ECOSYSTEMS

Uptake of Allochthonous Dissolved Organic Matter from Soil and Salmon in Coastal Temperate Rainforest Streams

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ABSTRACT

Dissolved organic matter (DOM) is an important component of aquatic food webs. We compare the uptake kinetics for NH_4–N and different fractions of DOM during soil and salmon leachate additions by evaluating the uptake of organic forms of carbon (DOC) and nitrogen (DON), and proteinaceous DOM, as measured by parallel factor (PARAFAC) modeling of DOM fluorescence. Seasonal DOM slug additions were conducted in three headwater streams draining a bog, forested wetland, and upland forest using DOM collected by leaching watershed soils. We also used DOM collected from bog soil and salmon carcasses to perform additions in the upland forest stream. DOC uptake velocity ranged from 0.010 to 0.063 mm s^{-1} and DON uptake velocity ranged from 0.015 to 0.086 mm s^{-1}, which provides evidence for the whole-stream uptake of allochthonous DOM. These findings imply that wetlands could potentially be an important source of DOM to support stream heterotrophic production. There was no significant difference in the uptake of DOC and DON across the soil leachate additions ($P > 0.05$), although differential uptake of DOM fractions was observed as protein-like fluorescence was removed from the water column more efficiently than bulk DOC and DON ($P < 0.05$). Moreover, PARAFAC analysis of DOM fluorescence showed that protein-like fluorescence decreased downstream during all DOM additions, whereas humic-like fluorescence did not change. This differential processing in added DOM suggests slow and fast turnover pools exist for aquatic DOM. Taken together, our findings argue that DON could potentially fill a larger role in satisfying biotic N demand in oligotrophic headwater streams than previously thought.

Key words: DOM; DOC; DON; Fluorescence; PARAFAC salmon; Wetlands; Nutrient uptake.

INTRODUCTION

Dissolved organic matter (DOM), which includes organic forms of carbon (DOC) and nitrogen (DON), is an important component of ecosystem food webs because it supplies C, N, and energy for heterotrophic bacteria. Most streamwater DOM in temperate forested watersheds is a complex mixture of terrestrially derived, organic compounds

that vary in their biological availability. Thus, the availability of labile DOM to stream communities can exert a large influence on stream heterotrophic activity (McKnight and others 1993; Fisher and others 2002) and subsequent DOM export. Because DOM often represents the largest pool of dissolved N loss from temperate forested watersheds (Hedin and others 1995; Campbell and others 2000), DOM may be a particularly important source of N in small streams where inorganic N concentrations are typically low.

Streams are important sites for the transport, removal, and transformation of nutrients. Streams vary in nutrient uptake rate due to a number of factors including the streambed surface area to volume ratio (Alexander and others 2000), gross primary production (Hall and Tank 2003), and stream temperature and discharge (Butturini and Sabater 1998). The lability of DOM can also influence nutrient dynamics within streams (Bernhardt and Likens 2002). As these factors change considerably throughout the day and over time, nutrient uptake can vary both diurnally (Mulholland and others 2006) and seasonally (Simon and others 2005).

Terrestrial inputs of DOM are particularly important in low-order, heterotrophic streams where allochthonous DOM is the dominant source of available energy for stream ecosystems. Wetlands are an important allochthonous source of DOM to streams and aquatic DOC concentrations are correlated with wetland coverage and wetland type (Xenopoulos and others 2003). Wetland contributions of DOM could be particularly important in southeast Alaska where wetlands comprise approximately 29% of the land area (USDA 1997), and the biodegradability of wetland DOM can vary seasonally and with wetland type (Fellman and others 2008a). Another important allochthonous source of DOM to southeast Alaskan streams is anadromous spawning salmon. Salmon carcasses not only contribute DOM to the stream but also can dramatically change the chemical quality of the bulk pool of streamwater DOM during spawning, as salmon contribute DOM that is protein-rich relative to terrestrial sources (Hood and others 2007). Therefore, understanding how DOM derived from wetland soils and salmon carcasses is utilized in aquatic ecosystems will allow us to evaluate the relative role of these common allochthonous DOM sources for supporting in-stream productivity within the temperate forest eco-region that extends from the Pacific Northwest through the Gulf of Alaska.

Conventional analyses of aquatic DOM commonly treat DOC and DON as individual pools, due primarily to the complex nature of DOM and the analytic and interpretive difficulties associated with characterizing DOM fractions (McDowell 2003). These bulk analyses reflect the combined result of DOC and DON production and removal processes and can hide the actual C and N transformations that are occurring. For example, amino acids are N-rich organic molecules contributing to both DOC and DON pools that can be removed from the water column more rapidly than other forms of DOM (Fiebig 1997; Findlay and Sinsabaugh 2003). Thus, a major challenge for ecosystem scientists is to develop techniques that will improve our understanding of DOM dynamics in aquatic ecosystems. Recent advances in fluorescence excitation–emission spectroscopy (EEMs) combined with parallel factor (PARAFAC) analysis modeling enable the rapid and precise characterization of DOM (Stedmon and others 2003). PARAFAC analysis of fluorescence EEMs has been used to trace changes in DOM production and consumption over short time periods in laboratory photodegradation experiments (Stedmon and Markager 2005).

Most studies of DOM dynamics in streams have examined C and N uptake separately (for example, Wiegner and others 2005), or have used simple organic compounds, such as glucose (Newbold and others 2006), acetate (Bernhardt and Likens 2002), and urea (Brookshire and others 2005). As the chemical quality of stream DOM varies depending on source and degree of transformation, DOC and DON uptake dynamics can only partially be elucidated using simple organic compounds. In this study, we examined the in-stream uptake of allochthonous DOM derived from watershed soils and salmon carcasses by performing DOM slug additions in headwater streams that drained three common landscape types in southeast Alaska: bog, forested wetland, and upland forest. Our goal was to compare the uptake kinetics for NH_4–N and different fractions of DOM by evaluating the uptake of DOC, DON, and proteinaceous DOM, as measured by PARAFAC modeling of fluorescence EEMs. We also used PARAFAC analysis of EEMs to evaluate downstream changes in the chemical quality of DOM during additions.

