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A stormflow/baseflow comparison of dissolved organic matter concentrations and bioavailability in an Appalachian stream

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Abstract. Patterns of dissolved organic carbon (DOC) and nitrogen (DON) delivery were compared between times of stormflow and baseflow in Paine Run, an Appalachian stream draining a 12.4 km^2 forested catchment in the Shenandoah National Park (SNP), Virginia. The potential in-stream ecological impact of altered concentrations and/or chemical composition of DOM during storms also was examined, using standardized bacterial bioassays. DOC and DON concentrations in Paine Run were consistently low during baseflow and did not show a seasonal pattern. During storms however, mean DOC and DON concentrations approximately doubled, with maximum concentrations occurring on the rising limb of storm hydrographs. The rapid response of DOM concentration to changes in flow suggests a near-stream or in-stream source of DOM during storms. Stormflow (4% of the time, 36% of the annual discharge) contributed >50% of DOC, DON and NO_3^- flux in Paine Run during 1997. In laboratory bacterial bioassays, growth rate constants were higher on Paine Run stormflow water than on baseflow water, but the fraction of total DOM which was bioavailable was not significantly different. The fraction of the total stream DOC pool taken up by water column bacteria was estimated to increase from 0.03 $\pm$ 0.02% h^{-1} during baseflow, to 0.15 $\pm$ 0.04% h^{-1} during storms. This uptake rate would have a minimal effect on bulk DOM concentrations in Paine Run, but storms may still have considerable impact on the bacterial stream communities by mobilizing them into the water column and by supplying a pulse of DOM.

Introduction

Dissolved organic matter (DOM) is a vital source of energy and nutrients for heterotrophic bacteria in streams (Kaplan & Newbold 1993). DOM that is incorporated into biomass plays an important role in determining the magnitude of system metabolism and regulating the development of stream food webs. In low-order forested watersheds, most of the DOM is terrestrially derived because in-stream photosynthesis is limited by low light levels due to overshadowing by the canopy (Fisher & Likens 1973).

Dissolved organic nitrogen (DON) concentrations in streams have been measured in relatively few studies, partly due to the difficulty of the measurement (e.g. Frankovich & Jones 1998), and partly because inorganic forms of nitrogen are considered to be ecologically more important and more reactive. Recent measurements in small undisturbed forested catchments have found mean stream DON concentrations ranging from 50% (Wondzell & Swanson 1996) to 95% (Hedin et al. 1995) of total dissolved nitrogen (TDN). Many studies have found that DON is also more ecologically relevant than previously thought (e.g. Bronk et al. 1994; Kroer et al. 1994). DON can support the nitrogen requirements of phytoplankton (Antia et al. 1991), though most research has focused on the use of free amino acids (e.g. Wheeler & Kirchman 1986). Many forms of DON can be utilized by heterotrophic bacteria (Kroer et al. 1994; Volk et al. 1997). Through degradation by light (Bushaw et al. 1996) or microbes (Goldman et al. 1987; Wondzell & Swanson 1996), DON can be mineralized to dissolved inorganic nitrogen (DIN) which is then readily utilized by both primary and secondary producers.

Several studies have shown stream dissolved organic carbon (DOC) concentrations to be at least modestly correlated with discharge (e.g. Meyer & Tate 1983; McDowell & Likens 1988; Kaplan & Newbold 1993; Ivarsson & Jansson 1994; Boyer et al. 1995). High discharge events typically result in increases in DOC concentration when they follow long periods with unsaturated/frozen soils, as in spring snowmelt conditions (Hornberger et al. 1994; Ivarsson & Jansson 1994; Boyer et al. 1995). On a shorter time scale, individual storm events result in increased DOC for a number of streams (Meyer & Tate 1983; Kaplan & Newbold 1995; David et al. 1992; Hinton et al. 1998). Research on stream DON-discharge relationships is limited, but a recent study of nitrogen dynamics in the Cascade Mountains found an increase in stream DON concentration during storm events (Wondzell & Swanson 1996). In a study of 20 streams in Sweden and Finland, a significant positive correlation was found between total organic-N concentrations and flow in most of the catchments (Arheimer et al. 1996), and in a recent study of forested watersheds in New England, DON increased with discharge for 5 of the 9 streams examined (Campbell et al. 2000). Increases in stream nitrate (NO_3^-) concentrations during both seasonal and event-based high discharge conditions have been well documented in a number of different regions (Hill 1993; Creed et al. 1996; Hyer 1997; Campbell et al. 2000).

When inorganic nutrients are abundant in aquatic systems, bacterial growth is often stimulated by the addition of organic carbon (e.g. Pomeroy 1974; Tranvik & Höfle 1987; Amon & Benner 1996). The fraction of the total

DOC that is biologically usable (bioavailable) on a short (minutes to hours) timescale is most important for determining bacterial growth, since much of the DOC pool is unusable (refractory) material (Leff & Meyer 1991). For a given total DOC pool, the size of the labile component may range from less than 1% to over 50% of the total DOC (Meyer 1994). Bioavailability is difficult to quantify (Perdue & Gjessing 1990), but it is related to the chemical structure of the organic molecules, and may be correlated with molecular weight (Amon & Benner 1996), degree of reduction (Vallino et al. 1996), or various elemental ratios such as H/C and N/C (Sun et al. 1997, Hopkinson et al. 1998, Hunt et al. 2000).

Because stream bacterial heterotrophic activity is often dependent on the supply of bioavailable DOM, bacterial growth may be affected by storms. This would occur if storm events flush out upper layers of soil or leaf litter which contain high levels of relatively fresh organic matter. However, few researchers have examined the effect of stormflow organic matter on bacterial growth, with mixed results. In high-DOC blackwater rivers in Georgia, Meyer et al. (1988) found that high-discharge events resulted in a lower concentration of carbohydrates, and a lower bacterial activity, per unit DOC. In White Clay Creek, a small Pennsylvania stream, Kaplan and Newbold (1995) found that concentrations of water column DOC tripled during stormflow, and the concentrations of biodegradable DOC increased by an even greater factor. This increase in bioavailable DOC was linked to an increase in readily utilizable monosaccharides and other carbohydrates (Volk et al. 1997).

The present study examines the watershed process of discharge-driven variations in DOC and DON delivery to Paine Run, a small forested stream in Virginia, coupled with potential in-stream effects of stormflow organic matter on heterotrophic bacterial growth in the water column. We set out to answer the question whether stormflow results in a change in stream DOM quantity and/or quality, with the hypothesis that there would be measureable changes in DOC and DON concentrations, DOM chemical character (measured as N/C), and DOM quality (measured as bacterial bioavailability).

Site description

Paine Run and a number of other streams (Figure 1) in the Shenandoah National Park have been studied extensively as a part of the Shenandoah Watershed Study (SWAS) program (Webb et al. 1995, Bulger et al. 1995) and the Virginia Trout Streams Sensitivity Survey (VTSSS). In Paine Run, inorganic solutes have been measured during stormflow episodes and during baseflow at a single outlet site at the Park boundary since 1992. Paine Run is a second order stream, and has a perennial length of approximately 5 km

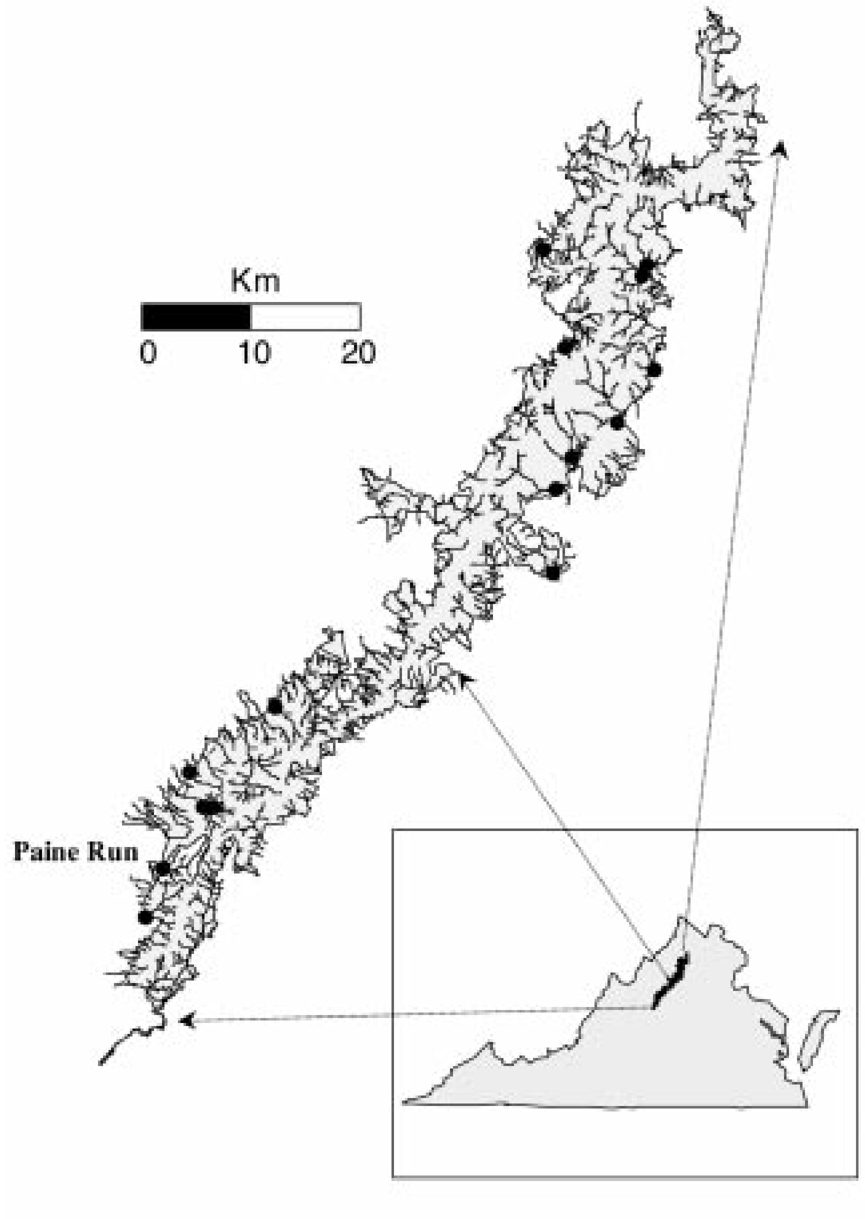

Figure 1. Stream network in Shenandoah National Park, Virginia. Streams outlets marked on the map (•) are those sampled for the Virginia Trout Streams Sensitivity Survey (VTSSS) quarterly survey of stream chemistry. Paine Run, the stream used intensively in this study, is located in the southwest quadrant of the Park.

from the headwaters (561 m altitude) to the gaged outlet (427 m altitude). Stream discharge at the Paine Run outlet ranges over 3 orders of magnitude, from approximately 0.005 to 10 m^3 s^{-1} in a typical year.

