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Patterns in the Chemical Fractionation of Organic Nitrogen in Rocky Mountain Streams

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Abstract

The intraannual dynamics of particulate organic nitrogen (PON) and two fractions of dissolved organic nitrogen (DON) were investigated in two Rocky Mountain streams draining watersheds with low rates of N deposition. Organic nitrogen accounted for over 60% of the total annual nitrogen export and consisted mostly of DON. Nitrate peaked during winter months and declined considerably during the growing season (less than 10 μg/L) suggesting the importance of biotic uptake. Concentrations of PON, total DON, and two DON fractions (humic and non-humic) peaked during spring runoff and were positively related to discharge, indicating hydrologic influence. Total DON and its two fractions showed significant inverse relationships to nitrate, indicating that DON and nitrate followed different intraannual patterns. Despite its seasonal fluctuations in concentration, PON showed a consistent carbon–nitrogen (C:N) ratio suggesting that it was relatively uniform in composition. Fractionation studies indicated that DON was primarily of non-humic origin, whereas dissolved organic carbon (DOC) was mainly derived from humic sources. The two DON fractions differed from each other in seasonal patterns of concentration and C:N ratio. The proportion of humic DON increased during snowmelt, and there were diverging seasonal patterns in the C:N ratio of the two fractions implying variations in bioavailability. Although organic nitrogen is commonly treated as a single pool in ecological studies, our results indicated that DON consists of fractions that undergo large intraannual changes in proportions and chemical composition. Treatment of DON as a single pool may be misleading from the viewpoint of understanding ecosystem processes directly related to changes in its sources and biological reactivity.

Key words: organic nitrogen; organic carbon; nitrate; nitrogen fractions; streams.

Introduction

Organic nitrogen can account for a substantial proportion of the total nitrogen in streams draining both undisturbed and disturbed watersheds (for example, Hedin and others, 1995; Lovett and others 1998; Lewis and others 1999; Perakis and Hedin 2002). Consequently, much recent work has been devoted to understanding the factors influencing interannual and intraannual variations in export of organic nitrogen from watersheds. Some studies have shown that hydrologic factors are important in influencing transport of dissolved (for example, see Campbell and others 2000; Williams and others 2001; Sickman and others 2001) and particulate (McDowell and Asbury 1994; Wollheim and others 2001) organic nitrogen. Other studies, 1994 (Seitzinger and Sanders 1997; Creed and Band 1998; Goodale and others 2000) have noted the importance of factors such as sorption in soils, topography, flow path, and processing in streams.

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Although the components of inorganic nitrogen often are analyzed separately in ecological studies, dissolved organic nitrogen (DON) typically is treated as a single pool because of discrepancies in methodology for its chemical characterization and the difficulty in fractionation of dissolved organic matter. Organic nitrogen, however, is likely to contain a mixture of diverse compounds such as humic substances, amino acids, amino sugars, and tannins. The distribution of these compounds in an ecosystem is likely to vary in time and space (Findlay and Sinsabaugh 1999) but may provide useful information on sources, mobilization, and biological reactivity of organic nitrogen in ecosystems.

We hypothesized that the chemical composition of organic nitrogen in montane streams varies temporally. Our primary objective was to document seasonal patterns in fractions of organic nitrogen and interpret them in the context of patterns of bulk DON, organic carbon, and inorganic nitrogen, all of which have been thoroughly studied. A second objective was to relate changes in the dynamics of organic nitrogen fractions to discharge and temperature, which show considerable seasonal fluctuations. Runoff is likely to affect both yield (transport per unit area of watershed) and concentration of organic nitrogen fractions because it controls both dilution and removal of nitrogen. Temperature may also affect dynamics of organic nitrogen by influencing uptake rates for nitrogen fractions during the growing season and production and release of organic nitrogen by microbial processes.

We separated organic nitrogen into four fractions: particulate organic nitrogen (PON), total DON, humic DON, and non-humic DON. DON was fractionated with XAD-8 resin (for example, see Qualls and Haines 1992; McKnight and others 1997). Organic matter of humic origin is hydrophobic, generally has a high molecular weight, and is derived from lignified plant remains (Guggenberger and others 1994). Organic matter of non-humic origin is hydrophilic, generally derived from microbial activity or plant sugars (Guggenberger and others 1994), and is thought to be more bioavailable (Qualls and Haines 1991).