METHODS

Study Sites

Dissolved organic matter additions were conducted in the Tongass National Forest, located near Juneau, Alaska (58.5° N, 134.5° W). Juneau has a maritime climate with a mean annual temperature of 4.7°C and a mean annual precipitation of

1,400 mm at sea level, most of which falls in the autumn as rain or as snow at upper elevations during the winter. The heavily glaciated, mountainous terrain of southeastern Alaska, cool climate, and abundant precipitation create a region of well-defined watersheds that includes well-drained, mineral soils interspersed with peatland soils.

The three streams examined in this study drained three distinct landscape types: bog, forested wetland, and upland forest within the McGinnis Creek watershed. The bog and forested wetland represent the dominant mapped wetland communities in southeast Alaska (USDA 1997). Soils in these wetland sites were histosols, and were mapped as a complex of deep, moderate to well-decomposed peat that has accumulated over glacial till. The bog was typical of the slope bog (NWWG 1988) wetland type with peat accumulations more than 2 m deep, and the forested wetland was typical of the raised peatland swamp (NWWG 1988) with 0.5–0.75 m deep peat overlaying glacial till. The upland forest site was predominantly spodosol (Typic Humicryod) and the soil was moderately deep, moderately well drained and was colluvial material derived from bedrock dominated by igneous intrusive material.

The stream channels were characterized by a sequence of riffles or small waterfalls and small pools. Channel gradient ranges from 1% to 2% in the bog to a high of approximately 6–8% in the upland forest. Stream channel widths vary between 25 and 50 cm and sediments were dominated by gravel and small pebbles. Stream discharge varies considerably throughout the year and the temporal patterns in precipitation combined with long summer days produce three distinct hydrological periods during the main runoff season: (1) spring snowmelt (May), (2) summer drawdown (June–July), and (3) fall wet season (August–November). Streamflow typically reaches a minimum in winter or during the summer drawdown and peaks during the autumn rainy season.

Experimental Design and DOM Additions

Dissolved organic matter slug additions were conducted during the primary runoff season of 2007 in the bog, forested wetland, and upland forest streams using DOM collected by leaching soils from each site. DOM uptake was measured once during the spring (May 29–31), summer (July 27–29), and fall (September 27–30) for all three sites. In addition, we used DOM leachate collected from the bog soil and salmon carcasses to perform seasonal additions in the upland forest stream. Additions of salmon DOM were only performed in July and September because salmon carcasses were not available in May. Salmon and wetland soils are two common allochthonous sources of DOM in southeast Alaska and our goal was to elucidate the metabolic significance of these DOM sources in freshwater aquatic ecosystems within the region. Because of the high ambient streamwater DOM concentrations observed in the two wetland streams, we used slug additions rather than the conventional steady-state technique to evaluate DOM uptake. DOM additions were conducted immediately following extractions because we wanted the amendments to contain the nutrient and chemical characteristics of freshly leached DOM commonly observed during episodic flushing events, such as stormflows.

For each addition, we established a 25-m reach in each stream with sampling stations located 10, 15, 20, and 25 m downstream from the site of the injection. The upstream injection site was selected to ensure rapid mixing of the injectate into the stream. Prior to each release, three replicate water samples were collected at each sampling station for background nutrients and spectroscopic analyses of DOM. Cl^- was used as a conservative tracer during the DOM additions and NaCl was added to the injectate to elevate streamwater Cl^- concentration 2–5 mg l^{-1} above background (Brookshire and others 2005). An approximately 20 l slug of leachate was added into the stream at the head of the 25 m injection reach. The exact volume of the slug varied slightly across the additions to prevent substantially elevating the DOM concentrations above background levels. During each addition, we tracked the downstream progression of the slug by monitoring specific electrical conductivity using two, YSI (model 85) handheld meters. Once maximum conductivity was reached at each sampling station, three replicate water samples were collected and placed into acid-washed, 60-ml polyethylene bottles. The slug was typically well mixed with the streamwater 5–7 m downstream from the injection site but water samples were not collected until the 10-m sampling station. All water samples were filtered through pre-combusted, glass fiber filters (nominal pore size 0.7 μm) and stored in the refrigerator until analysis for DOM, Cl^- concentration, and spectroscopic analyses of DOM.

The soil leachate used in the DOM additions was prepared by extracting soil from each of the three landscape types. Two to three locations were randomly selected within each site to excavate a

25-cm deep core of soil. The soil collected represented a composite from the Oi/Oe horizons in the bog and forested wetland and from the O and upper mineral horizons in the upland forest. Approximately 5 cm of the surface vegetation layer was removed from each core and the remainder was placed in a single 5-gallon bucket until the bucket was approximately half full with soil. The composited soil sample was hand-mixed using latex gloves to remove woody debris and large root masses. Streamwater collected from each site was added to the bucket, stirred vigorously for 2 min and allowed to equilibrate for 1 h. After equilibration, the leachate was filtered through 66-μm nylon mesh into another 5 gallon bucket and if necessary, additional streamwater was added to reach a final volume of approximately 20 l. The salmon carcass leachate was prepared by letting a frozen chum salmon (*Oncorhynchus keta*) thaw in 20 l of streamwater for approximately 24 h followed by filtration through 66-μm nylon mesh.

Discharge was measured in each stream during all additions using the slug-injection method. Specific conductivity probes (Solinst model 3001) were placed in the stream 10 and 25 m downstream from the addition site and measurements were recorded every 5 s. Discharge was calculated by calculating the area under the conductivity curve (Gordon and others 1992). The mean stream wetted-width was measured at 10 locations within each reach.

