Biogeochemistry (2005) 74: 303–321
DOI 10.1007/s10533-004-4723-5

Fate and transport of organic nitrogen in minimally disturbed montane streams of Colorado, USA

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Received 1 March 2004; accepted in revised form 8 October 2004

Key words: Bioavailability, Nitrate, Nitrogen cycling, Organic carbon, Organic nitrogen

Abstract. In two montane watersheds that receive minimal deposition of atmospheric nitrogen, 15–71% of dissolved organic nitrogen (DON) was bioavailable in stream water over a 2-year period. Discharge-weighted concentrations of bulk DON were between 102 and 135 μg/l, and the C:N ratio differed substantially between humic and non-humic fractions of DON. Approximately 70% of DON export occurred during snowmelt, and 40% of that DON was biologically available to microbes in stream sediments. Concentrations of bioavailable DON in stream water were 2–16 times greater than dissolved inorganic nitrogen (DIN) during the growing season, and bioavailable DON was depleted within 2–14 days during experimental incubations. Uptake of DON was influenced by the concentration of inorganic N in stream water, the concentration of non-humic DON in stream water, and the C:N ratio of the non-humic fraction of dissolved organic matter (DOM). Uptake of DON declined logarithmically as the concentration of inorganic N in stream water increased. Experimental additions of inorganic N also caused a decline in uptake of DON and net production of DON when the C:N ratio of non-humic DOM was high. This study indicates that the relative and absolute amount of bioavailable DON can vary greatly within and across years due to interactions between the availability of inorganic nutrients and composition of DOM. DOM has the potential to be used biotically at a high rate in nitrogen-poor streams, and it may be generated by heterotrophic microbes when DIN and labile DOM with low relative nitrogen content become abundant.

Introduction

Studies of the nitrogen cycle in streams have focused primarily on inorganic nitrogen, although dissolved organic nitrogen (DON) comprises a substantial proportion of dissolved nitrogen exported from minimally disturbed watersheds (Lewis 1986; Lewis et al. 1999; Perakis and Hedin 2002) and also is abundant in runoff from watersheds that have been enriched with inorganic N (Lajtha et al. 1995; Lovett et al. 1998; Campbell et al. 2000; Goodale et al. 2000).

A variable portion of the DON exported from terrestrial environments is biologically labile (Seitzinger and Sanders 1997; Stepanauskas et al. 2000; Wiegner and Seitzinger 2001). Seitzinger et al. (2002) showed that 0–73% of

DON in runoff from forests, pastures, and urban areas in the northeastern U.S was bioavailable to estuarine plankton over a period of 10–12 days; Stepanauskas et al. (2000) showed that 20–55% of DON in boreal streams of Sweden was available to heterotrophic microbes over a period of 14 days. The biologically reactive pool of DON is expected to consist mostly of peptides of high molecular weight, but also may contain amino acids and urea (Stepanauskas et al. 1999, 2002; Guldberg et al. 2002). Bioavailable DON can originate from the atmosphere (Peierls and Paerl 1997; Seitzinger and Sanders 1999), soils (Qualls and Haines 1992; Neff and Hooper 2002), and water (Bronk and Ward 1999). In montane streams, substantial amounts of bioavailable DON are likely to originate from microbial metabolism in soils during snowmelt (Brooks et al. 1999; Lipson et al. 1999; Neff and Hooper 2002).

The metabolism of DON in streams may be influenced by the concentration of inorganic N, the concentration of DON, and the chemical composition of DON. These variables are likely to change seasonally, but they may interact in predictable ways to regulate uptake and production of DON by heterotrophic microbes. The importance of these and other variables on the metabolism of DOC has been well studied (Findlay 2003). Because dissolved organic matter (DOM) contains both carbon and nitrogen, it might be expected that the mechanisms influencing their metabolism are coupled, but few studies have compared the metabolism of DOC and DON in streams. Some of this work suggests that DOC and DON are contained in different chemical fractions of DOM (Kaushal and Lewis 2003) and can be cycled at different rates (Wiegner and Seitzinger 2001; Caraco and Cole 2003), whereas other work suggests that they can be cycled similarly (Qualls and Haines 1992).

Because DON has the potential to contribute to eutrophication (Seitzinger and Sanders 1997; Seitzinger et al. 2002; Stepanauskas et al. 2002), much of our knowledge on DON bioavailability is derived from studies of the effects of anthropogenic disturbance on the metabolism of organic N (Findlay et al. 2001; Wiegner and Seitzinger 2001; Seitzinger et al. 2002). Human activity can increase the amount of DOC and DON exported from watersheds (Currie et al. 1996; McDowell et al. 1998), induce changes in the chemical composition of organic matter (Boyer and Groffman 1996; Kaushal and Binford 1999; Wolfe et al. 2002; McDowell et al. In press), and increase the biological reactivity of both DOC and DON transported to aquatic systems (Boyer and Groffman 1996; Wiegner and Seitzinger 2001; Seitzinger et al. 2002).