Study Sites

The two study sites, Spruce Creek (39°26′30″ N, 106°03′00″W) and McCullough Gulch (39°24′15″N, 106°03′30″W), are second-order streams draining watersheds in Summit County, Colorado, on the western slope of the Rocky Mountains. Spruce Creek drains an area of 1585 ha and McCullough Gulch drains an area of 1295 ha. Both watersheds are similar in aspect, slope, elevation, geology, soils, and vegetation. The watersheds drain to the east into the larger Blue River, a tributary of the Colorado River. Elevation ranges from 3200 to 4250 m in each watershed. Bedrock consists of biotitic gneiss, schist, and migmatite, and soils are classified as mixed typic cryoboralfs (Summit County Soil Survey 1980). Vegetation and land cover in the watersheds is characteristic of other small catchments in Summit County described by (Brooks and others 1999). Approximately 50% of the area in each watershed is above treeline and portions of the watersheds extend into alpine tundra. The highest elevations are characterized by alpine meadows with grasses, sedges, dwarf willows, and forbs interspersed with talus fields and exposed bedrock. Lower elevations contain primarily subalpine forests with pine–spruce–fir communities. Sampling for the project was conducted in forested sections of the streams (elevation about 3400 m a.s.l.).

The nitrogen cycles of the study areas are minimally perturbed (natural vegetative cover, no resident populations, and no roads). A detailed atmospheric deposition network study for the state of Colorado in the 1980s showed that atmospheric deposition of N in this area of Summit County (around. 3 kg/ha y NO_3^-–N plus NH_4^+–N) was among the lowest in the state (Lewis and others 1984a, 1984b); current data from the National Atmospheric Deposition Program show little subsequent change in deposition rates in this area of the western slope. Atmospheric deposition of N is substantially less than on the eastern slope of the Colorado Rockies, which may be progressing towards nitrogen saturation (Williams and others 1996; Baron and Campbell 1997; Rueth and Baron 2002), and over much of the eastern United States.

The hydrographs of small streams in Summit County are strongly controlled by snowmelt (Boyer and others 1997), which produces a peak of discharge that usually occurs in June. Concentrations of total N (TN) and N fractions change seasonally in montane streams of Summit County (Lewis and others 1984a) and in nearby montane watersheds (Lewis and Grant 1979, 1980).

Methods

Samples of stream water were collected from Spruce Creek and McCullough Gulch weekly (May to August) or biweekly (September to April) over two years beginning in 1999. Samples were stored in a dark cooler pending transport to the laboratory, where they were filtered within 12 h of collection

(Whatman GF/F; nominal pore size 0.7 μm), and frozen until analysis.

Ammonium was determined colorimetrically by a modified Solorzano method involving the production of indophenol blue (Grashoff 1976). Nitrate was measured with an ion chromatograph, and DON was measured by chemical oxidation with potassium persulfate (modified from Valderrama 1981) and subsequent determination of NO_3^- by ion chromatography (Davi and others 1993). DOC concentrations were measured on filtered water samples with a carbon analyzer. Analysis of particulate organic nitrogen and carbon involved filtration of 2–3 L of water onto a tared glass filter and combustion of filter subsamples in an elemental analyzer.

Beginning in October 2000, humic substances were isolated from approximately 20 L of water collected monthly until March and weekly from April to June. All water samples were filtered within 12 h of collection (Whatman GF/F) and acidified to pH 2 with sulfuric acid before fractionation in order to avoid chemical interference during subsequent analyses (Qualls and others 1991). Humic substances were concentrated from the filtered water samples by adsorption onto columns containing 400 mL of XAD-8 resin (Thurman and Malcolm 1981). The columns then were eluted with 0.1 N NaOH to produce approximately 1 L of concentrated humic substances. Concentrations of DON in the humic fraction were measured after chemical oxidation with potassium persulfate. Concentrations of DOC were measured in the humic fraction with a carbon analyzer. Concentrations of nonhumic DON and DOC in water samples were determined as the difference between total and humic DON and DOC.

Water temperature was measured with a thermometer at approximately 1300 h on all sampling dates, and discharge was estimated on each sampling date on the basis of current velocity as measured with a flow meter (Boyer and others 1997).