Analytical Methods

Streamwater concentrations of DOC and total dissolved N (TDN) were analyzed via high temperature combustion using a Shimadzu TOC/TN-V analyzer. Analytic precision for DOC ranged from 0.02 to 0.04 mg C l^{-1} (mean standard deviation for identical samples re-analyzed during analytical runs) for DOC concentrations below 5 and 0.1–0.2 mg C l^{-1} for samples of 5 mg C l^{-1} or above. Ion chromatography (Dionex ICS-1500 and 2500) was used to measure NH_4–N (lower detection limit 3 μg NH_4–N), NO_3–N (lower detection limit 2 μg NO_3–N), and Cl^- concentrations. DON was calculated as the difference between TDN and dissolved inorganic N (DIN = NH_4–N + NO_3–N) and the calculated error for DON values during analytical runs was 0.16 mg N l^{-1} (square root of the sum of the squared analytical errors of TDN and DIN). Soluble reactive phosphorus (SRP) was measured using the ascorbic acid method (Murphy and Riley 1962). Ash free dry mass (AFDM) was used to determine the sediment organic matter content for each stream. To determine AFDM, approximately 300 g of sediment was randomly collected from each stream and dried at 100°C for 24 h. Three replicate sediment samples were weighed, ashed at 500°C for 4 h, re-weighed, and AFDM (mg/cm^2) was determined by difference between the combusted and dried samples.

Spectroscopic Analyses and PARAFAC Modeling

The specific UV absorbance of DOC ($SUVA_{254}$), which is an indicator of aromatic C content, was measured on water samples allowed to warm to room temperature following the procedures of Weishaar and others (2003). $SUVA_{254}$ was calculated as the UV absorbance at 254 nm and is reported in units of mg-C l^{-1} m^{-1}. Fluorescence excitation–emission matrices (EEM) of DOM were measured on a Fluoromax-3 (Jobin Yvon Horiba) fluorometer with a xenon lamp following the procedures of Hood and others (2007). Because of high DOM concentrations, water samples were corrected for inner filter effects (Green and Blough 1994). Fluorescence EEMs were corrected for instrument bias and Raman normalized using the area under the water Raman peak at excitation 350 nm.

Parallel factor modeling of fluorescence EEMs was conducted with MATLAB using the PLS_toolbox version 3.7 following the procedures of Stedmon and others (2003). Our PARAFAC model identified a total of nine unique components within the fluorescence EEMs (Supplementary Appendix 1). However, we focus our analyses on three PARAFAC components: humic-like component 1 (ex = 240 nm/em = 450–460 nm), tryptophan-like component 8 (ex = 280 nm/em = 336 nm), and tyrosine-like component 9 (ex = 275 nm/em = 304 nm). These three components were selected because they were useful in evaluating changes in DOM composition during additions and have been previously identified in other studies (Baker 2001; Stedmon and others 2003; Cory and McKnight 2005). We validated our PARAFAC model using core consistency diagnostics followed by a split half validation (Supplementary Appendix 2). As each PARAFAC component likely represents a group of fluorophores with very similar fluorescence characteristics (Stedmon and others 2003); here, we refer to the humic component as ''humic-like'' and the tyrosine and tryptophan components as ''protein-like,'' which is the sum of tyrosine and tryptophan-like fluorescence.

DOM Uptake Metrics

Dissolved organic forms of carbon uptake length (S_w, m) was calculated as k^{-1}, where k is the longitudinal loss rate constant relating the slope of the natural log transformed regression of the DOC:Cl ratio at peak conductivity and distance downstream (Newbold and others [1981]). DOC uptake velocity ($V_f = Qk/w$, mm s^{-1}) was calculated because uptake length is strongly influenced by discharge and stream wetted-width, where Q is stream discharge (m^3 min^{-1}) and w is the mean stream wetted-width (Stream Solute Workshop [1990]). The areal uptake rate of DOC ($U = V_f N_b$, mg m^{-2} d^{-1}) was calculated to estimate the amount of DOC removed from the water column per square meter, where N_b is the background concentration of DOC (Stream Solute Workshop [1990]). We also calculated uptake kinetics for DON, NH$_4$–N and proteinaceous DOM (measured as protein-like fluorescence and is the sum of tryptophan and tyrosine-like components), using the same approach as described above for DOC. Although protein-like fluorescence is representative of amino acid content (Yamashita and Tanoue [2003]), it is not possible to convert PARA-FAC concentrations (defined as scores) to actual concentrations for complex mixtures such as for DOM. We, therefore, present the PARAFAC scores for protein-like fluorescence as an F_{max}, which is the fluorescence at the excitation and emission maximum (see Stedmon and Markager [2005]), to calculate uptake kinetics of protein-like fluorescence during the additions. Thus, the ratio between the F_{max} for protein-like fluorescence and Cl$^-$ concentration was used to calculate the uptake kinetics for proteinaceous DOM. In addition, we did not calculate areal uptake rate for protein-like fluorescence because PARAFAC scores cannot be converted to concentrations.

Statistical Analyses

We used a mixed-model (Proc Mixed; SAS Institute Inc. 2003), repeated measures analysis of variance with a compound symmetry covariance structure combined with a Tukey's pairwise differences test to evaluate DOM uptake for the soil leachate additions. Because there is considerable uncertainty in the DOM additions themselves (for example, different concentrations of DOM were added for each addition), we did not compare DOM uptake across the seasonal additions or across sites. Rather, we focus our analyses on within addition comparisons of DOM fraction kinetics by evaluating the uptake of DOC, DON, protein-like fluorescence, and NH$_4$–N. All values for different sample dates were considered repeated measurements.

RESULTS

Background Physical, Chemical, and Biological Parameters

Stream discharge was less than 2.5 l s^{-1} for all additions, and was lowest before the summer additions when streamwater temperature was warmest (Table 1). Background concentrations of DOC and DON showed considerable temporal variation for the two wetland streams, but concentrations varied only slightly in the upland forest stream. Background DOC:DON ratios were lowest for all three streams before the spring additions. DON was the dominant component of TDN for all streams and accounted for greater than 74% of TDN. Concentrations of SRP were low and showed little seasonal variation for all three streams (Table 1). AFDM content of stream sediment showed little variation across the three additions and was greatest in the forested wetland.