The biotic importance of DON in streams draining minimally disturbed watersheds is less well known. Organic N is the dominant form of N in streams draining watersheds with low rates of atmospheric deposition of inorganic N (Lewis 1986, 2002; Hedin et al. 1995; Lewis et al. 1999; Vanderbilt et al. 2003), and concentrations of inorganic N can be low in these streams (Sollins and McCorison 1981; Hedin et al. 1995; Perakis and Hedin 2002; Kaushal and Lewis 2003). Even streams that are enriched with N have the potential to develop low concentrations of inorganic N on a seasonal basis (Williams et al. 1996; Lovett et al. 1998; Campbell et al. 2000; Goodale et al. 2000; Mulholland et al. 2000). Inorganic N can be rapidly incorporated into organic matter (Peterson et al. 1997; Mulholland et al. 2000; Bernhardt and Likens 2002), and the biotic demand for labile fractions of DON may increase when inorganic N is scarce. In marine environments, portions of DON are quickly assimilated and regenerated by bacteria and algae (Seitzinger and Sanders 1997; Bronk et al. 1998; Bronk and Ward 1999).

The objectives of the present study were to quantify the degree to which DON exported from minimally disturbed watersheds can be used biologically over short time scales, to examine how biotic uptake of DON may change in response to fluctuations in concentration of inorganic N and the chemical composition of DON, and to compare seasonal patterns in metabolism of DON with DOC. We hypothesized that: (1) DON increases in relative and absolute contribution to N demand when availability of inorganic N is low, (2) DON can be generated by heterotrophic microbes when inorganic N and labile C are abundant, and (3) DON and DOC show different seasonal patterns in their bioavailability.

Methods

The two study sites, Spruce Creek (39°26′30″ N, 106°03′00″ W; 1585 ha) and McCullough Gulch (39°24′15″ N, 106°03′30″ W; 1295 ha), are second-order streams draining watersheds in Summit County, Colorado, on the western slope of the Rocky Mountains. The watersheds are similar in aspect, slope, elevation, geology, soils, and vegetation; both drain east into the Blue River, a tributary of the Colorado River. Elevation ranges from 3200 to 4250 m above sea level in each watershed. Sampling for the project was conducted in sections of the streams surrounded by communities of pine, spruce, and fir.

The study areas have natural vegetative cover, and there are no resident populations or roads. Atmospheric deposition of N in this area of Summit County is among the lowest in the state (ca. 3 kg/ha/y NO_3^--N plus NH_4^+-N) (Lewis et al. 1984a, b; Rueth and Baron 2002), and it is substantially less than on the eastern slope of the Colorado Rockies, which may be progressing toward nitrogen saturation (Williams et al. 1996; Baron and Campbell 1997; Rueth and Baron 2002; Sickman et al. 2002).

The hydrographs of small streams in Summit County are strongly controlled by snowmelt, which typically begins in early April and produces a peak of discharge in June (Figure 1). It may be assumed that the watersheds have a growing season from the beginning of snowmelt until the end of summer. This time span does not encompass all physiological activities of plants but typically represents changes in environmental conditions and the absence of snow accumulation. Concentrations of inorganic N (mostly nitrate) are highest during winter and can become undetectable (< 5 μg/l) throughout the growing season (Kaushal and Lewis 2003). Concentrations of DON peak during spring, and DON accounts for most of the total dissolved nitrogen (up to 90%) during

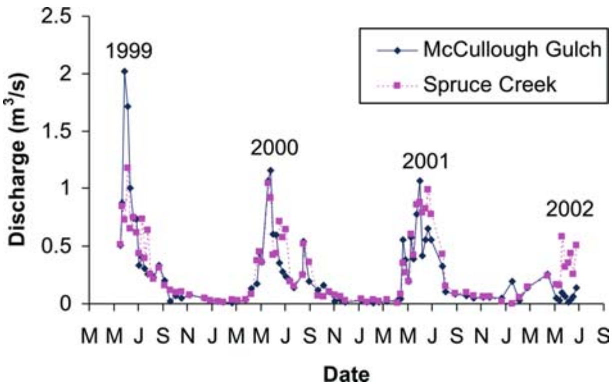

Figure 1. Seasonal changes in discharge of McCullough Gulch and Spruce Creek from June 1999 to June 2002.

the growing season (Kaushal and Lewis 2003). Annual net primary production within streams of this area is low (< 25 g dry mass/m^2/y: McCutchan and Lewis 2002).

Samples of stream water were collected from Spruce Creek and McCullough Gulch weekly (May to August) or bi-weekly (September to April) from June 1999 to June 2002. Samples were stored in a dark cooler, filtered within 12 h of collection (Whatman GF/F; nominal pore size 0.7 μm), and frozen until analysis.

Concentrations of DOC were measured on filtered water samples with a Shimadzu carbon analyzer. Ammonium was determined colorimetrically by a modified Solorzano method involving the production of indophenol blue (Grashoff 1976) and long pathlength spectrophotometry. Nitrate was measured with a Dionex ion chromatograph, and TDN was measured by chemical oxidation with potassium persulfate (modified from Valderrama 1981) and subsequent determination of NO_3^- by ion chromatography (Davi et al. 1993). Samples of water were oxidized in triplicate for analysis of TDN, and replicates typically differed < 10 μg/l in N concentration. DON was calculated as the difference between TDN and dissolved inorganic N. Soluble reactive phosphorus (SRP) was analyzed by the ascorbic acid–molybdate method of Murphy and Riley (1962) and long pathlength spectrophotometry. Total dissolved P (TDP) was determined by a modification of the oxidation method described by Lagler and Hendrix (1982) and Valderrama (1981). Samples of water were oxidized in duplicate for analysis of TDP, and replicates typically differed < 4 μg/l. Dissolved organic P (DOP) was calculated as the difference between TDP and SRP.