Stream transport of N was estimated using concentration and flow data. In the estimation of transport, daily concentration and discharge were assumed to remain constant before and after a sampling date up to the midpoint of the intervals between sampling dates. This approach could lead to error by missing storm events, which might occur between sampling dates. The relative contribution of storm events, however, is likely to be much smaller than snowmelt, which dominates the hydrograph of streams in Summit County. Discharge-weighted mean concentrations were calculated as the sum of the products of discharges and concentrations for all days of the year divided by the annual discharge. The discharge-weighted means were also expressed as export per unit of watershed area (kg/ha y).

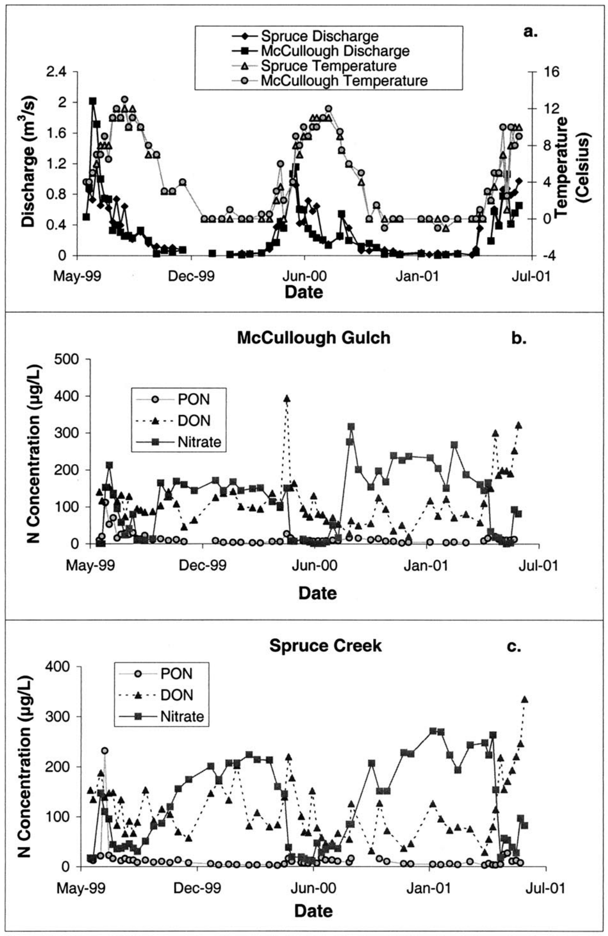

Figure 1. **a** Seasonal changes in discharge and water temperature at both study sites. **b** Seasonal changes in concentrations of major nitrogen fractions in McCullough Gulch over the sampling period. **c** Seasonal changes in concentrations of major nitrogen fractions in Spruce Creek over the sampling period.

Results

Over the study period, mean annual runoff was 440 mm and 502 mm for Spruce Creek and McCullough Gulch, respectively. There was a strong peak in discharge for both streams during June (Figure 1a) and a smaller peak in May. Water temperatures were close to zero throughout winter and reached a peak of 13°C in summer (Figure 1a). Between November and March, the streams and soils in the watershed were covered with ice and snow.

Concentrations of ammonium consistently were

Table 1. Nitrogen Fractions and Total Nitrogen for Spruce Creek and McCullough Gulch over a Two-Year Sampling Period[a]

	Discharge-Weighted Concentrations (μg/L)		Export (kg/ha/y)	
	Spruce	McCullough	Spruce	McCullough
NH_4^+	6	6	0.03	0.03
NO_3^-	83	80	0.36	0.40
DON	118	121	0.52	0.61
TDN	200	207	0.88	1.04
PON	19	17	0.08	0.09
TN	219	224	0.96	1.13

[a]*Concentrations are given as discharge-weighted means.*

very low (less than 10 μg/L). Nitrate, the dominant form of DIN, declined substantially during spring and late summer (less than 10 μg/L), and increased considerably in winter (Figure 1b and c). Concentrations of DON peaked sharply during early spring runoff but showed little evidence of a seasonal pattern in concentration at other times of the year. Proportionately, concentrations of DON were highest during summer, at which time DON accounted for 90% or more of total discharged nitrogen (TDN). Concentrations of particulate organic nitrogen were low except during peak discharge.