The 12.4 km^2 Paine Run watershed is underlain by mostly siliciclastic bedrock (91% Hampton and 9% Antietam formations) (Dise 1984). The catchment is uninhabited and almost entirely forested, primarily by chestnut oak and pine (96%) with the remainder in hemlock and yellow poplar (Dolloff & Newman 1998). Mean annual temperature (50-year average collected by the National Park Service at Big Meadows) for the region is 8.4 °C, ranging from a January mean of –4 °C to a July mean of 19 °C. Mean annual precipitation to the area is 133 $\pm$ 47 cm, measured for the last 17 years at the Big Meadows site VA28 (NADP/NTN 1998). Snowfall between November and March generally comprises less than 10% of this amount, and there is typically little lasting snowpack during the winter months.

Methods

Paine Run discharge and chemistry

Hourly stream discharge at the Paine Run outlet was calculated from stage height, which was continuously recorded (Stevens recorder) and digitized. A periodically updated rating curve, validated with direct velocity and cross-sectional area measurements, was used to relate height to discharge.

The annual study period (Jan. 1997–Jan. 1998) was classified into times of baseflow and times of stormflow. In the field, storms were operationally defined by a rise of approximately 6 cm in stream height. For Paine Run over the range of typical baseflow stage heights, this corresponds to an increase in discharge of approximately 5-fold. The events were considered to continue for either 2 or 3 days, depending upon their magnitude. This method divided the annual discharge into comparable-sized storm and baseflow compartments, based on rapid multiplicative changes in flow.

All streamwater samples were collected at the Paine Run outlet site (Figure 1) unless otherwise noted. Baseflow stream samples were collected at least weekly by hand in HDPE bottles, while stormflow samplings used a stream-height activated automated sampler (ISCO model 2900) equipped with teflon sample tubing and silicone peristaltic pump tubing. During the year-long study period, there were six discrete storm events that were sampled. After the onset of a storm, the ISCO sampler collected duplicate 500-mL samples every 3 hours, for 36 hours total. With the exception of one storm, ISCO samples were brought back from the field, filtered and preserved within 24 hours of the end of the sampling period. The samples were not preserved in the field, but tests on Paine Run streamwater samples stored for 72 hours in ISCO bottles showed no changes greater than the analytical uncertainty in any of the measured parameters (Buffam 1999). Periodic concurrent grab samples taken from the stream by hand were used to test comparability of sampling methods. While NO_3^- and DON concentrations were not significantly affected by sampling method, sampling via ISCO induced a variable NH_4^+ contamination (0–1.5 μM). The NH_4^+ contamination is accounted for in the TDN measurement and did not affect the calculation of DON concentration. However, due to the variable NH_4^+ contamination, direct comparisons of NH_4^+ levels in stormflow and baseflow were not made. ISCO sampling also introduced a fairly consistent DOC contamination of 27 $\pm$ 13 μM (n = 19). To correct for the DOC contamination, 25 μM-C was subtracted from the measured DOC concentrations of all ISCO samples in this study.

Streamwater bacterial samples were obtained in duplicate 20 mL glass scintillation vials, preserved with 2% v/v 0.2 μm-filtered formalin, and stored at 4 °C until analysis. Samples were counted by epifluorescence spectroscopy using acridine orange (Hobbie et al. 1977). For each slide, 5–10 fields of 20–200 cells were counted, ensuring that at least 200 bacteria total were enumerated per sample.

All water samples for chemical analysis were vacuum-filtered through precombusted (500 °C) and deionized water-rinsed Whatman GF/F glass fiber filters (nominal pore size 0.7 μm). Periodic filter blank checks showed that for all analyses, filtration did not induce detectable contamination. Following filtration, water samples were subsampled into 20 mL aliquots for each analyte and stored until analysis. Samples for NO_3^-, NH_4^+ and total dissolved nitrogen (TDN) analyses were stored at –20 °C in acid-washed HDPE scintillation vials, while samples for DOC analysis were acidified to pH 2 with concentrated H_2SO_4 and stored at 4 °C in pre-combusted, pre-rinsed 20 mL glass EPA vials with teflon septum liners.

TDN was analyzed with an Antek 7000B Nitrogen analyzer, by high-temperature (950 °C) combustion and chemiluminescent detection after oxidation to NO_2* (Walsh 1989; Frankovich & Jones 1998). Samples were delivered in triplicate 10-μL injections with a Dynatech Model GC311H autosampler. Nitrate standards were used since the streamwater samples contained more NO_3^- than other N-species, and NH_4^+ standards were analyzed each run to ensure acceptable oxidation efficiency. Although it is generally accepted that current methods of TDN analysis do not achieve quantitative recovery of all natural organic compounds, several studies (Frankovich & Jones 1998; Walsh 1989) have achieved 90–100% recovery for organic-N compounds using the same high-temperature oxidation method with the Antek instrument. During the current study, a test analysis of various organic compounds gave variable recoveries of 50%–90% depending upon compound type, so the analysis is expected to underestimate natural organic nitrogen concentrations, and thus underestimate calculated DON and N/C by as much as 50%. It is acknowledged that this methodological difficulty makes interpretation of small differences in DON and N/C tenuous, and we have taken this into account in the discussion of this study.

Nitrate was analyzed using a Dionex Model 14 Ion Chromatograph with HPIC AS4A separator column (Tabatabai & Dick 1983). A spot check of 15% of the water samples revealed NO_2^- levels always below detection limit (0.2 μM-N), so NO_2^- concentrations were assumed to be 0 for calculations of DIN and DON. Ammonium was analyzed either using the phenol-hypochlorite wet chemistry method (Solorzano 1969), or utilizing a Technicon Autoanalyzer II for colorometric detection by the indophenol blue technique (TIS 1973). An intercomparison of the two methods demonstrated that the method of analysis did not bias the results, with a mean difference of 0.03 ± 0.2 μM-N ($n = 35$).

Following sparging with ultrapure O_2 to remove dissolved inorganic carbon, DOC was analyzed with a Dohrman DC-80 carbon analyzer utilizing UV-assisted persulfate oxidation. Potassium hydrogen phthalate (KHP) standards were used, and the machine reactor fluid blank (2% persulfate) was used to calculate the DOC concentration in the DIW blank.

DIN was calculated as the sum of NO_3^- and NH_4^+ concentrations, and DON was calculated as the difference between TDN and DIN. The molar ratio N/C rather than C/N is used for calculations of mean values, since this minimizes denominator variance and uncertainty in the ratio (Atchley et al. 1976). When mean C/N is expressed for comparison to other studies, the value is obtained by taking the inverse of the final N/C value. Analytical uncertainties were estimated for all analyses as the standard deviation of standard quality control samples between different analytical runs.

Annual fluxes of DOC, DON, and NO_3^- were calculated for Paine Run for the study period by integrating the product of hourly stream discharge and time-interpolated stream concentrations. Time-interpolation appears to be adequate for times of baseflow, since solute concentrations were fairly constant over short time periods (days to weeks) except when storm events occurred. The rising limb of storms required a more complicated interpolation technique, because storm events caused rapid increases in the concentrations of DOC, DON and NO_3^-. In the case of an initial antecedent baseflow sample point (low concentration) and an initial storm sampling point (high concentration, usually near the peak in flow), the assumption was made that the concentration remained constant until flow began to increase, after which it increased in proportion to flow, up to the initial measured stormflow concentration.

Bacterial bioassay of organic matter bioavailability

For four of the six storms identified, streamwater samples were collected near the peak in flow, then filter-sterilized (0.2 μm Supor) to remove bacteria and other particulate matter. Sample water from each event was run in a standardized bioassay along with corresponding filter-sterilized baseflow streamwater, which was collected a few days before or after the event. The bacterial bioassay was developed in order to compare DOM quality between stormflow and baseflow samples in Paine Run.

There are a number of different methods used by researchers to assay microbial growth on dissolved organic matter in streams and rivers (e.g. Meyer et al. 1987; Moran & Hodson 1990; Leff & Meyer 1991; Qualls & Haines 1992; Kaplan & Newbold 1995; Sun et al. 1997; Volk et al. 1997), which makes direct comparisons between different studies difficult. The strategy used in this study was to control as many variables as possible in a laboratory bioassay, and to use a relatively simple protocol, similar to that utilized by Leff and Meyer (1991). The strength of this approach is that it is easily repeatable, and allows a direct comparison between the stormflow DOM and the baseflow DOM. The major weakness is that the bioassay does not approximate natural field conditions, and in particular does not contain sediment/surface area for bacterial colonization, such that extrapolation to the Paine Run system is difficult. However, the growth rates from the bioassay are thought to give a reasonable estimate of potential water-column activity.

A bacterial-dilution growth experiment (cf. Landry & Hassett 1982) demonstrated that growth rate constants (μ) estimated from dilutions of streamwater to 10–20% natural abundance were within 15% of $\mu_{\max}$, the growth rate constant estimated for infinite dilution (no grazing). Thus, a standardized bioassay with diluted inoculum can be used to calculate the approximate rate of carbon incorporation into bacterial biomass for this system. Initial experiments suggested that an incubation time of 2–3 days, with bacterial counts made at 0, 1.5, 2 and 2.5 days, should be sufficient to estimate bacterial production prior to any major grazing effects (Buffam 1999).