Annual transport of DOC, DON and DOP was estimated from data on concentration and discharge. Discharge was estimated on each sampling date from cross-sectional measurements of current velocity as measured with a flow meter. 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 (Kaushal and Lewis 2003), and also were expressed as export per unit of watershed area (kg/ha/y).

Beginning in October 2000, humic substances were isolated from approximately 20 l of water collected monthly through March and weekly from April to June. In the present study, humic substances are defined as the hydrophobic fractions of DOM (neutrals, bases, and acids) that adsorb to XAD-8 resin at a pH of 2 (similar to Thurman and Malcolm 1981). Humic substances also were isolated during the growing season of 2001 on dates coinciding with bioavailability assays. 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 prevent chemical interference during subsequent analyses (Qualls and Haines 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; McKnight et al. 2002). 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 using a Shimadzu Total Organic Carbon analyzer. Concentrations of non-humic DON and DOC in water samples were determined as the difference between total and humic DON and DOC. Mass balance analyses showed that recovery of DOC and DON was almost complete, with the sum of DOC and DON in the humic and non-humic fractions typically between 90 and 110% of the total DOC and DON in the original sample.

Bioavailability of DOC and DON was measured in three treatments of unamended stream water, stream water enriched with DIN, and stream water enriched with SRP. Assays were similar to those of Seitzinger and Sanders (1997) and Wikner et al. (1999). Water (12 l from each site) was collected during the growing seasons of 2001 and 2002, when concentrations of DON were highest, and was filtered initially through Whatman GF/F glass microfiber filters and then through polycarbonate filters of 0.22 μm pore size. For each treatment, 500 ml of filtered water was poured into triplicate Erlenmeyer flasks. Blanks consisted of 500 ml of sterile filtered deionized water poured into triplicate flasks.

Flasks containing stream water (unamended or with DIN or SRP) were inoculated with 5 ml of concentrated microbial suspension derived from a sediment slurry taken from the Blue River downstream of the two study sites. Sediment slurry was agitated by a vortexer to dislodge bacteria from particles, and it was then was gravity-filtered through polycarbonate filters of 0.6 μm pore size, which were used to remove grazers (Wikner et al. 1999). For the treatments amended with DIN, nitrate was added to raise the final concentration of NO_3^--N in stream water by 100 μg/l. For the treatments amended with P, orthophosphate was added to raise the final concentration of SRP in stream water by 20 μg/l. Subsamples of water were taken from the flasks prior to incubation and initial concentrations of DOC, DIN, and DON were measured. Flasks then were stored in the dark at 10 °C and the contents were stirred with a shaker table. After 14 days, the incubations were filtered again through polycarbonate filters of 0.22 μm pore size. Final concentrations of DOC, DIN, and DON were measured. Bioavailable DOC and DON were determined as the difference between initial and final concentrations. Changes in blanks following incubation were within the analytical variance of analyses.

Results

Concentration and yield of DOC and DON were lower in 2002 (Table 1), which was especially dry (Figure 1). Concentrations and yields of inorganic nutrients were less affected by interannual variations in runoff. DON accounted for 58–59% of TDN, and DOP accounted for 65–68% of TDP yield.

On an intraannual basis, DIN:SRP ratios were below the Redfield ratio (15:1, molar) during the growing season, which suggests the potential for co-limitation by inorganic N (Figure 2). At this same time, however, DON:-DOP ratios were well above the Redfield Ratio.

Chemical fractionation with XAD-8 resin showed that the proportion of DON in the humic fraction peaked during early snowmelt, and then declined throughout the growing season (Figure 3). DOC in most months was more

Table 1. Transport of dissolved fractions of carbon, nitrogen, and phosphorus for McCullough Gulch (M) and Spruce Creek (S) during 1999–2001.

	Site	Discharge-weighted concentrations[a]			Annual transport[b]		
		1999	2000	2001	1999	2000	2001
[a]DOC	M	2.16	2.22	1.32	11.66	11.94	5.82
	S	2.58	2.22	1.31	11.38	9.77	5.78
DIN	M	84.3	76.8	83.0	0.45	0.41	0.37
	S	82.1	96.1	90.7	0.36	0.42	0.40
DON	M	131	102	95.2	0.70	0.55	0.42
	S	135	107	120	0.60	0.47	0.53
TDN	M	214	179	178	1.15	0.96	0.78
	S	217	189	211	0.96	0.83	0.93
SRP	M	–	1.3	1.8	–	0.0068	0.0077
	S	–	1.7	1.4	–	0.0075	0.0061
DOP	M	–	2.1	4.0	–	0.0112	0.0176
	S	–	2.2	2.9	–	0.0093	0.0126
TDP	M	–	3.2	5.8	–	0.0171	0.0254
	S	–	3.6	4.3	–	0.0160	0.0189
Runoff	M				600	433	381
	S				491	408	359

[a]Units are mg/l for C and μg/l for N and P.
[b]Units are kg/ha/y for chemical fractions and mm for water yield.