Discharge-weighted mean concentrations of nitrogen fractions and total nitrogen were similar in the two streams (Table 1). Mean concentrations of DON were greater than nitrate and the other nitrogen fractions. Mean concentrations of PON and ammonium were very low for both streams. Over the sampling period, DON comprised 59% and 58% of the annual TDN exported in Spruce Creek and McCullough Gulch respectively. Particulate and dissolved organic nitrogen accounted for slightly over 60% of the total nitrogen exported from both watersheds.

The relationships of discharge and temperature to concentrations of organic nitrogen and other nitrogen fractions were explored with stepwise multiple regression analysis following log transformation of all variables and subsequent normalization of the data (Table 2). Discharge and temperature were related to each other in both streams (McCullough Gulch, $R^2 = 0.11$; Spruce Creek, $R^2 = 0.27$). Therefore, discharge was forced as the first variable in the analysis and temperature was included in the regression model only if it could explain significant additional variance. Ammonium was excluded from the statistical analysis because of its consistently low concentrations.

Discharge explained substantial variance in concentrations of PON in both streams. Concentrations of bulk DON were significantly related to discharge in both streams, but only a modest amount of variance was explained; temperature explained a small amount of additional variance in Spruce Creek but not in McCullough Gulch. Concentrations of DON fractions were more strongly related to discharge than bulk DON, and temperature explained no additional variance. Discharge explained no variance in concentration of nitrate in Spruce Creek and very little variance in McCullough Gulch; temperature explained considerable variance in nitrate concentration for both streams. Concentrations of TDN and TN in both streams were significantly related to discharge, but more variance could be explained by temperature than by discharge probably because of the strong relationship between nitrate and temperature.

Concentrations of PON were very strongly related to concentrations of particulate organic carbon (POC) (Figure 2a). The C:N ratio of particulate organic matter showed very small variation seasonally over the two-year period. The mean C:N ratio of particulate organic matter for McCullough Gulch was 9.96 (SE = 0.23) and the mean C:N ratio for Spruce Creek was 10.6 (SE = 0.29). Although concentrations of DON peaked close to the time that concentrations of DOC peaked (Figure 2b), there was only a weak (but significant) relationship between concentrations of DON and DOC (Figure 2c). The C:N ratio of dissolved organic matter was considerably higher than particulate organic matter and showed more seasonal variation. The mean C:N ratio of dissolved organic matter (DOM) for McCullough Gulch was 20.80 (SE = 1.96) and the mean C:N ratio for Spruce Creek was 20.88 (SE = 2.00).

Concentrations of bulk DON were negatively but

Table 2. Correlations of Nitrogen Concentrations with Discharge and Temperature (log–log)[a]

	Discharge(m^3/s)					Temperature (°C)				
	Site	Const.	Slope	SE Slope	Partial r^2	Const.	Slope	S.E. Slope	Partial r^2	Adjusted R^2
NO_3^-	S	—	—	—	—	2.39	0.78	0.17	0.43	0.41
	M	3.17	0.39	0.16	0.01	3.17	1.43	0.27	0.33	0.32
DON	S	2.38	0.31	0.08	0.14	2.38	0.28	0.12	0.07	0.18
	M	2.05	0.13	0.07	0.07	—	—	—	—	0.05
DON (Humic)	S	1.64	0.37	0.11	0.48	—	—	—	—	0.44
	M	1.54	0.29	0.16	0.20	—	—	—	—	0.14
DON (Non-humic)	S	2.33	0.46	0.12	0.52	—	—	—	—	0.48
	M	2.31	0.28	0.13	0.28	—	—	—	—	0.22
TDN	S	2.73	0.09	0.05	0.15	2.73	0.51	0.07	0.42	0.55
	M	2.89	0.19	0.05	0.00	2.89	0.56	0.09	0.44	0.42
PON	S	1.36	0.48	0.05	0.58	—	—	—	—	0.57
	M	1.52	0.50	0.05	0.66	—	—	—	—	0.65
TN	S	2.67	0.05	0.06	0.14	2.67	0.47	0.09	0.41	0.38
	M	2.79	0.14	0.05	0.01	2.79	0.49	0.08	0.43	0.41

[a] *Values for Spruce Creek (S) are listed first followed by those for McCullough Gulch (M). All values shown are significant at $p < 0.05$ in a stepwise multiple regression.*

weakly related to concentrations of nitrate in both streams (McCullough Gulch, R^2 = 0.16; Spruce Creek, R^2 = 0.07). Concentrations of DON in the humic and non-humic fractions also were negatively related to concentrations of nitrate and typically more strongly so than bulk DON (Figure 3). The non-humic fraction showed a greater rate of increase in concentration with decreasing concentration of nitrate. Analysis of covariance and a Tukey's test indicated that the slopes of the regressions for humic and non-humic substances were different.