The bioassays were run with 0.2 μm sterile-filtered sample water inoculated to a concentration of $0.48 \pm 0.14 \times 10^5$ cells mL^{-1} (approximately 25% natural abundance) with a standard pelagic bacterial community isolated from the stream. To create this inoculum, cells were combined from late summer high-flow and low-flow water samples from Paine Run, filter-concentrated, and resuspended in approximately 1/20 initial volume (72% recovery). Bacterivores were removed by filtration through a 1.0 μm nuclepore PC membrane, and the concentrated inoculum was then frozen and stored in aliquots at –20 °C to be thawed and utilized for the bioassays. Recovery of intact cells following freezing was 92+/–27%, based on abundances for the initial bioassay timepoint after inoculation. Inorganic nutrients (+5 μM NO_3^-, +5 μM NH_4^+, +1 μM PO_4^{3-}) were added to prevent nutrient limitation, and the bioassay was buffered to pH 6.4–6.7 with 60 μM $NaHCO_3$ to minimize effects of pH variability on microbial activity. For each stormflow/baseflow pair, a single bioassay was run with duplicates of each treatment. Samples were incubated in the dark at 20 °C in 1000-mL nitric-acid washed glass bottles with teflon-lined caps, and measurements of bacterial abundance (acridine orange direct count) were made at four timepoints: 0 h (initial), 36 h, 48 h, and 60 h. The growth curves generated were used to calculate growth rate constants (μ) for all treatments.

After pooling the results of each duplicate incubation, baseflow and stormflow were compared for each of three parameters: μ, norm-μ, %DOC consumed (paired t-test, $n = 4$ stormflow/baseflow pairs, $\alpha = 0.05$). Parameter explanations and calculation methods follow.

Bioassay calculations

Growth rate constants(μ) were calculated for each treatment by assuming exponential growth and fitting the exponential growth equation to the observed growth curves using two points. The initial lag phase (up to 36 h), was not included in the growth rate constant calculations, and typically the 36 h and 60 h timepoints were used.

$$A_t = A_o e^{\mu t} \quad \text{OR} \quad \mu = \frac{(\ln(A_t) - \ln(A_o))}{(\Delta t)}$$

where A_o = initial cell abundance (cells mL^{-1})
A_t = cell abundance at time t (cells mL^{-1})
Δt = time elapsed (h)
μ = growth rate constant (h^{-1})

Normalized growth rate constant (norm-μ)

The growth rate constant should be proportional to the concentration of bioavailable DOM, assuming DOM is limiting bacterial growth. To account for changes in μ based on changes in DOM concentration alone the parameter norm-μ was calculated for all bioassays.

$$norm - \mu = \frac{\mu}{[DOC]_{initial}}$$

where norm-μ = the normalized growth rate constant (h^{-1} μM-C^{-1})
[DOC]$_{initial}$ = the sample DOC concentration at the beginning of the bioassay (μM-C)

Estimated rate of in-stream DOC uptake

The uptake of DOC in the stream should be a function of three primary factors: the mean growth rate constant of the bacteria, the standing stock of bacteria, and the bacterial growth efficiency. The rate at which DOC would be taken up in the stream was estimated by applying the growth rate constant (μ) obtained from the standardized bioassays to the stream standing stock of bacteria at the time the water sample was taken, yielding a production estimate. This value, which is proportional to the rate of incorporation of C into bacterial biomass, is converted into DOC uptake rate using an assumed 30% bacterial growth efficiency (BGE). Studies performed with natural microbial assemblages and streamwater or lakewater DOM have found BGE values in the 17%–31% range (Tranvik & Höfle 1987; Mann & Wetzel 1995; Meyer et al. 1987; Kaplan & Bott 1983). The value of 30% was chosen an estimate for the Paine Run bioassays based on a study by Kaplan and Bott (1983), who observed a BGE of 28% on baseflow natural organic matter in a small, low-DOM creek in Pennsylvania.

a) estimated biomass increase in-stream:

$$M_t = M_o e^{\mu t}$$

where M_0 = stream bacterial biomass (μM-C)
An estimated mean value of 20×10^{-15} g-C per cell is used, after Lee and Fuhrman (1987).
M_t = biomass at time t (μM-C)
t = time elapsed (h)
μ = growth rate constant (h^{-1}) measured in bioassay

b) estimated rate of DOC uptake:

$$\%uptake = \frac{\left(\frac{M_t - M_o}{\Delta t \times E}\right)}{[DOC]_{STREAM}} \times 100$$

where % uptake = uptake rate of DOC as a % of available stream DOC (% h^{-1})
M_t = biomass at time t (μM-C)
Δt = time elapsed (used 1 h for rate calculations)
E = microbial growth efficiency (used 0.3 = 30%)
$[DOC]_{STREAM}$ = DOC concentration in the stream at the time the respective sample was collected for the bioassay

Results

Baseflow concentrations in Paine Run

Paine Run showed a consistent level of DOC (Table 1, Figure 2(a)) during baseflow throughout the sample period at 71 $\pm$ 4 μM-C (N = 85). Variations on the timescale of hours to days during times of increasing flow were clearly more extreme than any annual variability in the baseflow DOC level. The maximum DOC concentration measured during the 12-month study period was 260 μM-C during the rising limb of a large storm in early November 1997. Baseflow DON in Paine Run (Table 1, Figure 2(b)) was more variable, but again the highest concentrations occured during times of increasing flow. N/C of the DOM was highly variable, with a mean value and standard deviation of 0.023 $\pm$ 0.011 (equivalent to a mean C/N of 44), and no apparent seasonal trends.

The Paine Run baseflow nitrate concentration averaged 8.7 $\pm$ 4.0 μM, approximately 5 times the mean DON concentration, and over 50 times the mean NH_4^+ concentration. Ammonium was never measured above 2 μM, and was below 0.5 μM for 90% of the samples. Nitrate concentrations at baseflow were constrained throughout most of the study period (5–15 μM-N) but dropped to near 0 in late October (Figure 2(c)). Nitrate concentrations were also clearly influenced by flow conditions, reaching a peak of 41 μM-N during a storm in early June.

The 15 streams sampled quarterly in SNP (Figure 1) vary in aspect, underlying bedrock geology, altitude, catchment area, and forest type, and are fairly representative of SNP as a whole. Baseflow DOC concentrations in Paine Run were comparable to mean annual concentrations in the 15 VTSSS streams, while Paine Run NO_3^- and DON concentrations were below average but still well within the range of the other streams (Table 1).

Table 1. Mean water chemistry during 1997 for Paine Run at baseflow and at stormflow. Mean baseflow chemistry from several sampling frequencies demonstrate that increasing sampling frequency does not significantly improve the estimate of baseflow DOM concentrations in Paine Run. Both standard deviation and range of values are reported for complete datasets, and 'true' flow-weighted target mean values are included for comparison. The annual baseflow means for 15 different streams in SNP, included for comparison with Paine Run baseflow, are calculated from the means of 4 quarterly measurements in each stream.

	Mean DOC (μM)	*Mean DON (μM)*	*Mean NO_3^- (μM)*
Paine Run – Baseflow			
Quarterly (N = 4)	71.8 ± 6.4	2.4 ± 1.0	7.5 ± 5.2
Monthly (N = 11)	65.4 ± 7.2	1.4 ± 0.7	7.0 ± 3.5
All measurements (N = 85)	71 ± 14 (51–139)	1.7 ± 1.0 (–0.1–5.9)	8.7 ± 4.0 (0.0–20.0)
Flow-weighted mean	70	1.5	9.1
Paine Run – Stormflow			
All measurements (N = 67)	148 ± 46 (71–260)	3.0 ± 1.5 (–0.3–7.5)	18.4 ± 9.0 (0.0–41.0)
Flow-weighted mean	167	3.7	19.4
Paine Run – Overall			
All measurements (N = 152)	105 ± 49 (51–260)	2.3 ± 1.4 (–0.3–7.5)	13.0 ± 8.0 (0.0–41.0)
Flow-weighted mean	105	2.3	12.7
SNP streams			
Annual baseflow means (N = 15)	68.0 ± 14 (49–98)	2.2 ± 0.6 (1.1–3.2)	16.6 ± 10.2 (4.4–36.0)