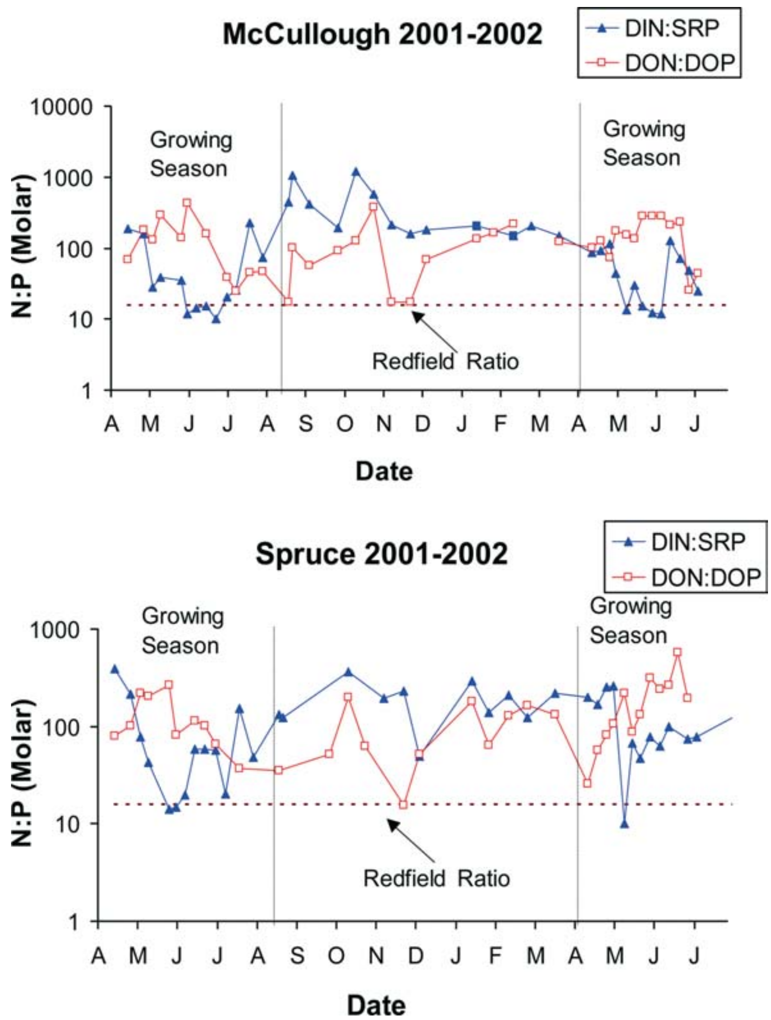

Figure 2. Seasonal changes in N:P ratios of organic and inorganic nutrients of the two study sites from April 2001 to July 2002.

humic than DON, and in 2001 peaked 1 to 2 months later than DON. In 2002, the differences in timing of peaks was less evident, possibly because of drought.

The percentage of bioavailable DON remained relatively high (usually > 20%) throughout growing seasons in both streams (range 20–65%: Figure 4a, b). In contrast, the percentage of bioavailable DOC was highest (15–40%) in early spring and then declined rapidly into the growing season (Figure 4c, d). The bioavailability of both DON and DOC was lower during the dry year of 2002 than in 2001, a year of more typical runoff.

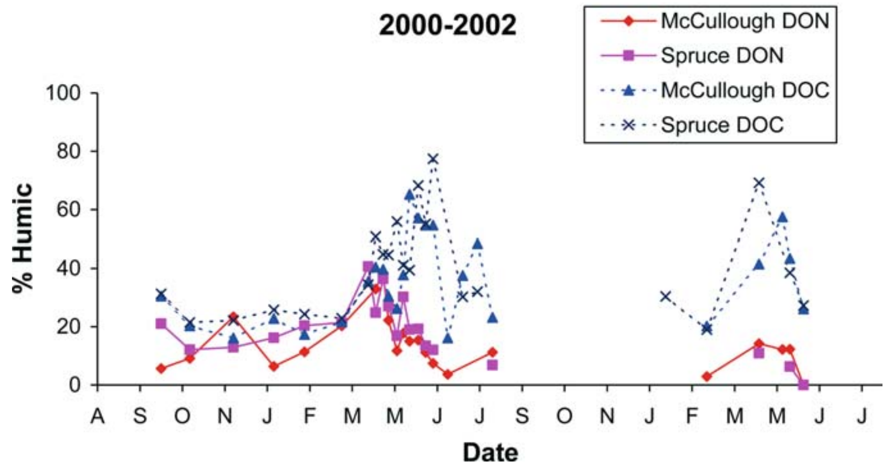

Figure 3. Changes in the proportions of humic DON and DOC for McCullough Gulch and Spruce Creek from October 2000 to June 2002.

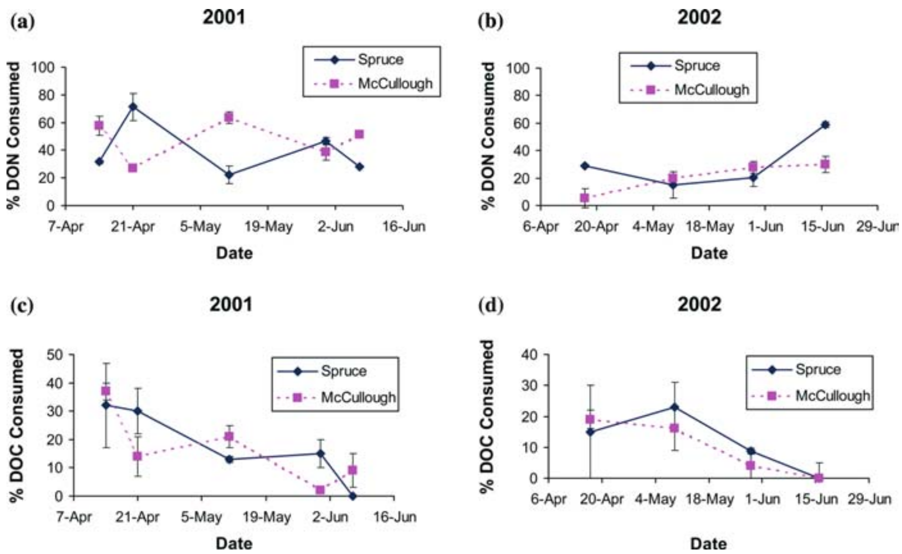

Figure 4. Seasonal changes in the bioavailability of DON (a, b) and DOC (c, d) in McCullough Gulch and Spruce Creek during the growing seasons of 2001 and 2002.