The chemical composition of dissolved organic carbon and nitrogen changed seasonally. Absolute concentrations of both fractions of DOC and DON peaked during early snowmelt (Figure 4a and b), but seasonal patterns for DON fractions were different than for DOC fractions. For the humic fraction, DOC showed a much greater relative increase during snowmelt than DON, which remained primarily of non-humic origin. The percentage of DOC that was humic increased from approximately 20% during base flow to 80% at peak discharge (Figure 5a). The percentage of humic DON increased from 20% during base flow to 40% on the ascending limb of the hydrograph and then declined at peak discharge (Figure 5b). Across all sampling dates, 60%–80% of DON consisted of non-humic substances. The C:N ratio of bulk DOM increased slightly during runoff (Figure 6a). The C:N ratio of the humic and non-humic fractions also changed seasonally but showed diverging patterns (Figure 6b). The C:N ratio of humic substances increased from approximately 25 at base flow to 75 at peak flow (Figure 6b). The C:N ratio of non-humic substances showed the opposite pattern: it decreased from 20 at base flow to below 10 during peak discharge (Figure 6b).

Discussion

Organic nitrogen accounted for a substantial proportion (over 60%) of the total nitrogen transported in the two streams. Other studies in forested watersheds receiving low rates of N deposition have also reported that organic N can account for a considerable proportion of N export (for example, see Hedin and others 1995; Lewis and others 1999; Perakis and Hedin 2002; Vanderbilt and others 2003). Exports of nitrogen fractions and total nitrogen in the present study were comparable to other minimally disturbed watersheds that have similar amounts of annual runoff (Lewis and others 1999).

PON and DON showed considerable seasonal variations in concentration. Concentrations of PON were strongly related to discharge, as expected in view of the role of shear stress in moving particles. Concentrations of bulk DON and DON fractions also were positively related to discharge, indicating hydrologic influence on their mobilization.

Concentrations of nitrate at the study sites de-

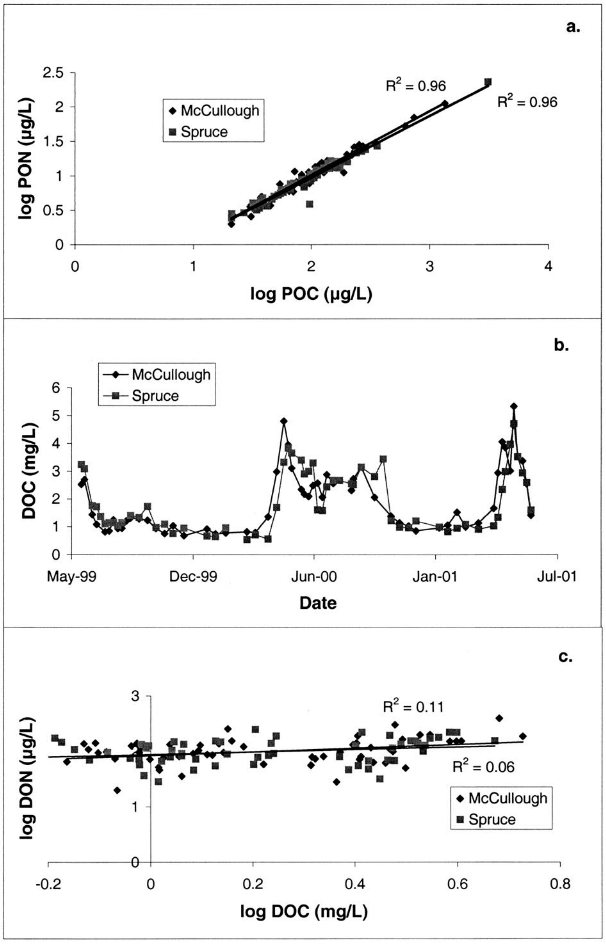

Figure 2. **a** Relationship between concentrations of PON and POC. **b** Seasonal changes in concentrations of DOC during the sampling period. **c** Relationship between concentrations of DON and DOC.