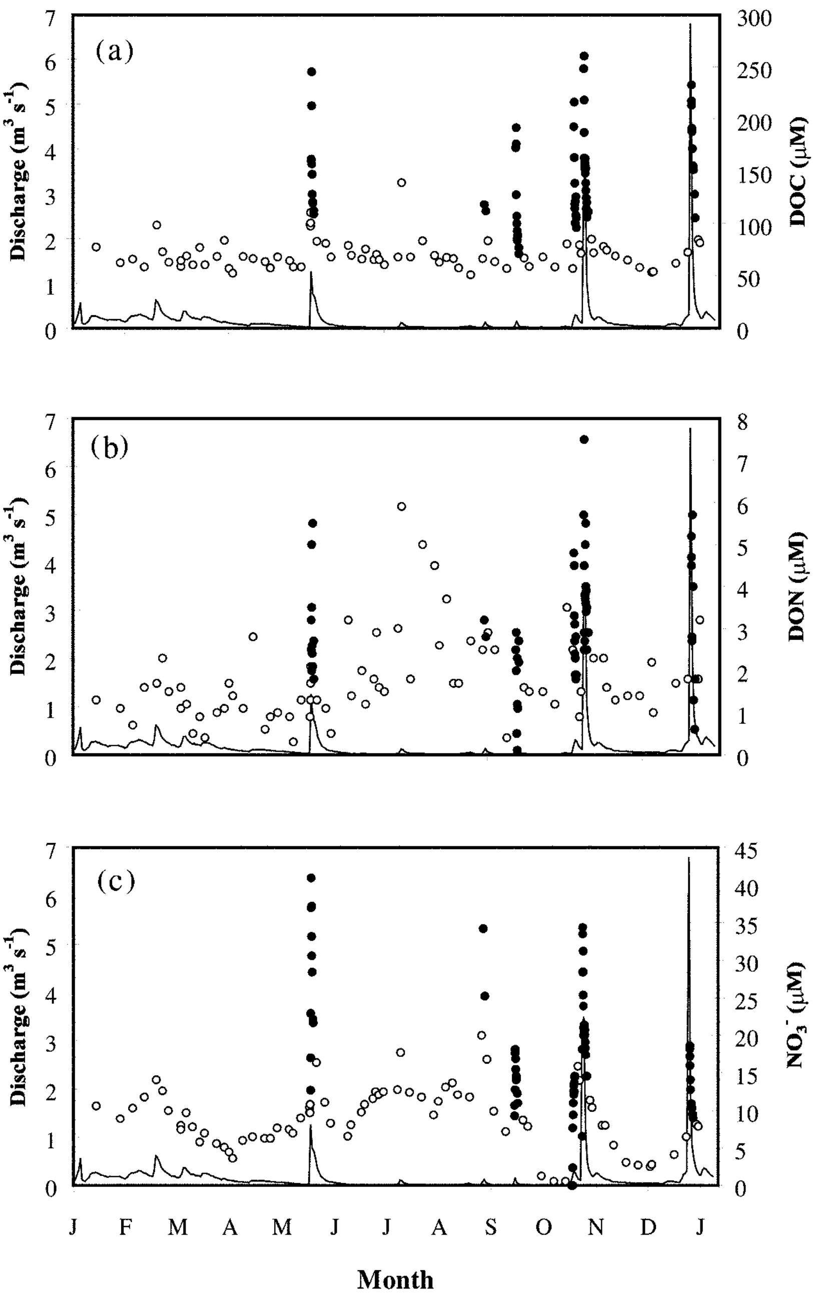

Figure 2. Paine Run stream solute concentrations (circles) and discharge (–) for the study period, Jan. 1997 to Jan. 1998. Open circles (○) represent samples taken during times of baseflow, typically on a weekly collection routine. Closed circles (●) represent stormflow samples, with a collection strategy emphasizing times of changing flow. (a) DOC concentration and daily mean discharge; (b) DON concentration and daily mean discharge; (c) NO_3^- concentration and daily mean discharge.

Table 2. Flux calculations for major carbon and nitrogen species in Paine Run, Jan. 15, 1997 – Jan. 14, 1998.

	Baseflow	Storm	Total
Time (days)	350	15	365
Surface runoff (cm)	27.2	14.9	42.1
Discharge (%)	65	35	100
n (# samples taken)	85	67	152
DOC (mol ha^{-1} yr^{-1})	187	243	430
DON (mol ha^{-1} yr^{-1})	3.9	5.4	9.3
NO_3^- (mol ha^{-1} yr^{-1})	24.1	28.2	52.3

Effect of storms on DOM and NO_3^- concentrations in Paine Run

Hourly mean discharge at the Paine Run outlet varied from a low of 0.001 m^3 s^{-1} during late summer low-flow, to a high of 21.4 m^3 s^{-1} during a storm on Jan. 8, 1998, a range of well over 4 orders of magnitude during the course of the year. There were six storms during the 12-month study period, five of which were intensively sampled (Table 3). Concentrations of DOC and DON increased significantly during storms ($P < 0.001$, t-test), with storm means approximately twice baseflow means (Table 1, Figure 2). Mean stormflow C/N was slightly higher than baseflow (49 vs. 41), but this was not a significant difference due to variability in the measurement. Concentrations of NO_3^- also increased significantly ($P < 0.001$, t-test) during storms (Table 1, Figure 2), with the average stormflow concentration slightly more than double the average baseflow concentration. Storms constituted only 4% (15 days) of the annual study period but contributed 36% of the annual flow and over half of the flux of DOC, DON and NO_3^- (57%, 58% and 54%, respectively) in Paine Run in 1997 (Table 2). Total fluxes were 430 mol ha^{-1} y^{-1} DOC, 9.3 mol ha^{-1} y^{-1} DON, and 52.3 mol ha^{-1} y^{-1} NO_3^-.

Although baseflow DOC concentrations were fairly constant throughout the year, regardless of baseflow discharge (Figure 2(a), Table 1), DOC rapidly increased during the onset of storm events, often within a few hours of the first observed increase in discharge (Q) (sample storm shown in Figure 3(a)). The maximum DOC concentration reached during Paine Run storms was relatively consistent (200–300 μM, or 3–4x the baseflow concentration) regardless of event magnitude. DOC was much higher at a given Q on the rising limb than at the same flow on the falling limb, giving rise to a clockwise C-Q (concentration-discharge) hysteresis loop for all four intensively sampled storms. The most rapid increase in DOC occurred at the

Table 3. Description of the six storms identified during the study period, of which five were sampled intensively. Antecedent conditions are referred to as dry, intermediate (med.) or wet, which is a qualitative assessment made by the researcher of relative ground moisture level in the catchment prior to the event. The Jan. 8, 1998 event consisted of a melting snowpack in addition to the new rain.

Date of onset	Length of event (days)	# of samples taken during event	Antecedent Q (m^3 s^{-1})	Precipitation (cm)	Maximum Q (m^3 s^{-1})
June 1, 1997	3	11	0.02 (med.)	9	1.97
Sept. 10, 1997	2	2	0.002 (dry)	5	0.15
Sept. 28, 1997	2	11	0.008 (dry)	4	0.20
Nov. 1, 1997	2	12	0.02 (med.)	3	0.31
Nov. 7, 1997	3	20	0.11 (wet)	11	4.33, 5.06 (double peak)
Jan. 8, 1998	3	10	0.27 (wet, rain on snow)	7	21.4
Totals:	*15*	*66*	*Mean = 0.07*	*Mean = 6.5*	*Mean = 4.8*

earliest measured point on the rising limb, while the peak in DOC occurred during the rising limb prior to maximum Q (e.g. Figure 3(d)). DOC levels recovered rapidly to the baseflow concentration, typically to within 15 μM-C of baseflow level within 1–3 days.

During individual storms, DON (e.g. Figure 3(b), 3(e)) showed similar behavior to DOC, though not as clearly, probably due to analytical imprecision. The maximum DON concentration occurred during the rising limb, and DON always showed a similar clockwise hysteresis to DOC. There were typically a few outliers (e.g. peak at center of hysteresis loop in Figure 3(e)), but the response was consistently a clockwise hysteresis loop.

Nitrate showed a clear pattern of increase during storms (Figure 3(c), 3(f)), but the pattern was different from that of the organic matter. The most striking difference between the DOM and NO_3^- storm pattern was that the NO_3^- concentration maximum followed the peak in flow rather than preceding it. The lag between peak Q and peak NO_3^- concentration varied from 4–24 h, resulting in a counterclockwise C-Q hysteresis (Figure 3(f)), opposite that observed for DOC and DON.

Bioassay results

Samples were collected in Paine Run for bioassay experiments during four storms, and four corresponding times of baseflow. The flow at the time of sampling varied from 0.008 m^3 s^{-1} (baseflow, Aug. 23, 1997) to 6.0 m^3 s^{-1} (stormflow, Jan. 8, 1998). Stream DOC, DON and NO_3^- concentrations and

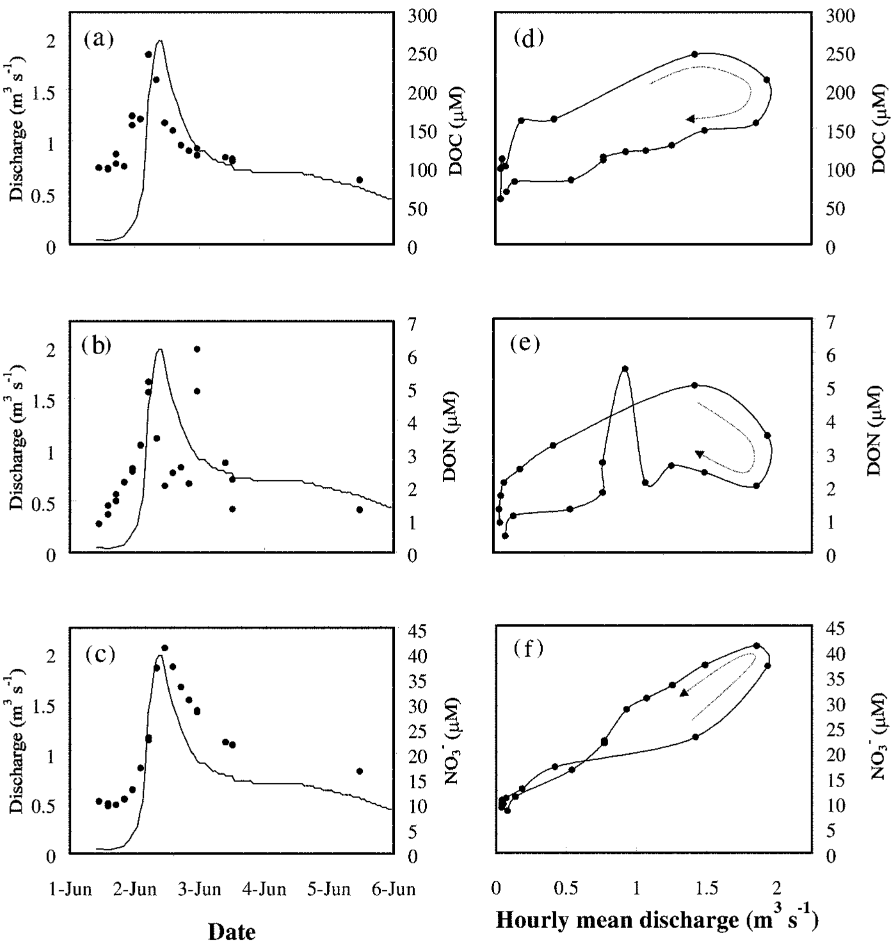

Figure 3. Timecourse of solute concentrations and discharge (a–c), and solute concentrations as a function of discharge (d–f) at the Paine Run outlet during the June 1, 1997 storm. Behavior of solutes during this storm was typical of the four storms which had a single defined hydrograph peak (of six storms total). (a–c): Hourly mean discharge (–) and solute concentration (•) for a) DOC, b) DON, and c) NO_3^-; (d–f): Solute concentrations vs. hourly mean discharge for: d) DOC, e) DON, and f) NO_3^-.

bacterial abundance always were higher for the stormflow samples than the corresponding baseflow (Table 4).

The bioassays resulted in linear increases in bacterial abundance over time from an initial abundance of $0.48 \pm 0.14 \times 10^{-5}$ mL^{-1}, after a lag phase of up to 36 h. Growth curves were generated for the four stormflow/baseflow bioassay pairs (example for one storm shown in Figure 4).