The percentage of DON consumed showed no significant relationship to the C:N ratio of the non-humic fraction in unamended incubations; it remained relatively high (ca. 50%) regardless of substrate quality (Figure 5a). The percentage of DON consumed showed a negative relationship to the C:N ratio of the non-humic fraction, however, when inorganic N or P was added

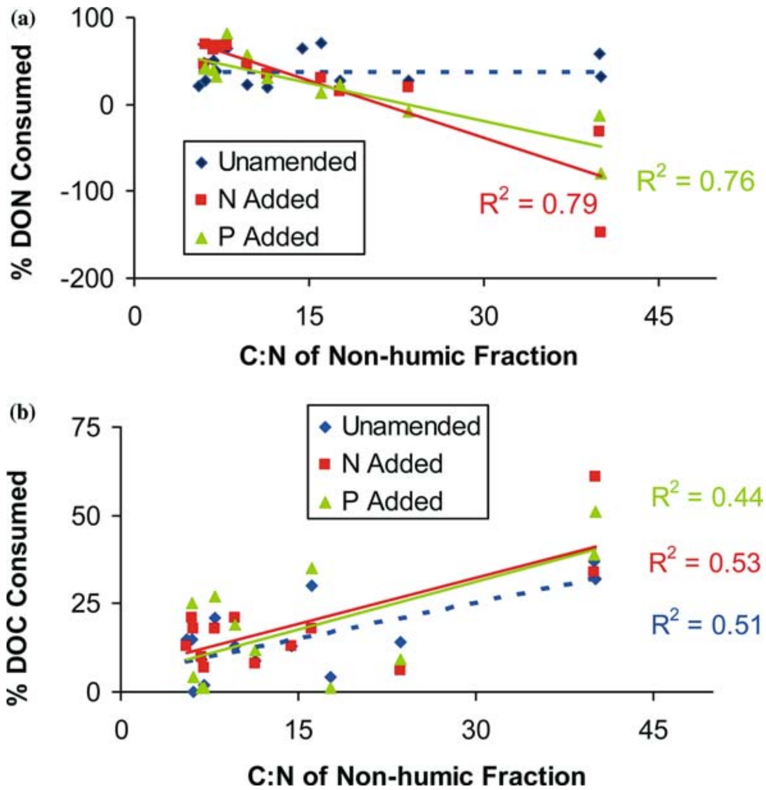

Figure 5. Relationship of the C:N ratio of non-humic fractions with % DOC (a) and % DON (b) consumed in incubations.

(Figure 5a). At the highest C:N ratios, the addition of inorganic N or P caused a net generation of DON (shown as negative consumption in (Figure 5a). In contrast, percentage of DOC consumed showed a significant positive relationship to C:N ratio of the non-humic fraction, and was unaffected by the addition of inorganic nutrients (Figure 5b).

Concentrations of bioavailable DON in stream water were higher than those of inorganic N in both streams during the growing seasons, but lower prior to the growing seasons (Figure 6). Maximum uptake of DON occurred when concentration of DIN in stream water was lowest. A time-course incubation for water collected during the middle of summer (June 16, 2002) showed that approximately 40% of the DON in both streams could be consumed over two days (Figure 7).

The uptake of DON in all incubations was strongly related to the concentration of non-humic DON (Figure 8a). The rate of DON uptake per unit of non-humic DON was significantly lower for the unamended treatment than for treatments involving addition of N or P (ANCOVA, $p < 0.05$). The overall

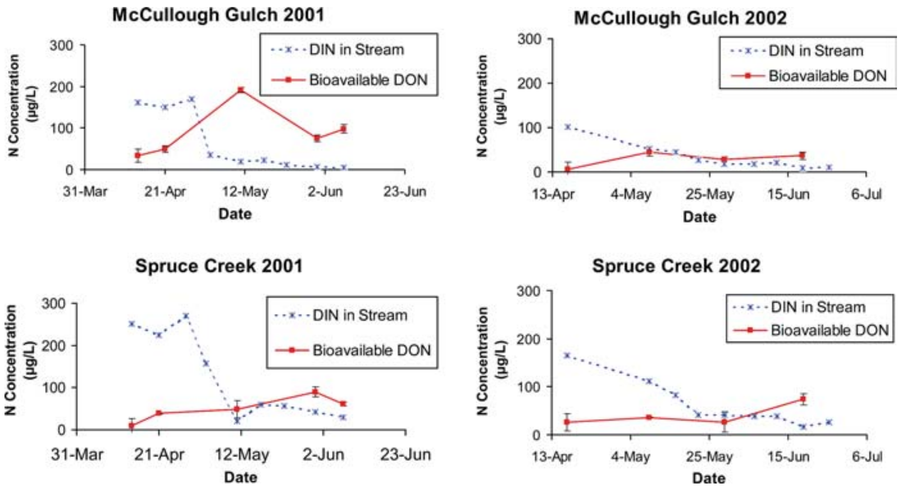

Figure 6. Seasonal changes in absolute concentrations of biologically available DON relative to DIN in stream water of McCullough Gulch and Spruce Creek during the growing seasons of 2001 and 2002.