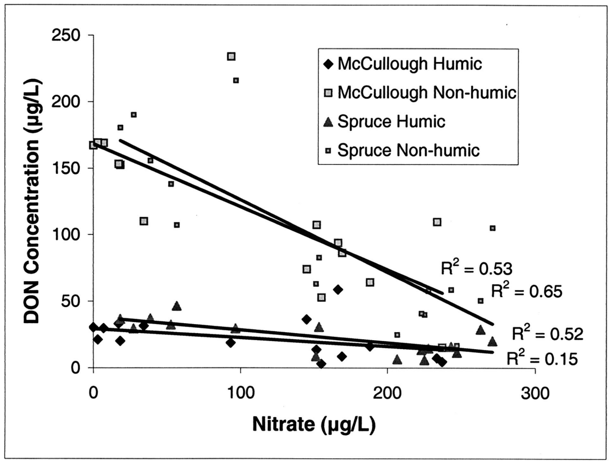

Figure 3. Relationship of humic and nonhumic DON concentrations to nitrate concentrations.

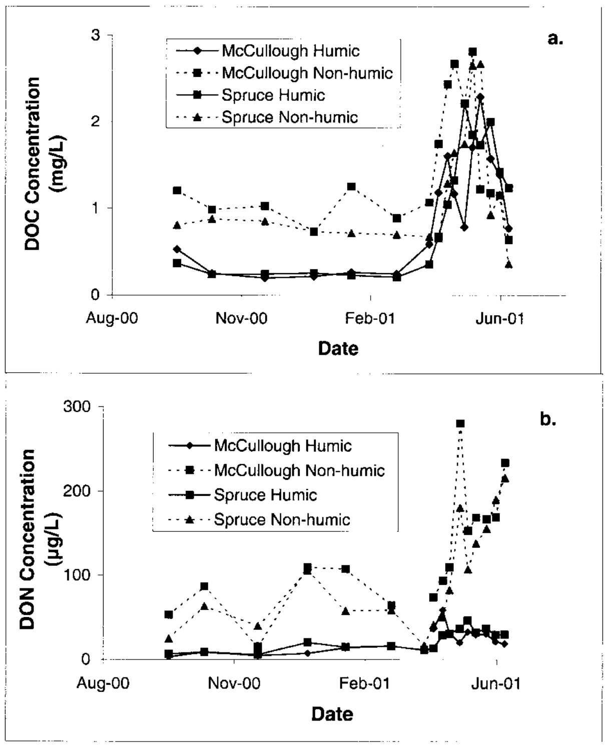

Figure 4. **a** Seasonal changes of DOC in the humic and nonhumic fractions. **b** Seasonal changes in concentrations of DON in the humic and nonhumic fractions.

creased substantially as temperature increased during the growing season, suggesting the importance of biotic uptake. The watersheds are likely to be in the earliest stages of N saturation because there was some leaching of inorganic N during the growing season (Williams and others 1996; Aber and others 1998), even though concentrations were generally near detection limits (5 µg/L). Other studies of streams on the eastern slope of the Colorado Rockies have also documented declines in concentrations of nitrate during the growing season (Williams and others 1996; Baron and Campbell 1997), but minimum concentrations of nitrate in this region have been increasing due to N deposition from anthropogenic sources (Williams and others 1996; Williams and Tonneson 2000). It is difficult to estimate retention of inorganic nitrogen at the study sites because current measurements of N inputs from deposition were not available. A rough estimate of deposition at 3 kg/ha y NO_3^-–N plus NH_4^+–N can be obtained, however, using data from Lewis and others (1984a, 1984b), and this rate is within the current range reported for similar watersheds in this region of Colorado (Sickman and others 2002; Stottlemeyer and others 1997). Exports of DIN at Spruce Creek and McCullough Gulch were 0.39 kg/ha y and 0.43 kg/ha y, respec-