Table 4. Paine Run stream chemistry data at the time of sample water collection for the four stormflow/baseflow bioassay pairs. Samples were collected for bioassays near the peak in flow during four of the six storms identified.

Bioassay #	Collection Date	Q ($m^3\ s^{-1}$)	stream bact ($x10^5\ mL^{-1}$)	DOC (μM)	DON (μM)	NO_3^- (μM)
1 – Base	Aug. 23, 1997	0.01	2.4	66.4	1.9	13.7
1 – Storm	Sept. 10, 1997	0.07	9.4	118.4	3.3	34.1
2 – Base	Oct. 3, 1997	0.02	1.1	67.2	1.6	8.7
2 – Storm	Sept. 28, 1997	0.06	7.1	173.1	4.0	10.5
3 – Base	Nov. 12, 1997	0.21	1.2	72.0	2.3	10.4
3 – Storm	Nov. 7, 1997	2.75	6.5	175.3	3.3	28.4
4 – Base	Jan. 12, 1998	0.39	1.1	84.5	1.8	8.2
4 – Storm	Jan. 8, 1998	6.00	9.1	205.5	3.1	18.7
Mean Base		0.16	*1.5*	*72.5*	*1.9*	*10.3*
Mean Storm		2.21	*8.0*	*168*	*3.4*	*22.9*

Bacterial abundance after 60 h was considerably higher for the stormflow samples as compared to the baseflow in bioassays 2, 3, and 4, while in bioassay 1, the abundance was approximately the same on both types of sample water. Samples without the nutrient amendment gave mean 60h abundances of 14% lower (baseflow samples) and 36% lower (stormflow samples) than their respective nutrient-addition samples, suggesting secondary nutrient limitation. However, since the target parameter for this study is the amount of bioavailable DOM, nutrient-addition results only are presented.

Growth rate constants were calculated from the abundance curves (e.g. Figure 4) assuming exponential growth, and varied from 0.005 to 0.065 h^{-1} (Figure 5(a)). The growth rate constants were greater for stormflow sample water as compared to baseflow water (α = 0.05, paired t-test). There was a trend for higher growth rates on samples with higher initial DOC and DON (univariate linear regression, R^2 = 0.58 and 0.47 for DOC and DON, respectively), but the results were not significant when adjusted for multiple comparisons made. Growth rate was not significantly affected by N/C.

Based on the operational definition of bioavailable DOC (BDOC), the mean growth rate constant of bacteria, for a given bacterial culture, should be proportional to the amount of organic matter substrate which is bioavailable on a short timescale. This assumes that other nutrients or environmental conditions are not limiting (e.g. Felip et al. 1996). Thus, μ normalized to initial DOC concentration should be proportional to [BDOC]/[DOC], the

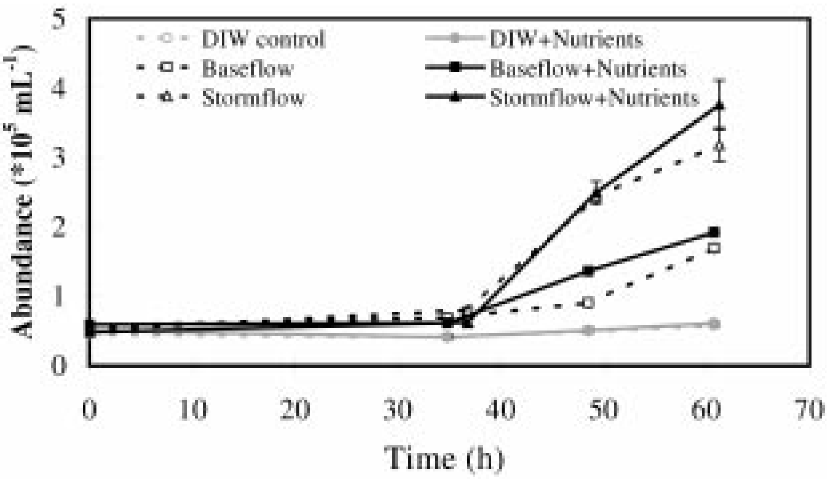

Figure 4. Sample bioassay growth curves, comparing high-flow vs. low-flow samples, and nutrient addition vs. unamended samples. The bioassay shown here (Bioassay #2), with baseflow sample from Oct. 3, 1997 and stormflow sample from Sept. 28, 1997, is typical of the patterns observed. The growth curves were used to calculate growth rate constants (Figure 5) using the timepoints 36 h and 60 h. Error bars represent the range of bacterial abundance from duplicate bottle incubations. The control was an incubation run with filter-sterilized deionized water instead of filter-sterilized Paine Run sample water, with all other inoculation and incubation characteristics identical.

fraction of the total DOC which is bioavailable. This parameter, 'norm-μ' (units of h^{-1} μM-C^{-1}) was slightly greater in the presence of nutrients, but varied inconsistently between baseflow and stormflow samples, with no significant difference (Figure 5(b)). Norm-μ was not significantly correlated (univariate linear regression) with DOC, DON or N/C.

The estimate of %DOC utilized per hour in stream (Figure 5(c)) is based on stream DOC concentration, stream bacterial abundances, and growth rate constants from the bioassay, and assumes a mean biomass per cell and a constant bacterial growth efficiency. Estimated rates of DOC utilization ranged from 0.003% to 0.2% per hour, and were significantly increased in stormwater (α = 0.05, paired t-test), but were not significantly correlated (univariate linear regression) with DOC, DON or N/C. The fraction of the total stream DOC pool taken up by water column bacteria was estimated to increase from 0.03 $\pm$ 0.02% h^{-1} (0.02 μM-C h^{-1}) during baseflow, to 0.17 $\pm$ 0.04% h^{-1} (0.25 μM-C h^{-1}) during storms. Actual loss of DOC (presumed to be uptake by bacteria) was measured for a continuation of some of the bioassays. Using water from baseflow/stormflow pairs from November 1997 and January 1998, observed DOC losses after 192 hours were 4 and 6 μM for stormflow bioassays (2.6% and 2.7% of initial DOC) and 5 and 2.5 μM

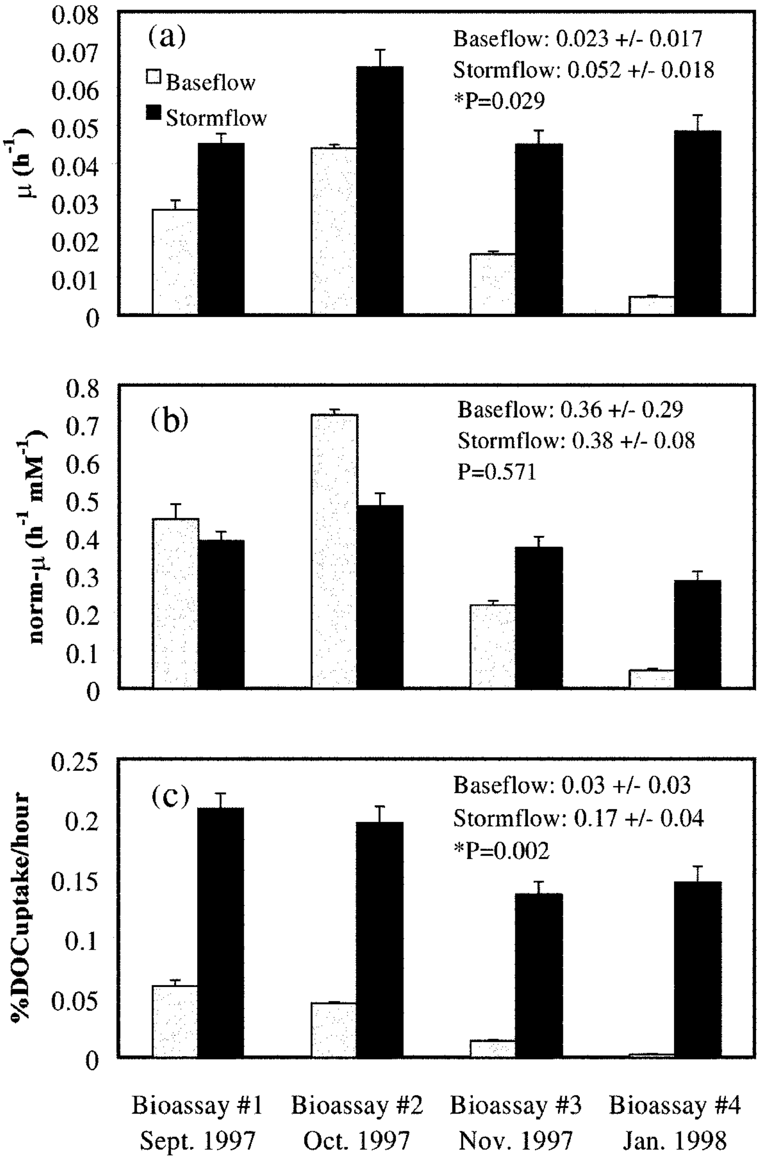

Figure 5. Summary of bioassay results, calculated from rates of bacterial abundance increase during the standardized bioassays with nutrient addition (e.g. Figure 4). Error bars represent the coefficient of variation propagated from the standard deviation of bacterial abundance from duplicate bottle incubations. Each graph is separated into four sections, one for each bioassay. (a) growth rate constant (μ) calculated for each treatment; (b) growth rate normalized to initial [DOC] ('norm-μ'); (c) estimated percentage of stream DOC consumed per hour by microbes in the water column.

for baseflow bioassays (6.8% and 2.5% of initial DOC). These correspond to mean uptake rates of 0.01–0.03 μM-C h^{-1} (0.01–0.04% h^{-1}).

Discussion

Baseflow DOM and NO_3^- concentrations

Mean baseflow concentrations of DOC and DON in Paine Run were at the low end of values reported for rivers and streams worldwide (Tables 5, 6) but similar to other small forested catchments in the Appalachian mountains (e.g. Qualls & Haines 1991; Meyer & Tate 1983) and to the concentrations measured in other streams sampled quarterly in SNP for this study (Tables 5, 6). Low DOM concentrations are typical for these streams, probably due to the high mineral content in the watershed soils, and the fact that the streams are underlain directly by bedrock rather than by an organic matter source. Paine Run and the other SNP watersheds have a low fraction of dissolved nitrogen in the organic form (Table 5), which is due both to low stream DOM concentrations, and to moderately elevated DIN concentrations. Nitrate in many of the streams in SNP has been high in recent years due to a gypsy moth infestation which resulted in substantial N-leakage (Webb et al. 1995).