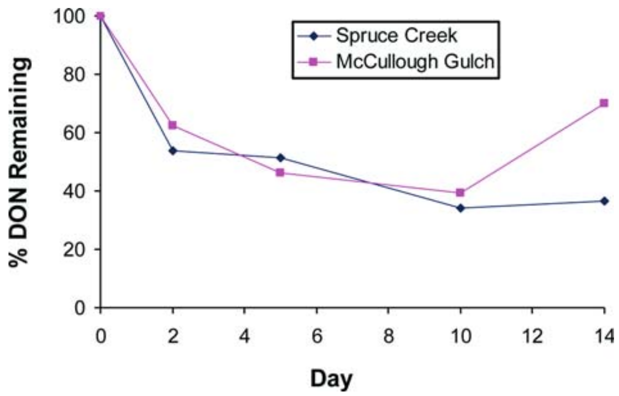

Figure 7. Percentage of DON remaining at designated intervals during a 14-day, unamended incubation using stream water collected on June 16, 2002.

mean consumption of DON and change in DIN over the two years did not differ statistically among treatments (Figure 8b). There was substantial temporal variability across sampling dates. On average, net production of DIN (net mineralization) in incubations accounted for 49% (McCullough) and 51% (Spruce) of DON consumption in unamended incubations and in incubations amended with SRP. Production of DIN accounted for 91% of DON consumption in incubations amended with nitrate.

During the growing season, approximately 40% of the DON export was biologically available, and this bioavailable fraction of DON comprised approximately 30% of the total dissolved nitrogen export. Concentration of

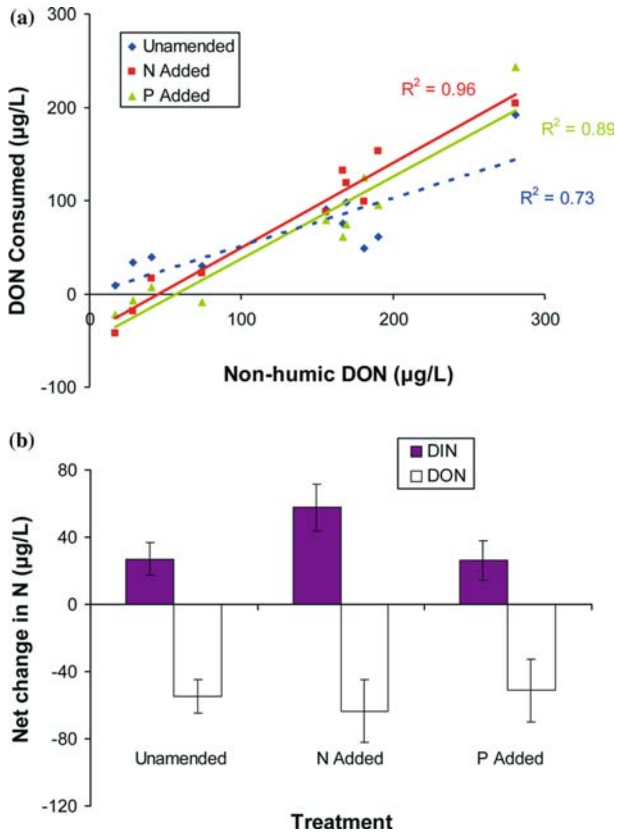

Figure 8. Relationship between uptake of DON in incubations and the concentration of non-humic DON in stream water (a), and mean net change of DON and DIN in incubations over two growing seasons (b).

the non-humic fraction of DON increased in stream water as the concentration of DIN decreased on a seasonal basis (Figure 9a). DON uptake was highest in incubations when DIN was scarce in stream water, and declined logarithmically as DIN increased in concentration (Figure 9b). Concentration of non-humic DON was consistently greater than the concentration of DON that could be consumed in incubations over the entire range of DIN concentration present in stream water.

Discussion

On an annual basis, approximately 60% of soluble nitrogen was exported as DON. Export of DON and DOC differed over the years, mainly because of drought in 2002. Amount and quality of DON and DOC are known to vary with amount of runoff (McKnight et al. 1997; Hood et al. 2003; Kaushal and Lewis 2003).