Table 1. Stream Experimental Conditions and Ambient Nutrient Concentrations for Each of the DOM Additions

	Bog			Forested wetland			Upland forest		
	Spring	Summer	Fall	Spring	Summer	Fall	Spring	Summer	Fall
Discharge (l s^{-1})	0.8	0.4	1.1	1.5	0.6	2.0	1.2	0.5	1.6
Stream temp. (°C)	7.5	10.5	8.8	5.6	9.7	7.7	5.7	9.5	7.7
DOC (mg C l^{-1})	14.4	27.4	15.7	22.4	32.5	23.0	3.3	3.9	4.3
DON (mg N l^{-1})	0.4	0.5	0.3	0.3	0.4	0.3	0.1	0.1	0.1
NO$_3$–N (µg N l^{-1})	8.0	<DL	<DL	6.8	<DL	<DL	15.6	21.6	14.5
NH$_4$–N (µg N l^{-1})	<DL	3.7	5.3	3.2	3.9	5.5	<DL	<DL	<DL
SRP (µg P l^{-1})	5.1	6.9	4.7	5.4	6.7	5.1	2.0	2.8	2.4
AFDM (g/cm^2)	0.38	0.35	0.34	0.69	0.62	0.64	0.53	0.46	0.51

<DL indicates below instrument detection limit.

DOC Injectate

The DOC concentration of the soil leachates ranged from 24 to 74 mg C l^{-1}, and showed very little seasonal variation (Table 2). In contrast, soil leachate DON concentrations showed considerable variation across the additions with the highest concentrations during the spring injection. The greater N content of the spring leachate was reflected in DOC:DON ratios, as the spring leachates had an average C:N of 13 followed by a ratio of 39 and 23 during the summer and fall, respectively. The salmon leachates had substantially greater concentrations of NH_4–N and SRP than the soil leachates, and there was little variability in dissolved N, P, and C concentrations between the summer and fall salmon leachates.

The percent relative contribution of protein-like fluorescence ranged from a low of 10% in the forested wetland to a high of 33% in the bog leachate added to the upland forest stream, and was generally highest in the spring (Table 2). The highest soil leachate $SUVA_{254}$ values were observed in the summer with the forested wetland leachate having the highest aromatic carbon content (Table 2). The high DON concentration in the salmon leachates were reflected in their spectroscopic properties as the relative contribution of protein-like fluorescence averaged 63% for the two leachates.

Nutrient Uptake

Dissolved organic matter leached from watershed soils and salmon carcasses was removed from the water column during all additions with average uptake lengths ranging from 66 to 452 m for DOC and from 47 to 242 m for DON (Table 3). The variation in DOC uptake length was not significantly related to the DOC concentration increase for all additions taken together ($P > 0.5$; Figure 1). There was no significant difference in the uptake velocity of DOC and DON across the soil leachate additions ($P > 0.05$). However, proteinaceous DOM was removed from the water column at a significantly higher rate than bulk DOC ($P < 0.05$) and DON ($P < 0.05$), as average protein-like fluorescence uptake velocities (mean of all soil additions) were 1.8 times greater than for DOC and 1.6 times greater than for DON (Table 3). There was efficient uptake of NH_4–N during the soil leachate additions, which resulted in significantly higher uptake velocities than for DOC and DON ($P < 0.05$), but not than for protein-like fluorescence ($P > 0.05$; Table 4). Concentrations of NO_3–N changed very little during the additions, thus NO_3–N uptake kinetics were not evaluated.

Similar to the soil leachates, differential uptake of DOM fractions was observed during the salmon leachate additions as protein-like fluorescence uptake velocity was 1.3 times greater than for DOC. In contrast to the soil additions, the uptake velocity for protein-like fluorescence was similar to that of DON during the salmon leachate additions. Overall, we found no significant relationship between background nutrient concentrations and DOM uptake kinetics for all additions taken together. However, the contribution of protein-like fluorescence in the DOM injectates was significantly

Table 2. Soil and Salmon Leachate Characteristics for Each of the Fourteen Additions

| Site	Season	DOC (mg C l^{-1})	DON (mg N l^{-1})	NH_4–N (µg N l^{-1})	SRP (µg P l^{-1})	PLF (%)	SUVA (l mg-C^{-1} m^{-1})
Bog	Spring	66.4	7.1	30.2	23.1	32	2.9
Bog	Summer	69.7	2.3	27.2	25.2	21	3.3
Bog	Fall	68.0	4.2	32.2	21.6	29	3.0
For wetland	Spring	72.3	5.0	24.8	22.6	17	3.9
For wetland	Summer	74.2	1.4	30.2	24.5	10	4.2
For wetland	Fall	65.6	2.9	28.2	22.8	14	3.8
Upland forest	Spring	25.2	1.3	21.3	21.3	20	3.1
Upland forest	Summer	28.8	0.6	20.2	22.6	16	3.2
Upland forest	Fall	24.6	0.7	22.6	20.5	22	3.0
Upland/bog	Spring	51.7	5.9	29.1	20.2	33	2.8
Upland/bog	Summer	52.4	2.0	32.4	24.1	23	3.1
Upland/bog	Fall	58.3	3.2	25.6	22.9	31	2.8
Upland/fish	Summer	49.6	6.8	2523.0	1027.8	66	2.4
Upland/fish	Fall	46.3	7.5	3068.0	1103.2	60	2.2

Protein-like fluorescence (PLF) is the sum of tyrosine and tryptophan-like PARAFAC components and the units are in percent relative contribution.
Upland/bog is the DOM leachate collected from the bog soil and Upland/fish is the DOM leachate collected from the salmon carcass that were added to the upland forest stream.