DOC did not show a seasonal pattern, but several high DON values in mid-late summer (Figure 2(b)) were suggestive of increased N-content of organic matter at that time. Paine Run DON/DOC was highly variable, but was highest during the low baseflow period of August and September, as was PON/POC (Buffam 1999). In-stream processes are expected to be strongest relative to watershed processes during times of low flow (Mulholland & Hill 1997), and elevated DON during late summer in Paine Run may represent the byproducts of in-stream production. Small amounts of algae (C/N = 9) and moss (C/N = 27) were present on the streambed and may have contributed to DON due to their high N-content relative to terrestrial sources. The observed variability in DON (Figure 2(b)) is due in part to the analytical imprecision, which is ± 1.6 μM-N for any given sample. The mean DON value for all samples taken in Paine Run was 2.3 μM, so the analytical uncertainty is on the order of 50–100% of individual sample values. This precludes the ability to make strong statements about DON concentrations or N/C ratios for individual samples or small numbers of samples.

Nitrate gave a clear seasonal pattern, with values dropping to near 0 in the autumn. This coincides with leaf-fall, and the seasonal pattern in NO_3^- may be due to changes in flow path, soil chemistry, or in-stream uptake rates. Typically, new organic matter inputs from leaf-fall stimulate the uptake of NO_3^- by soil microbes (e.g. Creed et al. 1996; Mulholland & Hill 1997; Campbell et al. 2000), resulting in lower concentrations in the soil, and ultimately in the stream. Mulholland and Hill (1997) reported high in-stream net nutrient uptake in the autumn in Walker Branch and White Oak Creek, Tennessee. This primarily heterotrophic uptake was accentuated in times of low flow, when in-stream controls on water chemistry were emphasized. Leaf-litter input in streams has also been implicated in nutrient uptake (Tank & Webster 1998), and the combination of low flow and leaf-litter input into Paine Run in the fall could explain the very low values for October baseflow NO_3^-.

Stormflow DOM and NO_3^-: Patterns observed and possible sources

The hypothesis that streamwater DOC, DON and NO_3^- concentrations would change during storms is linked implicitly to the assumption that hydrologic source areas and flow paths are different between baseflow and stormflow. Likewise, the hypothesis that the chemical character or bacterial bioavailability (quality) of stream DOM would change during storms is linked to the same assumption, that differing flow paths during storms would entrain a different type of organic matter. Based on the assumption that stormflow is likely to accentuate the contribution of DOM sources near the organic-rich soil surface (by throughfall, leaf-litter entrainment/leaching, saturated overland flow or return flow), we expected storms to result in increased concentrations of DOC and DON, increased C/N (Qualls & Haines 1991), and increased bacterial bioavailability (Kaplan & Bott 1983; Qualls & Haines 1992). However, the source DOM pools were not measured directly for Paine Run, adding a degree of uncertainty to any hydrologic analysis.

The results of this study support earlier observations in other streams of elevated DOC concentration with increasing flow during individual events and during seasonal floods (Table 6). Most stream DON studies represent baseflow samples only, although a few recent studies in small watersheds found an elevated mean concentration of DON during storms (Wondzell & Swanson 1996; Jordan et al. 1997). During a study of coastal plain rivers in California, DON was moderately positively correlated with flow (R^2 = 0.2), with an increase of about 20% for every order of magnitude increase in flow (Smith et al. 1996). In the current study, Paine Run showed a significant increase in DON during storms in conjunction with DOC (Figure 3). Paine Run showed a trend (not significant) for slightly increasing C/N during storms, which is suggestive of a new source emphasizing high C/N material during high flow. Campbell et al. (2000) reported similar behavior for New England streams, with discharge having a greater positive effect on DOC concentrations than on DON concentrations. This phenomenon could be explained by the contribution of throughfall or the leaching of leaf-litter or surface DOM during overland flow, since this organic matter is expected to

Table 5. DON concentrations, DON as a % of TDN, C/N of organic matter, and annual DON fluxes from several stream studies.

Type of system	Mean DON (μM)	DON % of TDN	Mean C/N (molar) of DOM	Annual DON flux (moles ha^{-1})	Reference
15 streams (2–22 km^2 forested catchments) in the Blue Ridge Mountains, SNP, Virginia	2.2 (range 1.1–3.2)	16% (range 4–37%)	30 (range 22–53)		This study
Paine Run, small low-ANC stream in forested catchment, Shenandoah National Park, VA	(range 0.0–7.5) 1.7 ± 0.1 (baseflow)	15% (baseflow)	41 (baseflow)	9.3	This study
	3.0 ± 0.2 (stormflow)	15% (stormflow)	49 (stormflow)		
9 small forested watersheds in New England	(range 7–27)	19–90%	17–51	36–171	Campbell et al. 2000
39 small forested watersheds in the Catskill Mountains, NY (baseflow)	4.9 ± 1.3[a] (range 2.8–7.7)	7–73%[a]	21 ± 37[a]	50 (mean)[a]	Lovett et al. 2000
61 upland catchments in Britain	12.5 (range 0–54)	14–69%			Chapman et al. 1998
Very small forested (hardwood) watershed #110, in Rhode River basin, long term study	23	67%		46	Correll & Weller 1997

Table 5. Continued

Small forested catchments in Chesapeake Bay watershed, different geological regions	12.4 (range = 8.4–20.1)	11–57%			Correll et al. 1996
Walker and Lagunitas Creeks, Tomales Bay watershed, California, mixed land types	25 ± 11	35–53%	21	43	Smith et al. 1996
McCrae Creek, 4th-order forested stream in H.J.Andrews experimental forest	approx. 1.5[b] (baseflow) approx. 2.5[b] (stormflow)	60–70% (range 30–90%)			Wondzell & Swanson 1996
Small watersheds in old-growth temperate forest ecosystems, Chile	11 (range 6–30)	95%	48		Hedin et al. 1995
Various relatively unpolluted Rivers	19 (range 1.8–71)	Typically 30–70%	20	95 (approx.[c])	Meybeck 1982, Meybeck 1993
Small forested reference watershed WS-2 in Coweeta, North Carolina	1.9		35		Qualls & Haines 1991

[a]Unfiltered samples used, yielding TON instead of DON.
[b]Mean DON concentrations estimated from graphs of seasonal means.
[c]DON flux estimated from DOC flux, using median C/N of 20 (Meybeck 1993).

Table 6. Stormflow and baseflow concentrations of DOC and annual DOC flux for several stream studies.

Type of system	Mean DOC (μM)	Annual DOC flux (moles ha^{-1})	Reference
15 streams (2–22 km^2 forested catchments) in SNP, Virginia	68 (range 49–98)		This study
Paine Run, small forested watershed, SNP, Virginia	(range 51–260) 71 ± 1.5 (baseflow) 148 ± 5.6 (stormflow)	430	This study
Snake River and Deer Creek, alpine catchments, Colorado	50–100 (baseflow) 100–250 (spring snowmelt high flow)	340–800	Hornberger et al. 1994; Boyer 1998
Small forested hillslope catchments, Ontario	approx. 400 (baseflow) approx. 520 (stormflow)	2000–3600 (estimate)	Hinton et al. 1998
Walker and Lagunitas Creeks, California, mixed land types	530 ± 450	909	Smith et al. 1996
White Clay Creek, Pennsylvania	approx. 100 (range 60–333) (higher during stormflow than baseflow)		Kaplan & Newbold 1995
Humic Swedish river	1300 (higher during summer/ fall storms than baseflow)	5000	Ivarsson & Jansson 1994
Various relatively unpolluted rivers	350 (median) (range 40–3000)		Meybeck 1982; Meybeck 1993
Bear Brook, small forested watershed, New Hampshire	180 (range 63–630) (approx. 150 low flow) (approx. 400 high flow)	1100–2100	Fisher & Likens 1973; McDowell & Likens 1988; David et al. 1992
Small forested watershed WS-14 North Carolina	42–108 (baseflow) 83–420 (stormflow)	1240	Meyer & Tate 1983

be relatively low in %N as compared to groundwater DOM (e.g. Qualls & Haines 1991).

Paine Run exhibited a clockwise concentration-discharge (C-Q) hysteresis loop for DOC and DON (Figure 3(d), Figure 3(e)) that is typical of rivers during times of flood (Meybeck 1993). Some small forested Appalachian watersheds had equally high DOC levels on the falling limb as on the rising limb during storms (Meyer & Tate 1983), but others like Bear Brook in New Hampshire exhibited a peak in DOC concentration on the rising limb, giving rise to the same clockwise C-Q hysteresis loop (McDowell & Likens 1988).

Nitrate had peak concentrations in the stream later than DOM, during the falling limb (e.g. Figure 3(f), see also Hyer 1997), emphasizing the contribution of a new groundwater and/or soilwater source due to the rising water table. Maximum NO_3^- concentrations were variable between events, probably as a result of seasonal variability in soil NO_3^- concentrations (e.g. Creed et al. 1996) or stream uptake rates (Mulholland & Hill 1997). The opposite hysteresis patterns for NO_3^- and DOM illustrate that their primary source areas were effectively distinct: High-DOM areas contributed flow to the stream during the first several hours of a storm, while high-NO_3^- areas contributed flow later in the hydrograph. This timing difference could be useful in distinguishing between inorganic and organic sources of acidity during storms, both of which have been shown to contribute to a loss of alkalinity and low pH in freshwaters (Galloway et al. 1983; Eshleman & Hemond 1985; Bishop et al. 1990; Hyer 1997). In Paine Run, increases in nitric acid were the major contributor to acidification during storms in 1994 (Eshleman & Hyer 1998), but organic acids also increased during all events, averaging 7 μeq l^{-1} at the point of lowest ANC in Paine Run storms.

