the plot included unidentified components C1, C6, C10, and the terrestrially derived Q1 component. Lake Albion and Como Creek samples with positive scores on axis 1 of the fulvic acid PCA were from early season snowmelt samples at both sites as well as one late summer sample at Lake Albion.

4. Discussion

4.1. Differences in Spectral Characteristics of DOM Between Sites

[36] Our spectroscopic results indicate a difference in the source and chemical quality of DOM at the three sites. The decrease in SUVA\(_{254}\) and increase in FI values of the whole water and fulvic acid fractions during snowmelt at Green Lake 4 is consistent with the trends observed in the fulvic acid fraction of the DOM by Hood et al. [2003, 2005]. However, the oxidation of microbially derived nonhumic quinoline-like fluorophores and production of microbially derived nonhumic amino acid-like fluorophores at Green Lake 4 during the summer growing season has not been previously demonstrated. This result suggests that in-lake biogeochemical processes, such as production of nonhumic DOM by phytoplankton, during the summer in this alpine lake are important drivers of DOM quality in the alpine reaches of the catchment.

[37] The large seasonal differences in spectral characteristics of the DOM at Green Lake 4 were not as prominent at the subalpine sites. For example, there was little seasonal change in the oxidation state of the quinoline-like fluorophores at Lake Albion and Como Creek. The slight increase in FI at Lake Albion and Como Creek was less than the difference of 0.1 indicative of a change in source [McKnight et al., 2001]. The contribution of nonhumic amino acid-like fluorophores throughout the season at Lake Albion suggests that this fraction of the DOM may be produced in lake ecosystems in both alpine and subalpine environments. While amino acid-like fluorophores were produced in the subalpine lake during the summer months, any autochthonous DOM signal from this site may be masked by flushing of terrestrial DOM into the system. Likewise, the subalpine stream was dominated by terrestrial inputs throughout the summer months.

4.2. Comparison of Spectral Characteristics of Whole Water DOM With the Fulvic Acid Fraction

[38] Comparison of the spectral characteristics of the whole water DOM with the fulvic acid fraction indicates that the greatest heterogeneity in DOM source and quality between DOM pools (whole water versus fulvic acid) occurred during the summer at Green Lake 4. In general, spectral characteristics of DOM in the whole water and fulvic acid fractions were similar at the subalpine sites during both seasons, as well as at Green Lake 4 during snowmelt. On the other hand, the source (FI) and redox state (RI) of the whole water and fulvic acid fraction of the DOM during the summer at Green Lake 4 deviated from one another. In a similar comparison of DOM fractions, Cory et al.

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Table 2. PCA Weightings for Each of the 13 PARAFAC Components Identified by Cory and McKnight [2005]*

<table>
<thead>
<tr>
<th>Component</th>
<th>Molecular Association</th>
<th>Source</th>
<th>Whole Water PCA Weighting</th>
<th>Fulvic Acid PCA Weighting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Axis 1</td>
<td>Axis 2</td>
</tr>
<tr>
<td>C1</td>
<td>unknown</td>
<td>T</td>
<td>−0.03</td>
<td>−0.28</td>
</tr>
<tr>
<td>C2</td>
<td>quinoline-like (Q2)</td>
<td>M</td>
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<td>−0.04</td>
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<tr>
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<td>M</td>
<td>0.11</td>
<td>0.12</td>
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<tr>
<td>C4</td>
<td>hydroquinone-like (HQ)</td>
<td>both</td>
<td>−0.79</td>
<td>0.49</td>
</tr>
<tr>
<td>C5</td>
<td>semiquinone-like (SQ1)</td>
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<td>−0.31</td>
</tr>
<tr>
<td>C6</td>
<td>unknown</td>
<td>M</td>
<td>−0.07</td>
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</tr>
<tr>
<td>C7</td>
<td>semiquinone-like (SQ2)</td>
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</tr>
<tr>
<td>C8</td>
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<td>0.15</td>
<td>0.48</td>
</tr>
<tr>
<td>C9</td>
<td>semiquinone-like (SQ3)</td>
<td>M</td>
<td>0.16</td>
<td>0.03</td>
</tr>
<tr>
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<td>T</td>
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</tr>
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<td>quinone-like (Q3)</td>
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<tr>
<td>C13</td>
<td>amino acid-like (tyrosine)</td>
<td>M</td>
<td>0.41</td>
<td>0.44</td>
</tr>
</tbody>
</table>

*The weightings for the PCA run on the whole water samples as well as the PCA run on the fulvic acid samples are shown.