Table 3. Mean and Standard Error (±1 SE) of DOC, DON, and Protein-Like Fluorescence Uptake Metrics for the DOM Additions

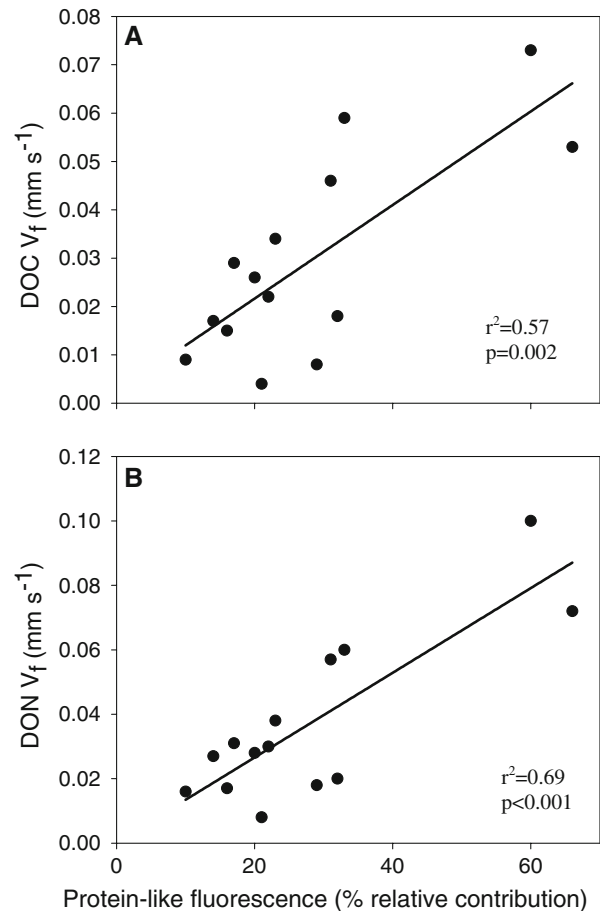|	DOC S_w	DON S_w	PLF S_w	DOC V_f	DON V_f	PLF V_f	DOC U	DON U	PLF U
Bog	452 (167)	242 (76)	141 (39)	0.010 (0.01)	0.013 (0.01)	0.026 (0.01)	2427 (385)	504 (105)	NA
Forest wetland	320 (84)	212 (36)	151 (38)	0.018 (0.01)	0.019 (0.01)	0.037 (0.01)	3474 (58)	686 (65)	NA
Upland forest	204 (33)	170 (15)	119 (22)	0.021 (0.01)	0.024 (0.01)	0.039 (0.01)	1642 (118)	218 (36)	NA
Upland/bog	94 (18)	82 (11)	67 (12)	0.046 (0.01)	0.053 (0.01)	0.068 (0.01)	2400 (234)	448 (59)	NA
Upland/fish	66 (16)	47 (10)	52 (12)	0.063 (0.01)	0.086 (0.01)	0.082 (0.01)	2907 (434)	762 (140)	NA

PLF is the sum of tyrosine and tryptophan-like PARAFAC components. Uptake length S_w (m), uptake velocity V_f (mm s^{-1}), and areal uptake rate U (mg m^{-2} d^{-1}). Upland/bog is the DOM leachate collected from the bog soil and Upland/fish is the DOM leachate collected from the salmon carcass that were added to the upland forest stream. NA indicates non-applicable.
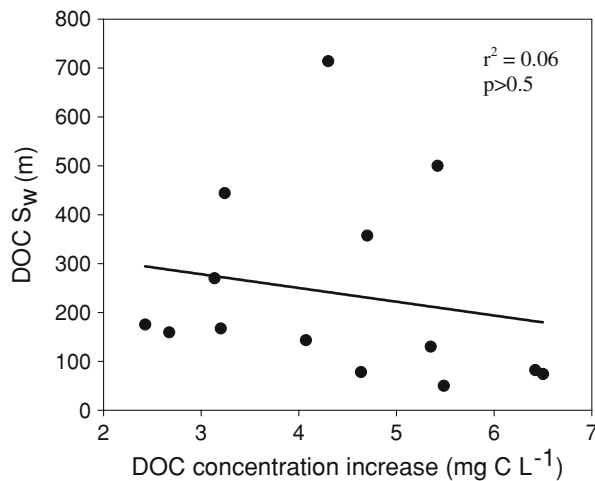
Figure 1. Regression model describing the relationship between the DOC concentration increase above background during the additions and DOC uptake length (S_w) for all additions taken together.

Table 4. Mean and Standard Error (±1 SE) of NH$_4$–N Uptake Metrics for the DOM Additions

	NH$_4$–N S_w	NH$_4$–N V_f	NH$_4$–N U
Bog	192 (33)	0.024 (0.01)	6.9 (1.9)
Forested wetland	114 (24)	0.041 (0.01)	11.8 (3.1)
Upland forest	105 (18)	0.044 (0.01)	9.6 (2.6)
Upland/bog	75 (19)	0.063 (0.01)	17.9 (3.2)
Upland/fish	58 (15)	0.080 (0.02)	23.1 (2.8)

Uptake length S_w (m), uptake velocity V_f (mm s^{-1}), and areal uptake rate U (μg N m^{-2} min^{-1}). Upland/bog is the DOM leachate collected from the bog soil and Upland/fish is the DOM leachate collected from the salmon carcass that were added to the upland forest stream.

Figure 2. Regression model describing the relationship between protein-like fluorescence (sum of tyrosine and tryptophan-like PARAFAC components) in the DOM leachates and **A** DOC and **B** DON uptake velocity for the spring, summer and fall additions.

Changes in the Chemical Quality of DOM During Additions

The high-uptake velocities for protein-like fluorescence were associated with dramatic changes in the chemical quality of DOM with distance down-

correlated with uptake velocities for DOC ($r^2 = 0.57$; $P = 0.002$) and DON ($r^2 = 0.69$; $P < 0.001$; Figure 2).

stream during all additions. To illustrate, we present the downstream changes in fluorescence EEMs from the summer bog soil addition in the upland forest stream where some of the most pronounced changes in fluorescent components occurred. Visual analyses of the EEMs from this addition reveal a primary fluorescence peak at approximately 240 nm excitation and 450–460 nm emission (Figure 3). This fluorophore has been attributed to humic-like material of terrestrial origin (Stedmon and others 2003), and it is the dominant fluorophore present at all four downstream sampling locations. The 10-m EEM has two additional fluorophores with one at approximately 275 nm excitation and 306–308 nm emission, and the other at 280 nm excitation and 334 nm emission (Figure 3). These fluorophores, which have been linked to the amino acids tyrosine and tryptophan (Stedmon and others 2003), are very prominent in the 10-m EEM but are less well developed in the 15 and 20 m EEM and are virtually non-detectable in the 25-m EEM.