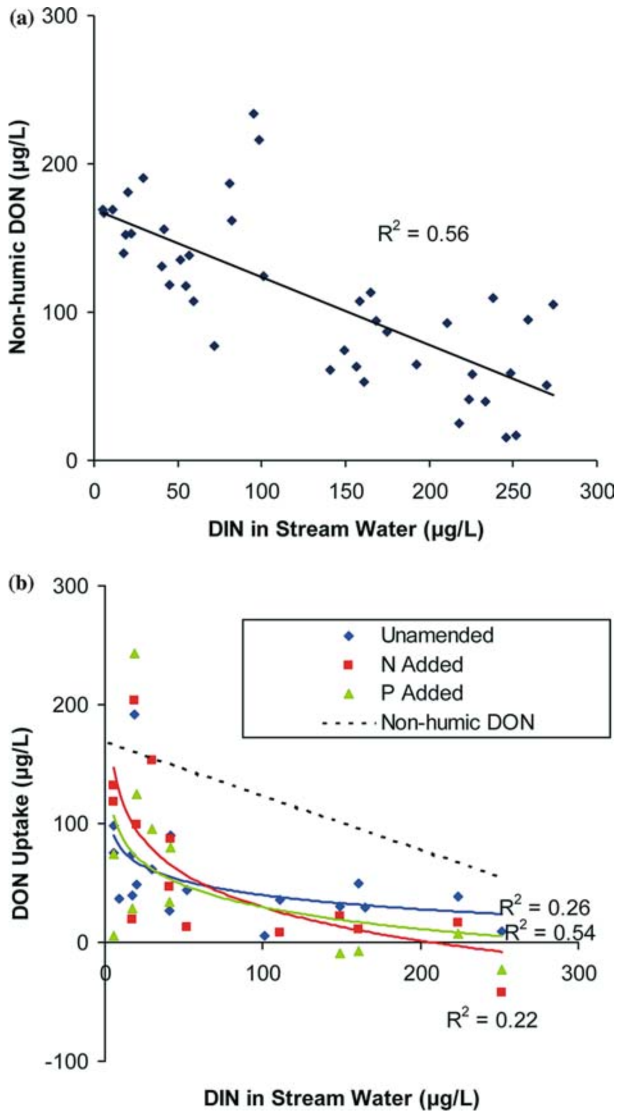

Figure 9. Dashed line shows relationship of non-humic DON concentrations to DIN concentrations in stream water over a two-year period (a), and logarithmic decline in uptake of DON during incubations as the availability of inorganic N increases in stream water over seasonal cycles (b).

During growing seasons, the N:P ratio of DOM was high (200–500) indicating that organic N, if bioavailable, could offset low N:P ratios (< 15) in the inorganic fractions. It has been argued that DON should be considered as a source of N (in addition to DIN) when attempting to predict the relative use of all nutrient resources by aquatic food webs Seitzinger and Sanders 1997;

Berman 2001; Dodds 2003). The importance of DON as an N source has been difficult to determine, however, because its bioavailability typically is not known. Results from the present study suggest that DIN is consumed preferentially when it is abundant, but organic N can be used biotically when concentrations of DIN are sufficiently low. Organic N may need to be considered as a biotic supply of N, particularly in stream ecosystems with low availability of DIN. Further integration of bioavailable DON into the N:P stoichiometric requirements of living organisms may be useful in predicting when N limitation occurs in some environments (Berman 2001).

Uptake of bulk DON has been measured experimentally in only a few studies involving incubations of water taken from streams and rivers (Qualls and Haines 1992; Seitzinger and Sanders 1997; Stepanauskas et al. 2000, 2002; Buffam et al. 2001). The present study shows that: (1) the proportion of bioavailable DON from natural sources can vary greatly within and across years, (2) DOC and DON can be concentrated in different fractions of DOM and undergo different patterns in metabolism, and (3) that the uptake and generation of labile DON may be related to the availability of inorganic N.

Bioavailability of DON was typically higher than DOC on a seasonal basis. The percentage of bioavailable DON fell within a range (15–71%) similar to that reported for boreal streams in Sweden (Stepanauskas et al. 2000). In contrast, the bioavailability of DOC peaked sharply during early spring at 30% and then declined rapidly to almost 0%. Bioavailability of DOC within this range has been reported previously for both boreal and montane environments (Wikner et al. 1999; Baker et al. 2000). Labile DOC is likely to be delivered to mountain streams primarily during snowmelt (Baker et al. 2000), and it may be respired over relatively short time scales and removed as CO_2. Instead, labile DON may represent an important supply of biotic N, which can be generated by heterotrophs and autotrophs within a N-poor ecosystem throughout seasons of high biotic activity (Bronk and Ward 1999) and be recycled via re-mineralization and transformation back to organic forms (Bronk et al. 1998).

Differences in the bioavailability of DON and DOC may also be partially explained by the distribution of carbon and nitrogen within fractions of organic matter (Wiegner and Seitzinger 2001; Kaushal and Lewis 2003). DON was mostly non-humic (up to 80%) during snowmelt and the growing season, but a substantial proportion of DOC was comprised of humic substances. Previous work in mountain streams has shown that the absolute concentration and relative proportion of humic DOC increases during snowmelt suggesting increased delivery of DOC from terrestrial sources (Hood et al. 2003; Kaushal and Lewis 2003). DOC may be primarily derived from humic substances formed by lignified plant materials (Guggenberger et al. 1994). In contrast, we speculate that DON may largely originate from non-humic substances derived from microbial metabolism (Kaushal and Lewis 2003). Because DOC tends to be more concentrated in humic (hydrophobic) fractions than DON, the two organic nutrients can be transported through soils and watersheds at different rates (Kaiser and Zech 2000; Kaushal et al. 2003; Lajtha et al. in review).

Previous work shows that non-humic DOM consists of peptides, proteins, and amino acids and is usually more bioavailable than humic DOM Moran and Hodson 1990; Qualls and Haines 1992; Michaelson et al. 1998). Differences in the origin, transport, and chemical composition may explain why DOM fractions that are rich in nitrogen can be used at a different rate than DOM fractions rich in carbon.