*Molecular associations for components as identified by Cory and McKnight [2005].

Cory et al.
[2007] found that the spectroscopic characteristics of whole water DOM and fulvic acid fractions in Arctic surface waters were similar, suggesting that interpreting trends in the fulvic acid fraction of DOM may be applicable to trends seen in whole water DOM. Our results indicate that while the spectroscopic characteristics of whole water and fulvic acid DOM may be similar in Arctic or subalpine environments, the source and quality of these two pools differ from one another during the summer growing season in an alpine lake. These findings suggest that in systems dominated by allochthonous inputs, where there is a dominance of high molecular weight, aromatic materials, there is a strong relationship between whole water DOM and fulvic acid fractions. On the other hand, in systems dominated by autochthonous algal production, there is the potential for the source and quality of the whole water DOM and fulvic acid fractions to deviate from one another as the percent fulvic acid decreases.

The slight increase in fulvic acid FI observed at Green Lake 4 during the summer is consistent with the findings of Hood et al. [2003, 2005] that fulvic acid DOM is produced in alpine lakes during the summer growing season. In addition to autochthonous production of fulvic acid DOM, our results provide support for the hypothesis that microbially derived nonhumic DOM is also produced in the alpine lake during the summer growing season. While both the Green Lake 4 and Lake Albion samples had FI values as high as 1.7, and midrange chlorophyll a concentrations (Figure 4d) are also produced in the alpine lake during the summer months. The finding that the Green Lake 4 summer samples had FI values as high as 1.7, and midrange chlorophyll a concentrations (Figure 5a), suggests that lower concentrations of chlorophyll biomass are required to produce a microbiologically derived DOM signal in the small alpine lake as compared to the larger volume subalpine lake. Similarly, while both the Green Lake 4 and Lake Albion summer samples are composed of organic molecules that have low aromaticity (i.e., low SUVA254), the whole water DOM at Green Lake 4 was more strongly derived from a microbial source (Figures 5a and 5b). The primary production in Green Lake 4 during the summer months, indicated by the increase in concentrations of chlorophyll a, likely contributed to the in-lake production of the microbially derived nonhumic pool of quinone-like and amino acid-like fluorophores. Indeed, reactive transport modeling results have shown that the production of nonhumic DOM in Green Lake 4 is dependent on chlorophyll a concentrations [Miller et al., 2009a]. Additionally, it has been demonstrated that there is a link between the production of nonhumic DOM and primary productivity in lake [Gondar et al., 2008] and marine [Davis and Benner, 2007] ecosystems.

4.3. Spectral Characterization of Low DOC Samples

Any bleed from the XAD-8 resin likely contributing to the fulvic acid fraction was accounted for by correcting for DOC bleed prior to calculating the SUVA254 values of the fulvic acid fraction of the samples. The low ferric iron concentrations (<0.05 mg L⁻¹) in surface waters in the Green Lakes Valley and the relatively low nitrate concentrations (2–3 mg L⁻¹) suggest that it is unlikely that these species were interfering with the absorption spectra. The whole water and fulvic acid SUVA254 values were influenced by the uncertainty in the DOC measurements (coefficient of variance is 0.2 mg L⁻¹). Additionally, the fulvic acid SUVA254 values were influenced by the uncertainty associated with the repeated XAD-8 bleed measurements (±0.2 mg L⁻¹). Taken together these errors could explain the observation that the SUVA254 value measured in the whole water sample was greater than the SUVA254 value of the fulvic acid sample collected on 29 June 2006 at Green Lake 4. While limitations on the accuracy of DOC measurements has the potential to influence the calculation of percent fulvic acid, the percent fulvic acid values reported here are comparable to those reported by Hood et al. [2003, 2005].