There was no downstream change in the contribution of PARAFAC components and DOC:DON ratio before the summer bog soil addition in the upland forest stream (Figure 4A). PARAFAC analysis of the fluorescence EEMs presented in Figure 3 showed that the humic-like component 1 did not change during the addition (Figure 4B). In contrast, the F_{max} for protein-like fluorescence decreased downstream from 0.29 to 0.09 during the addition. This decrease in protein-like fluorescence was accompanied by a slight increase in the DOC:DON ratio as the C:N ratio increased from 20 to 28 during the addition (Figure 4B). An evaluation of the other four leachate additions showed no downstream change in the three measures of DOM composition before the additions; however, during the additions, we found similar changes in DOM composition as those observed for the bog soil addition in the upland forest stream where the proteinaceous fraction of DOM was removed, whereas the humic-like component 1 did not change with distance downstream (Figure 4C–J). Interestingly, the most dramatic changes in C:N ratio and protein-like fluorescence were observed
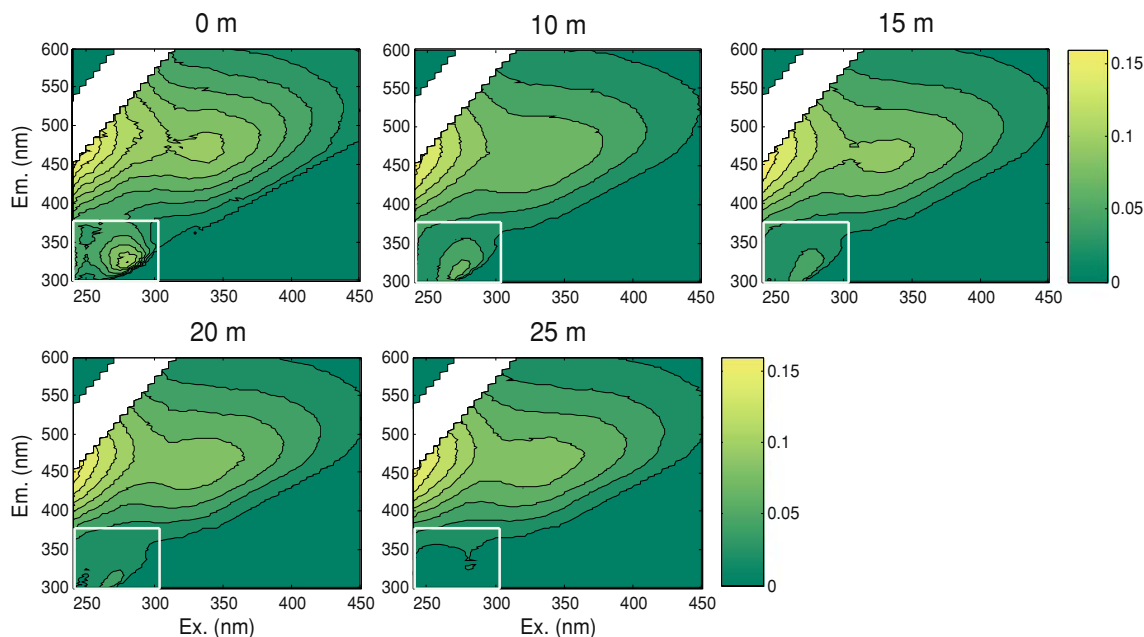
Figure 4. Change in the chemical properties of DOM▶ with distance downstream (m) before and during the summer additions. The F_{max} values are in Raman units and protein-like fluorescence is the sum of tyrosine and tryptophan-like PARAFAC components. Bog soil/Upland is the DOM leachate collected from the bog soil and Salmon/Upland is the DOM leachate collected from the salmon carcass that were added to the upland forest stream. Analytic precision for humic-like fluorescence was 0.08 (mean standard deviation for the F_{max} of identical sample EEMs re-analyzed) and 0.05 for protein-like fluorescence.

Figure 3. Fluorescence EEMs of DOM for the 0 and 10–25 m downstream sample stations during the summer bog soil addition in the upland forest stream. The white rectangle in each EEM indicates the region where protein-like fluorescence occurs. Excitation (Ex.) wavelengths range from 250 to 450 nm and emission (Em.) wavelengths range from 300 to 600 nm. Fluorescence intensities are in Raman units.