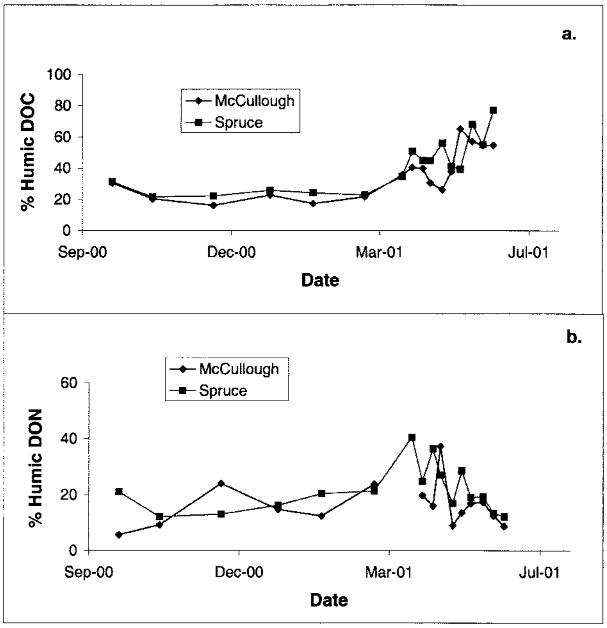

Figure 5. a Seasonal changes in the percentage of DOC from humic sources. b Seasonal changes in the percentage of DON from humic sources.

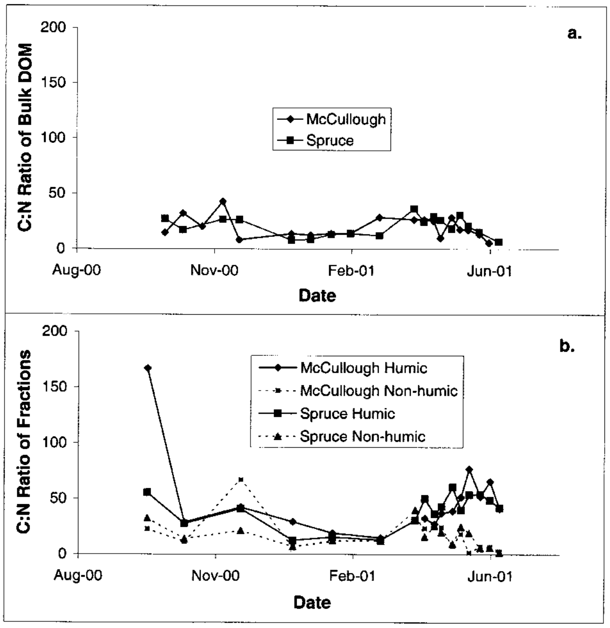

Figure 6. a Seasonal changes in the C:N of bulk dissolved organic matter. b Seasonal changes in the C:N ratios of humic and nonhumic fractions.

tively, and retention of DIN was estimated at 87% for the Spruce Creek watershed and 86% for the McCullough Gulch watershed. The values of DIN export for the study sites are lower than those typically reported for montane watersheds on the eastern slope of the Colorado Rockies but higher than some other minimally disturbed watersheds in the Sierra Nevada (Sickman and others 2002). Previous work has shown that watersheds on the western and eastern slopes of the Colorado Rockies can differ in the cycling of nitrogen in soils, vegetation, and streams as a result of differences in N deposition between the two regions. (Rueth and Baron 2002; Stottlemeyer and others 1997). Other factors, such as landscape heterogeneity, hydrologic flow paths, and climatic extremes (for example, see Campbell and others 2000; Clow and Sueker 2000; Sickman and others 2002), can also lead to variability in N exports between montane catchments over broad geographic areas.

Seasonal patterns of DIN and DON in montane streams are likely to be influenced by microbial activity and flow paths through soils. Heterotrophic activity by microbes during winter months has been shown to produce DOM (Brooks and others 1999) and consume inorganic nitrogen in soils at the plot scale (Brooks and others 1998; Heuer and others 1999). These findings are consistent with our observation that DON and its fractions (particularly the non-humic fraction) were inversely related to concentrations of nitrate in stream water at the watershed scale. Previous work (Lovett and others 1998; Goodale and others 2000; Williams and others 2001) has shown that organic and inorganic N can follow different seasonal patterns due to anthropogenic disturbance. Because of the strong inverse relationship between DON fractions and nitrate in the present study, we speculate that other factors, such as direct linkages between the uptake of inorganic N and production of organic nitrogen (for example, see Bronk and others 1994; Perakis and Hedin 2001), may not be ruled out in streams and watersheds experiencing low rates of N deposition.