4.4. Comparison of Principal Components Analysis of Whole Water and Fulvic Acid DOM

The separation of samples along the PCA axes reflects the within-site variability in DOM quality at Green Lake 4 on the seasonal timescale and the intersite variability between subalpine stream and lake sites. Specifically, the separation of the Como Creek samples and Green Lake 4 snowmelt samples from the Lake Albion samples is due to the seasonality of DOM production, where the Green Lake 4 snowmelt samples and the Como Creek samples support the hypothesis of Hood et al. [2003] that during high-flow conditions (e.g., snowmelt) the alpine lake acts like a wide point in the stream. Additionally, the strong influence of the terrestrially derived semiquinone-like component, SQ1, on both the whole water and fulvic acid samples at Green Lake 4 during snowmelt and the entire season at Como Creek is consistent with SQ1 being associated with DOM from higher plant matter in both DOM fractions [Cory and McKnight, 2005]. On the other hand, the finding that the most reduced quinone-like component, HQ, is a dominant driver in determining the quality of the fulvic acid fraction of the Green Lake 4 snowmelt and Como Creek DOM, but not the whole water DOM, suggests that HQ is predominantly associated with fulvic acid DOM at these sites.

Similar to the clumping of the Como Creek samples on the PCA axes, the clumping of the Lake Albion samples indicates that there is little temporal variability in the fluorescent fraction of the whole water or fulvic acid fractions of DOM at this site over time. The continuous contribution of
nonhumic amino acid-like fluorophores to Lake Albion and Green Lake 4 (Figure 4d) suggests that autochthonous production of DOM in these lake ecosystems is important. The importance of autochthonous production at these sites is further supported by the similarity in PCA scores between the Lake Albion points and the summer Green Lake 4 samples, which tended to be influenced by microbiologically derived quinone-like components (Q3) and amino acid-like components (tryptophan and tyrosine).

The separation of the two whole water samples collected at Green Lake 4 on 17 and 24 August, when the ΔFI was greatest and ΔRI was most negative, from the other Green Lake 4 points along both of the PCA axes suggests that the lake acts as a biogeochemical hot spot [McClain et al., 2003] for short periods of time during the summer phytoplankton bloom. Specifically, biogeochemical processes taking place in the lake produce nonhumic, oxidized quinone-like DOM during the peak in autotrophic activity.

While the PCA results clearly indicate that there are differences between the chemical quality of the fluorescent fraction of the whole water and fulvic acid fractions of the DOM at Lake Albion and Como Creek, the seasonal differences at the subalpine sites with respect to DOM source and redox state were not as substantial as those at Green Lake 4. Our results indicate that any microbial contribution of nonhumic DOM to the subalpine sites may have been offset by large inputs of terrestrially derived DOM from the surrounding catchment. On the other hand, the fulvic–whole water comparison data and the PCA results presented here suggest that nonhumic DOM is the dominant DOM pool influencing bulk DOM properties (e.g., source and oxidation state) during the summer phytoplankton bloom at Green Lake 4. Moreover, our results suggest that the nonhumic DOM produced during the summer in Green Lake 4 includes both protein- and quinone-like components.

5. Conclusions and Implications

Spectral characterization of whole water DOM and fulvic acid indicates that different types of water bodies with differing residence times, mixing processes and depths produce different patterns in DOM quantity and quality. Our results demonstrate that DOM quality is highly variable on a seasonal timescale in an alpine lake. Specifically, the small, clear alpine lake is strongly influenced by snowmelt processes early in the summer and then, as the summer progresses, the dominant DOM pool changes from one dominated by allochthonous sources to one dominated by autochthonous sources. The subalpine reservoir responds modestly to snowmelt flushing and does not provide a large autochthonous source of DOM. Likewise, the subalpine stream is dominated by terrestrial inputs throughout the summer months. Moreover, the rapid production of nonhumic amino acid-like and quinone-like fluorophores during the summer phytoplankton bloom in the alpine lake provides an example of the role of inland waters as hot spots of biogeochemical activity, which has recently been identified as an emerging research question for limnology [Wurtsbaugh et al., 2002; McClain et al., 2003].

[47] Acknowledgments. This work was funded by the NSF’s Niwot Ridge LTER Program (DEB-0423662) and Critical Zone Observatory project (EAR-0724960). We thank R. Cory for helpful comments on interpretation of the spectral data and B. Simone for helpful comments on the manuscript. We are grateful for comments provided by three anonymous reviewers. Funding was also provided by the University of Colorado Environmental Engineering Research Experience for Undergraduates Program.

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