Background conditions — During additions
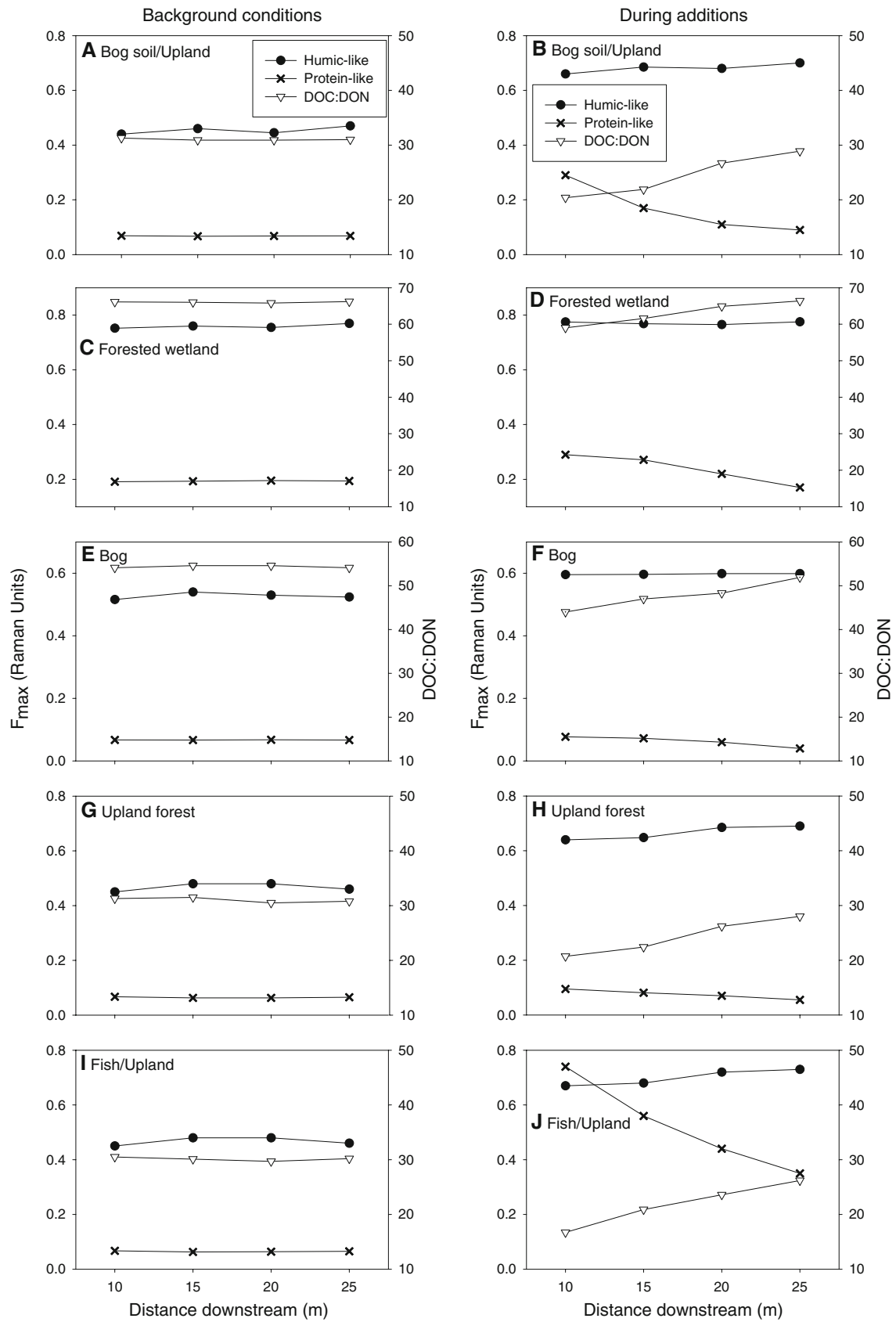
for the salmon additions, indicating greater removal of N-rich DOM during the salmon carcass than the soil leachate additions.

DISCUSSION

DOM Uptake and Changes in Chemical Quality

Dissolved organic matter derived from wetland soil leachate was removed from the water column during all additions providing evidence for the whole-stream uptake of DOM by the aquatic heterotrophic community present in streams draining each of the three landscape types. Because wetlands occupy such a large portion of the land area in southeast Alaska, our findings imply that wetlands could potentially be an important source of C and N to support stream heterotrophic production. Moreover, as DOM processing occurs during transport in headwater streams, it is modified in composition by the selective degradation of the proteinaceous fraction of DOM, whereas the humic-rich fraction did not change with distance downstream. This differential processing in added DOM suggests slow and fast turnover pools exist for aquatic DOM (for example, Kaplan and Newbold 2003), which is consistent with the more efficient uptake of protein-like fluorescence compared to bulk DOC and DON we observed during our additions.

From a methodological standpoint, short-term nutrient addition experiments are frequently used for measuring uptake length under ambient nutrient concentrations. Experimental evidence has largely confirmed that nutrient addition experiments can overestimate uptake length if nutrient concentrations are substantially elevated above background levels during additions (Mulholland and others 2002). Thus, nutrient additions should be kept as close to ambient concentrations as is analytically feasible when performing uptake studies. In our study, we recognize the fact that DOM concentrations were substantially elevated above background levels during several of the additions adds a level of uncertainty to our findings. However, we found no significant relationship between the concentration DOC increased and DOC uptake length during the additions.

The DOC and DON uptake velocities (DOC $V_f = 0.03$ mm s^{-1}, DON $V_f = 0.04$ mm s^{-1}, mean from all additions) reported in this study are similar to urea and glutamic acid additions in forested watersheds in southwest North Carolina (Brookshire and others 2005) and ^{13}C–DOC tree tissue leachate experiments in recirculating mesocosms

(Wiegner and others 2005). However, our uptake estimates for DOC are lower than for glucose additions in forested watersheds of the New York City region (Newbold and others 2006). As our estimates for DOC uptake kinetics are similar to other studies that have used steady-state additions to examine DOC uptake in streams, we suggest slug additions can be used as an alternative to the conventional steady-state approach in small streams with long water residence times.

During all injections, we observed changes in the chemical quality of DOM indicating that certain compounds are selectively removed while others remain. The downstream increase in DOC:DON ratio supports this finding, suggesting the DOM became more N poor and recalcitrant with distance downstream during the injections. This is consistent with other studies that have shown certain fractions of DOM, such as low molecular weight compounds (Kaplan and Bott 1983) or glucose (Battin and others 2003), are removed more rapidly by the stream community. Results from these studies support the idea that the chemical quality of allochthonous DOM combined with the selective processing of certain fractions of DOM over short distances can exert a large influence of the magnitude and lability of DOM exported from watersheds. However, it is worth noting that our DOC additions occurred during periods of low discharge, and the extent to which stream heterotrophs are capable of utilizing episodic inputs of labile DOM likely depends on the period of time necessary for the microbial community to adapt to the change in DOM composition and stream discharge.