In this study, the availability of inorganic nutrients also affected the bioavailability of DON and DOC differently. In contrast to DON, DOC was always consumed in incubations and was never generated. The proportion of bioavailable DOC was positively related to the C:N ratio of the non-humic fraction and was not significantly altered by the addition of inorganic nutrients. This positive relationship was likely caused by a predominance of carbohydrates, which have been shown to increase both the C:N of non-humic compounds and the bioavailability of DOM during early snowmelt (Michaelson et al. 1998). The percentage of bioavailable DON consumed showed a negative relationship with C:N ratio, but only when inorganic nutrients were added. Differences in the proportion and absolute amount of bioavailable DON were largely caused by a net production of DON in amended incubations when concentrations of non-humic DON were low and the C:N ratio of the non-humic fraction was high (during early snowmelt). Formation of organic nitrogen by heterotrophic microbes is recognized to be an important mechanism that prevents N leakage from soils and increases the N content of organic matter (Groffman et al. 1993; Zogg et al. 2000), and work in aquatic systems suggests that immobilization of DIN and transformation of DIN to DON by heterotrophic microbes can be important in aquatic environments when organic matter with high C:N ratio is abundant (Bronk et al. 1998; Caraco et al. 1998; Bronk and Ward 1999; Caraco and Cole 2003). Increased availability of DIN during times when labile DOM with low initial N content was available (e.g. during early snowmelt) may have resulted in the production of DON by microbes in the present study. In mountain watersheds, heterotrophic microbes may be important transformers of inorganic N to labile fractions of organic N, particularly throughout snowmelt and the growing season.

Overall, results showed that both relative and absolute uptake of DON could be related to three factors: (1) concentration of inorganic N, (2) concentration of labile non-humic DON, and (3) the C:N ratio of the non-humic fraction. Exposure to sunlight (Bushaw et al. 1996; Wiegner and Seitzinger 2001; Qualls and Richardson 2003; Vahatalo et al. 2003) and differences in microbial community composition (Guldberg et al. 2002; Findlay 2003) may also influence metabolism of organic nitrogen, but their effects were not quantified in this study.

From an ecosystem perspective, the uptake and production of inorganic and organic nitrogen were out of phase on a seasonal basis. Inorganic N was released from the watershed during early spring snowmelt, when the DIN:SRP was high. As the growing season continued, nitrogen in stream water was found in biologically labile pools of non-humic DON. This bioavailable DON was used at a rate inversely related to the availability of inorganic N in streams. Biotic demand for organic N in small, mountain streams may be greatest when inorganic N is present in low concentration (< 50 μg/l), and this demand may rapidly decline when inorganic N is more available before and after the growing season.

In the present study, approximately 70% of DON export occurred over the period of snowmelt and almost 40% of this DON was biologically available. Concentrations of nitrate became very low (< 5 μg/l) in both streams during summer months, and bioavailable DON was sometimes more abundant than DIN. These findings are counterintuitive because undisturbed forests are expected to retain biologically available forms of N (Hedin et al. 1995; Vitousek et al. 1998), although substantial inorganic N (Lewis 1986; Lewis et al. 1999) and DON may also leave such forests (Lewis 1986; Hedin et al. 1995; Vitousek et al. 1998; Vanderbilt et al. 2003). Previous work in mountain watersheds suggests that DON may be generated in watersheds along hydrologic flowpaths from soils to streams (Hood et al. 2003). Other work using isotopic tracers has shown that inorganic N can also be rapidly converted to organic matter via biological processing within streams (Peterson et al. 1997; Mulholland et al. 2000). During some seasons, DON may be generated by heterotrophic and autotrophic processes faster than it is consumed leading to a net export from the system. For example, the magnitude of DON production is greater than gross N uptake in other N-poor environments, even at times when DON can be an important biotic source of N (Bronk and Ward 1999). We found that only a fraction of organic nitrogen was biologically available, but this variable fraction was sometimes equivalent to or greater than the amount of inorganic nitrogen that was present.

The ecological significance of organic nitrogen appeared to change in response to DIN availability. Related work in soils has shown that long-term fertilization with inorganic N can increase both the amount of bulk DON in soil solution and alter the chemical composition of this DON (McDowell et al. in press) Enrichment of streams with DIN from atmospheric or agricultural sources may have the potential to alter the amount and reactivity of DON transported through aquatic systems. Bulk DON is comprised of labile and recalcitrant fractions, and the dynamics of these fractions should be considered separately when investigating changes in its relative and absolute contribution to N demand (Kaushal and Lewis 2003; Neff et al. 2003) and mass transport Qualls and Haines 1992; Qualls et al. 2002).

The present study shows that DON can be used biotically at a high rate in nitrogen-poor environments. Also, experimental enrichment with inorganic N and P caused the microbial communities in the streams of this study to become net producers of DON when labile DOM with low relative nitrogen content was abundant. The lability of DOC was uncoupled from the lability of DON in these unenriched environments. The results suggest that anthropogenic enrichment with N through atmospheric deposition or other mechanisms would be expected to suppress uptake of labile DON, and induce substantial additional production of DON by maximizing the conversion of inorganic N to DON when labile carbon is abundant. Further elucidation of mechanisms related to increases in the amount and bioavailability of organic N may be useful in predicting changes in the ecological significance of organic N in streams.

Acknowledgements

This work was primarily supported by Grant NA17RJ1229 from NOAA. Additional funding was provided by the Department of Environmental and Evolutionary Biology and the Cooperative Institute for Research in Environmental Sciences at the University of Colorado. Tim Covino, Blake Audsley and Jeremy King assisted with field work and laboratory analyses. Jim Saunders provided logistical support. Stuart Findlay, Kate Lajtha, Diane McKnight, and three anonymous reviewers provided comments that improved this manuscript.

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