There are several watershed source pools and delivery processes for organic matter which vary spatially and temporally, and could contribute to the observed variability in stream DOM concentrations in Paine Run. First, hydrologically distinct reservoirs can have different DOM concentrations. For instance, when the source pools in forested North American catchments have been measured, often throughfall [DOC] > overland flow [DOC] > soilwater [DOC] > groundwater [DOC] (e.g. Hinton et al. 1998). Second, a given reservoir may vary spatially in DOC concentration. For example, Qualls and Haines (1991) found that soilwater leachable DOC concentrations decrease with depth in a Coweeta, North Carolina catchment. Third, DOC concentrations can vary over time, even at the same spot in the same reservoir. For example, Hornberger et al. (1994) and Boyer (1998) used a flushing mechanism, in which soilwater DOC concentration was depleted over time while the soil was saturated, to explain DOC patterns in a Rocky Mountain stream.

Variability at any of these three scales could result in complicated hysteretic relationships between stream flow and DOC concentration. The most straightforward way to explain the observed hysteresis is by simple mixing between three constant concentration reservoirs. Evans and Davies (1998) developed a three component mixing model for stream storm events, which explains various types of C-Q hysteresis based on the relative concentrations of the solute of interest in three source reservoirs. Analysis by this method revealed that stormflow discharge/solute relationships in Paine Run could be explained by contributions from the following three reservoirs: (1) a high DOM, low NO_3^- surface runoff reservoir, occurring during the rising limb of storms only; (2) an intermediate DOM, high NO_3^- soilwater reservoir, contributing during storm events for an unknown period of time; (3) a low DOM, low NO_3^- groundwater zone, contributing at all times, but increasing during periods of high flow.

Although neither DOM nor NO_3^- were measured in any of these source reservoirs in Paine Run, the relative values assigned by this analysis fit well with conceptual models of DOM dynamics in forest soils, as well as with observations for forested catchments in the Appalachians (e.g. Qualls & Haines 1991). Leachable DOM typically decreases with depth, with the highest concentrations in the litter layer (which would influence surface runoff chemistry, reservoir #1) decreasing down through the soil horizons (soilwater, reservoir #2) to a low in bedrock (groundwater, reservoir #3). Nitrate is expected to build up in unsaturated soils as a byproduct of microbial mineralization of organic matter and subsequent nitrification, so that soilwater concentrations of NO_3^- could be higher than those in groundwater or surface water. The 3-component end-member mixing analysis model, then, is not inconsistent with observed behavior in Paine Run, and the observed hysteresis could be explained by contributions to streamwater from all three reservoirs.

However, it is important to note that DOM concentrations are expected to vary both temporally and spatially, even within source reservoirs, so that a simple mixing model is an oversimplification and may be of limited utility for DOC and DON. The storm on Nov. 7, 1997, which consisted of two distinct peaks in discharge within a 24-hour span (Table 3), gave evidence for a more complicated delivery pattern. In that storm, DOC, DON and NO_3^- concentrations were all considerably lower during the second peak, despite the fact that it was higher in flow than the first (Buffam 1999). This behavior is suggestive of the flushing-out of soluble DOM and DIN from the respective source reservoirs, similar to that observed for DOC during seasonal snowmelt in a Rocky Mountain catchment (Hornberger et al. 1994; Boyer 1998), but on a shorter temporal scale. Also, seasonal variation in baseflow and peak stormflow NO_3^- concentration suggests that the reservoirs are not static over the long term with respect to DIN.

During times of baseflow, groundwater and low-DOM lower B-horizon soils are expected to contribute a relatively constant level of DOM to a stream (cf. McDowell & Wood 1984). This pattern was observed for Paine Run, and stormflow DOM concentrations also were well constrained around a certain maximum value, seemingly with little dependence on storm magnitude. Responses of stream DOM concentrations to changes in flow were both rapid and transient, and stormflow chemographs suggest that relative change in flow rather than the absolute magnitude of discharge is more closely linked with increasing organic matter concentrations in Paine Run. Because of the rapid response to flow or rainfall observed, both in increasing and receding concentrations of DOM, the most important processes controlling DOM delivery to the stream during storms must be near-stream or in-stream processes. Near stream sources include direct throughfall, saturated overland flow, and the flushing of upper soil layers in the riparian zone (e.g. Bishop et al. 1994). In-stream DOM generation may also contribute to observed concentrations. The hyporheic zone is not expected to be a major sink or source, since the stream is generally underlain directly by bedrock or boulders, with only small sections of sediment/gravel. However, the leaching of leaf litter and other particulate organic matter in the stream could contribute to Paine Run DOM at all times, and especially during storms. In a small stream in Coweeta, North Carolina, leaf litter leachate contributed on the order of 30% of the daily export of DOC, and represented a particularly significant source of DOC during times of increasing flow (Meyer et al. 1998). The flushing of upper soil layers further from the stream may maintain moderately increased levels of stream DOM in the days following a large event (e.g. Hornberger et al. 1994). Groundwater flow and soilwater flow from outside the riparian zone are not likely to be major contributors to peak stormflow DOM, because their response times would not be rapid enough to explain the observed concentration jumps.

Annual fluxes of DOM in Paine Run, relative contribution of stormflow

DOM fluxes from Paine Run were 430 moles ha^{-1} carbon and 9.3 moles ha^{-1} nitrogen in 1997 (Table 2), somewhat low for undisturbed first and second order upland streams in the U.S. (Tables 5 & 6). The low mean DOM concentrations and low runoff (42 cm during 1997) resulted in the low flux in Paine Run. The NO_3^- flux of 52.3 moles ha^{-1} for 1997 is the lowest in recent years for Paine Run (SWAS unpublished data), and continues a decreasing trend (from 150 mol ha^{-1} in 1993 to 58 mol ha^{-1} in 1996) linked to forest recovery from a gypsy moth infestation in the early 1990s (Webb et al. 1995). Average annual atmospheric wet deposition was 387 ± 56 moles ha^{-1} yr^{-1} of

DIN (NADP/NTN 1998), as measured at the Big Meadows (VA28) collection site. This is an underestimate of total N input, since it does not include dry deposition or the contribution of DON. DON is on the order of 20% of total N in precipitation in this region, and can constitute as much as 60% of total N during individual events (Scudlark et al. 1998; Russell et al. 1998). In spite of chronic acid nitrate and sulfate deposition, the Paine Run catchment is at present still fairly retentive of nitrogen, with stream outflux of N amounting to a small percentage of the N inputs. The high DOC/DON (mean 44) in Paine Run may also be an indication that the Paine Run watershed is not N-saturated. Campbell et al. (2000) found that stream DOC/DON was negatively correlated with stream DIN export for nine forested watersheds in New England, and suggested that stream C/N might be an effective indicator of susceptibility to N-saturation. In their study, streams with a C/N > 40 exported less than 0.75 kg ha^{-1} (54 mol ha^{-1}) annually, while streams with a C/N < 30 exported up to three times that amount. With a DIN export of 52.3 mol ha^{-1} in 1997, Paine Run fits into the same envelope of values.

Although storms as defined for this year-long study occurred for only 15 days in Paine Run, they accounted for over one-third of the yearly discharge and over half of the flux of DOC, DON, and NO_3^- (Table 2), due to the increased concentrations during stormflow (Table 1). Consequently, using baseflow chemistry concentrations would underestimate annual flux of DOC, DON, and NO_3^- by 33%, 35%, and 28% respectively. Even weekly stream chemistry measurements failed to capture the most extreme variations in organic matter concentrations, which occur during a short period of a few hours during the onset of storm events. This behavior should be taken into account for stream budget studies, and underscores the importance of frequent sampling during storms.

In contrast to stormflow, there was little systematic variation in baseflow DOM concentrations in Paine Run, either seasonal or weekly (Table 1, Figure 2). Neither the accuracy nor the precision of baseflow measurements improved much with increased sampling frequency. More information is gained by the concentration of sampling efforts during times of varying flow, especially during the rising limb of the hydrograph and the time of peak stormflow. However, frequent baseflow sampling is still useful in detecting seasonal patterns, and increasing the precision of the estimate of annual flux.

Bioavailability of stormflow vs. baseflow organic matter in Paine Run

Given that there were differences in Paine Run DOM concentrations between baseflow and stormflow and given the complicated loading patterns during storms, it is likely that there are distinct pools of organic matter feeding into the stream. If this is the case, the different source pools should vary in their degree of microbial processing prior to entry into the stream, and as a result their bioavailability would be expected to differ.

Mean growth rate constants in the Paine Run bioassays were 0.023 $\pm$ 0.017 on baseflow water, and 0.052 $\pm$ 0.018 on stormflow water (Figure 5(a)). These growth rate constants correspond to mean generation times of 30 hours in baseflow water and 13 hours in stormflow water. Though typical of the range of generation times for bacterial growth on natural substrate, these generation times were still on the order of ten times the residence time of water in Paine Run. The low (1–2 $\times$ 10^5 mL^{-1}) abundance of bacteria in the Paine Run water column at baseflow is probably the result of the lack of a large bioavailable pool of DOM, or grazing (unlikely to be a major effect at such low abundance). Increased bacterial abundances during storms may be generated from the flushing of leaf-litter or riparian zone soil reservoirs, or the resuspension of biofilms on the streambed. Additionally, increases in bacterial abundance and growth rate during storms would heighten water column bacterial productivity, further increasing bacterial abundances.

Increased growth rate constants on stormflow vs. baseflow DOM (Figure 5(a)) suggest that there were higher concentrations of bioavailable DOM during storms than during baseflow in Paine Run. A similar result was found in another small stream system, White Clay Creek in Pennsylvania (Kaplan & Bott 1983; Volk et al. 1997). However, when normalized to the initial DOM level (norm-μ), growth on Paine Run stormflow DOM was not significantly different than growth on baseflow DOM (Figure 5(b)). Apparently, the *fraction* of bioavailable DOM was not a consistent function of flow regime. Increases in potential microbial growth rate constants during storms were due to increased concentrations of DOM, not to a change in the ‘quality’ of the organic matter. This result is consistent with a cross-system review of labile DOC in which a number of different systems had a similar fraction of bioavailable DOC (mean 19%), with the exception of blackwater rivers which had very low levels of labile DOC regardless of the total concentration (Søndergaard & Middelboe 1995).