The C:N ratio of particulate organic nitrogen remained relatively constant despite seasonal fluctuations in its concentration. Another study (McDowell and Asbury 1994) reported increasing C:N of particulate matter as discharge increased in two tropical watersheds due to a shift in transport of predominantly detrital particles at low flow to sand and clay at high flow. In the present study, visual inspection of filters indicated that detrital particles consistently were the main source of PON in streams. The C:N ratio of POM was considerably lower than DOM, suggesting differences in the composition and bioavailability of PON and DON. Other studies have also shown that POM generally has a lower C:N (McDowell and Asbury 1994; McKnight and others 1997) and higher concentrations of hydrolyzable amino acids and carbohydrates

(McKnight and others 1992; Aufdenkampe and others 2001) than DOM. Previous interpretations of these differences have either suggested that POM is less degraded or that labile amino acids selectively adsorb onto the surfaces of fine sediments (Aufdenkampe and others 2001).

Unlike POM, the relationship between DON and DOC showed great temporal variability. Previous studies have shown relationships of varying strength between DON and DOC (for example, see Campbell and others 2000; Goodale and others 2000; Hedin and others 1995). Concentrations of DOC in some streams increase rapidly during snowmelt (Boyer and others 1997) due to flushing of upper horizons of soil, which can accumulate substantial amounts of DOM due to heterotrophic activity in soils during winter months (Brooks and others 1999). This mechanism may lead to similar timing in peak concentrations of bulk DON and DOC during snowmelt. Our results indicate, however, that DON is primarily of non-humic origin, whereas DOC is mainly derived from humic sources, especially during snowmelt. Humic and non-humic fractions differ in origin, biological lability, and sorption characteristics (Guggenberger and others 1994). Humic substances are usually derived from plant remains (Guggenberger and others 1994) and have a mean molecular weight of 500–1000 daltons (Aiken and others 1992) and very low nitrogen content; their biological lability is expected to be low. In contrast, non-humic substances are strongly related to microbial activity or the release of plant-derived sugars (Guggenberger and others 1994); this fraction of organic matter is likely to be more labile (Qualls and Haines 1991). Its components may include protein fragments, free amino acids, carbohydrates, and pigment degradation products; its expected mean molecular weight is lower (100–500 daltons) (Aiken and others 1992).

DON and its fractions varied temporally in composition. Both the DOC:DON ratios and the DON fractionation data support this conclusion. The proportion of humic DON increased during snowmelt, and the absolute concentrations of humic and non-humic fractions also increased at this time. Intraannual changes in flow path through upper soil horizons (Easthouse and others 1992) and transport in streams (McKnight and others 2002) have been shown to influence the proportions of humic substances in DOM. The bulk DOC:DON ratio, which is commonly reported in literature, increased during runoff, but fractionation of DON revealed the presence of at least two chemically distinct pools which diverged in their temporal pattern. The substantial decrease in C:N of the non-humic fraction during spring runoff indicates a qualitative shift to potentially more labile compounds within this fraction of organic matter. Studies have shown that nitrogen from amino compounds can account for 59%–78% (Yu and others 2001) and 77%–82% (Michalzik and Matzner 1999) of DON in the upper horizons of soils in forests. The increase in C:N ratio of the humic fraction may reflect mobilization of humic substances from the upper soil horizon that have not been substantially degraded (Qualls and Haines 1992). These chemical changes in DOM may be helpful in explaining previous findings which show that DON (Stepanauskas and others 2000) and DOC (Baker and others 2000) can be biologically labile during snowmelt.

Further work involving identification and quantification of the compounds that comprise DON would be useful in providing information on the ecological significance of organic nitrogen. Large temporal changes in the concentrations and C:N ratio of two DON fractions in our study suggested that treatment of DON as a single pool could be misleading. Temporal changes probably are caused by differences in the production (Kaiser and others 2001) and transport (Kaiser and Zech 2000) of humic and non-humic organic matter. Temporal variations in the quantity and quality of organic nitrogen fractions may be important in attempting to elucidate mechanisms regulating the transport and processing of organic nitrogen in streams and their watersheds.

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