Protein-like fluorescence was removed at a significantly greater rate from the water column than bulk DOC and DON, despite efficient uptake of added NH$_4$–N. This result is consistent with the idea that amino acids are a readily available source of C, N, and energy for aquatic heterotrophs (Ellis and others 2000). Previous studies of forest soils (Kuzyakov 1997) and of stream sediments (Fiebig 1997; Findlay and Sinsabaugh 2003) have shown that amino acids are consumed more rapidly than other DOM compounds. Soil organic matter fractionation experiments have also shown that the turnover rate of amino acid N in dissolved humic material is two to three times greater than for C (Kuzyakov 1997). These findings suggest that amino acids play an important role in the removal of DOM from the water column, particularly in temperate headwater streams where DON often represents the largest pool of N loss. The extent to which amino acid N could satisfy biotic N demand in oligotrophic headwater streams is uncertain be-

cause amino acids typically account for a small portion of DOM in surface waters. However, our finding that protein-like fluorescence was removed from the water column as efficiently as NH_4–N leads us to suggest that DON could potentially fill a larger role in satisfying biotic N demand in head-water streams than was previously thought.

The protein-like fraction of DOM removed during soil leachate additions is likely associated with the peripheral parts of humic molecules because of the greater N content compared to that of the central part of the molecule (Kuzyakov 1997). These results further imply that certain DON fractions can have different uptake kinetics than the bulk pool of DOM. On the contrary, bulk DOC and DON uptake of added DOM were not significantly different across the soil additions suggesting a tight linkage between C and N removal. This pattern is consistent with the findings of Brookshire and others (2005) that streamwater DON is composed of two pools: a small labile pool of DON that is rapidly removed and a second, more recalcitrant pool of DON where its removal is tightly linked to the removal of DOC. Our finding that protein-like fluorescence was removed more efficiently than bulk DON further supports this idea of fast and slow turnover pools of DON. Therefore, treatment of DON as a single pool in ecological studies can be misleading when evaluating the extent N demand in streams is met through DON.

Our experimental approach for examining DOM uptake in streams does not provide us the ability to identify specific DOM removal processes. For example, stream DOC additions have shown abiotic sorption processes can remove substantial DOC (McDowell 1985; McKnight and others 2002) and NH_4 (Triska and others 1994) from the water column. In-stream flocculation processes could also remove substantial DOM, particularly in the stream reach immediately downstream from the release site. In addition, quenching of DOM fluorescence by dissolved metals (Yamashita and Jaffe 2008) could also have occurred during our additions resulting in a decrease in proteinaceous DOM with distance downstream. However, the preferential uptake of protein-like fluorescence combined with the lack of change for humic-like fluorescence strongly suggests biotic removal processes were modifying DOM composition during transport in the stream. Moreover, laboratory DOM incubations with soil pore water have shown biodegradation processes selectively remove protein-like fluorophores while others increase in relative abundance (Wickland and others 2007). Overall, our results provide highly consistent indirect evidence that biotic uptake is removing DOM from the water column. However, it is likely that multiple factors including biotic uptake and abiotic sorption are acting in concert to regulate C and N uptake in streams as DOM is transported through the watershed from its source in the soils to the watershed outlet.

The uptake of DOM varied dramatically across the seasonal additions, and uptake kinetics were strongly related to the chemical quality of the DOM leachates, rather than by physical factors such as stream temperature and the AFDM content of stream sediments. This finding is similar to other stream studies that found variations in the composition of the carbon supply can yield differing metabolic responses by the bacterial community (Findlay and others 2003) as well as N uptake (Sobczak and others 2003). The fact that aquatic heterotrophs appear to adapt to changes in terrestrial DOM inputs in our streams suggests that DOM composition can drive the structure and function of aquatic microbial communities (Judd and others 2006). Furthermore, previous laboratory incubations have shown a strong relationship between protein-like fluorescence and DOM bioavailability (Fellman and others 2008a), and the findings presented here provide in situ evidence that proteinaceous DOM is a good indicator of DOM lability in aquatic ecosystems. Thus, allochthonous contributions of DOM to streams that are rich in protein-like fluorescence, such as from salmon carcasses, are likely to be an important source of C and N for aquatic food webs.

Our finding of rapid DOM uptake during the peat and salmon carcass additions to the upland forest stream indicates that like salmon, wetland soils could be important for the biogeochemistry and function of stream ecosystems in coastal temperate rainforest watersheds. This finding is somewhat surprising because wetland inputs of DOM to aquatic ecosystems have conventionally been considered recalcitrant and largely unavailable for bacterial degradation (for example, Amon and Meon 2004). In southeast Alaska, both of these allochthonous sources of DOM are transiently available, either through seasonal or episodic inputs during storms (Fellman and others 2008b) and spawning (Hood and others 2007). Interestingly, the uptake velocity for bulk DON and proteinaceous DON was similar for the salmon leachate additions indicating salmon-derived DOM likely contains a much larger pool of labile DON that is readily available to aquatic heterotrophs compared to soil DOM. As anadromous salmon spawn in many of the small streams common to coastal

temperate rainforest watersheds, the complimentary inputs of labile DOM from salmon and wetlands could sustain stream ecosystem productivity throughout the primary runoff season that extends from May through October.

Since the development of the small watershed-concept by Bormann and Likens (1967) to study watershed ecosystem-function, many studies have shown the importance of terrestrial processes in influencing stream biogeochemistry (for example, Vitousek and Reiners 1975); and in fact, stream-water DOM concentrations reflect the combined effects of both production and selective removal processes throughout the watershed. Our finding of the selective removal of certain fractions of DOM suggests that over time and distance downstream, the chemical characteristics of streamwater DOM derived from different terrestrial source pools will become more similar to each other. As the spatial extent of small, headwater systems comprises a large portion of total watershed area, small streams can be important metabolic and chemical regulators of the downstream transport of DOM and nutrients. Results from this study, therefore, support the idea that the low concentrations of labile DOM typically observed in surface waters are at least partially a function of high-internal biotic demand for labile DOM by the stream community in lower order streams.

ACKNOWLEDGEMENTS

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