Qualls and Haines (1992) found that for soil solution in another Appalachian catchment, biodegradability declined vertically from throughfall to the A horizon and then increased with depth. Storms thus might contribute DOM of both increased and decreased bioavailability to the stream, resulting in a complicated response for the DOC-normalized growth rate. In the present study, the DOC-normalized growth rate changed more between the different baseflow bioassays than between the different stormflow bioassays (Figure 5), suggesting that there are long-term/seasonal factors at work which alter DOM biodegradability more than short-term differences in flowpath. Summer/autumn low-flow (<0.03 m^3 s^{-1}) baseflow DOM supported elevated growth relative to winter medium-flow (>0.20 m^3 s^{-1}) baseflow DOM. However, due to the low number of storm bioassays (n = 4) and the lack of samples from spring/early summer, it is not possible to make a full estimation of seasonal patterns in the bioavailable fraction of DOM. Furthermore, the standard bacterial inoculum used was collected during August, and this bacterial community may have been able to respond more rapidly to DOM substrate from that time of year (bioassays #1 and #2). For that reason, the bioassays in this study (particularly the winter bioassays) could give a longer lag time and lower apparent %BDOC (μ-norm) than studies which use DOM and bacteria collected at the same time and place.

Mean %DOC utilization during storms in Paine Run was estimated to be higher than that at baseflow (0.17% h^{-1} vs. 0.03% h^{-1}) (Figure 5(c)), mostly due to increased water column bacterial abundance during storms. Measured DOC uptake from the bioassays was lower still, totalling between 2–7% of the initial DOC after 192 hours (measurement made for Nov. and Dec. bioassays only). However, the bioassays were inoculated with a low concentration of bacteria which had been collected at low/intermediate flow. Actual uptake in the stream during storms would be higher since concentrations of bacteria are high and presumably represent a diverse assemblage of microbes from stream water column, riparian zone, biofilm, and soil which may be able to more fully utilize the DOM. The measured drop in DOC of 2–6 μM is a minimum estimate of bioavailable DOC, since it is unknown how much DOM would be utilized over longer time periods or at high concentrations of stormflow bacteria.

Estimates of %bioavailable DOC in natural aquatic systems vary greatly, from <1% to >75% (Sun et al. 1997), depending upon organic matter source and type of bioassay. In a comparison of stormflow and baseflow streamwater similar to the present one, Kaplan and Newbold (1995) and Volk et al. (1997) found that the fraction of DOC that was bioavailable (%BDOC) was moderately higher for stormflow DOC than baseflow DOC. Their results, for White Clay Creek in Pennsylvania, utilized a different type of bioassay: a flow-through column biofilm reactor which contained a large surface area colonized by native stream bacteria. With inflow DOC concentrations similar to those in the present study, and elevated levels of DOC during storms, these studies observed a high removal of DOC, giving a baseflow %BDOC of 20–30%, with stormflow %BDOC up to 34%.

The measurements of %BDOC in the 20–30% range for White Clay Creek came from a bioassay system which mimicked hyporheic zone conditions, maintaining a favorable environment for the formation of biofilms. Under these conditions, as long as inorganic nutrients are not limiting to growth, bacteria can remove a significant portion of the stream DOC in just a few hours (Kaplan & Newbold 1995). Hyporheic zones can effectively process DOM at higher rates than microbes in the stream water column, due to increased surface to volume ratio and increased hydraulic residence times. Adsorption of stream DOM in hyporheic sediments may also make DOM available to hyporheic zone microbes on a longer timescale than the hydraulic residence time (Findlay 1995). Although most of Paine Run is underlain by bedrock, there are some gravel beds which could provide zones of DOM processing, and the importance of this process to DOC and DON removal is unknown at this time. Therefore, it is important to note that the estimate of %DOC uptake is an estimate for the water-column only. Based on this estimate, DOM in Paine Run is not expected to be substantially depleted by in-stream bacteria, either during baseflow or during storms.

Potential effects of stormflow DOM on in-stream bacterial populations

Despite the lack of apparent effect of water column microbes on the bulk carbon cycle in the stream, DOM levels are still thought to be important for bacterial growth. Changes in DOM during storms would appear to have an impact on bacterial growth in the stream, which in turn has implications for the level of secondary productivity. During the storm bioassays, growth rate constants were on average twice those of the corresponding baseflow value (Figure 5(a)). This means that more than twice the amount of organic matter would be assimilated by bacteria during stormflow than would occur in an equivalent amount of time during baseflow, assuming cell size and growth efficiency are comparable between times of baseflow and stormflow. Due to increased bacterial abundance in the water column during storms (mean approx. 5-fold higher than at baseflow, Table 4), and the doubling of μ during storms, the calculated assimilation of DOC by bacteria is nearly 10 times as fast during storms as during baseflow (Figure 5(c)), on a mass per unit water volume basis. Although this is still a small fraction of total DOC, it represents a considerable increase in bacterial biomass. These bacteria generated from resuspension and from growth on stormflow organic matter will act to concentrate nutrients in response to the increased source of carbon. After the storm, they are a new potential source of food for flagellates and other bacterivores. Note that the increased concentration of stream bacteria during storms is transient, and they may be transported downstream relatively rapidly or deposited in the near-stream zone during large floods.

Many other stream characteristics, aside from organic matter concentrations and/or quality, are different during storms than during baseflow. It is difficult to predict the actual in-system ecological effect of the observed increase in DOM and bacterial concentrations, which resulted in an increase in potential bacterial growth rates and an increase in calculated %DOC uptake, respectively. It is not known whether this heightened potential productivity translates into actual productivity in the natural system. Recent research (Gremm & Kaplan 1998) in a small Piedmont stream found that although bioavailable DOC increased during storms, the productivity may not be realized locally due to the scouring of bacteria from biofilms on rocks and sediments. In Paine Run, scouring of bedrock during storms was visually observed. The occurrence of this process was supported by the observation of increased water column bacterial abundance during storms, which may have been resuspended from sediment and biofilms on the bedrock underlying the stream. Depending upon whether one considers water-column or hyporheic/biofilm activity, production may be either increased or decreased during storms.

The bioassay results show that the DOM entering during storms was not significantly different in %bioavailability than that entering during baseflow. One interpretation, which contrasts the DOM concentration data during storms, is that source pools were similar at all times for Paine Run. Another possible explanation is that if there were distinct new sources of DOM to the stream during storms, those sources were similar in bioavailability and DOC/DON to the baseflow groundwater DOM pool. In any case, DOC, DON, NO_3^- and bacterial abundance all increased in the water column during storms, resulting in an increased potential for secondary productivity and DOM uptake. This potential occured transiently in the water column in Paine Run, and may in part even represent the displacement of DOM and bacteria from streambed sediment and biofilms. Whatever the ecological dynamics during the stormflow period in a small catchment like Paine Run, there is clearly delivery of a large pulse of DOM and bacteria to downstream ecosystems in larger bodies of water. The ecological impact of this pulse of potential energy will depend upon such factors as settling rates, residence times, and ecological makeup of the downstream systems.

Conclusions

This study demonstrates one aspect of the potential ecological impact of storms in Paine Run, via the effect of stormflow DOM on bacterial production. DOM concentrations in Paine Run were consistently low (71 $\pm$ 14 μM DOC, 1.7 $\pm$ 1.0 μM DON) during baseflow and did not show a seasonal pattern, but during storms mean DOC and DON concentrations approximately doubled. The maximum DOC and DON concentrations occurred on the rising limb, averaging 228 $\pm$ 31 μM-C and 4.8 $\pm$ 1.2 μM-N. Flow-related changes in DOM concentrations necessitated sampling every few hours during storms to achieve an accurate estimate of stream solute fluxes, and baseflow sampling alone would significantly underestimate DOM and nitrate flux. The rapid response of DOM suggests a near-stream or in-stream source of organic matter during storms, which is most likely surface flow or channel expansion, perhaps releasing DOM from leaf litter and other particulate matter entrained during stormflow. All of these are thought to contribute to some degree to increased DOM during storms, with varying contributions depending upon the season, antecedent hydrological conditions, and type of storm. Hysteresis analysis of storm DOC, DON and NO_3^- suggests that the major source pools of DOM to the stream could be described by three distinct reservoirs. These reservoirs are a transient high DOM surface runoff source during the rising limb, an intermediate DOM soilwater source during and immediately following events, and a constant low DOM groundwater source. The three-component mixing model provides an initial simplistic way of attributing DOM to source areas during storms, but it is clear that solute concentrations in the reservoirs vary in more complicated spatial and temporal ways. In particular, DOM in the surface runoff reservoir appeared to exhibit a rapid flushing behavior (cf. Hornberger et al. 1994), while NO_3^- in the soilwater and groundwater reservoirs appeared to vary over a longer-term seasonal cycle.

In a standardized bioassay, growth rate constants were significantly higher in stormflow water than baseflow water, due to increased stormflow DOM concentrations. The fraction of DOM which was bioavailable, however, as measured by the growth rate constant normalized to sample DOC concentration, was not consistently different for stormflow as compared to baseflow water in Paine Run. This result suggests that stormflow and baseflow sources of DOM are similar, conflicting the stormflow chemograph analysis which points to distinct source pools. It is possible that there are spatially distinct source pools which differ in DOM concentration but have been degraded to a similar level of bioavailability. Alternately, DOM loading may be a more complicated process involving the mixture of many source pools which differ in bioavailability in less predictable ways, leading to inconsistent differences in %bioavailable DOM between baseflow and stormflow DOM (e.g. Qualls & Haines 1992).

As observed in other stream systems (McDowell & Wood 1984; Likens & Bormann 1977; Meybeck 1982), baseflow organic matter concentrations in Paine Run are stable compared to those of most inorganic solutes. This behavior of organic matter delivery is thought to stabilize heterotrophic aquatic ecosystems (Wetzel 1995). However, because of the increases at times of high discharge, there is an irregular pulsing behavior in organic matter flux from the stream. These pulses could result in bursts of increased productivity followed by increased consumption. The water column DOM uptake rate was estimated to increase 10-fold during storms, but this would have a minimal impact on bulk DOC and DON concentrations in Paine Run, since water column residence times are short (1–3 h) in the catchment. However, storms are still expected to have considerable impact on the bacterial stream communities, by mobilizing them into the water column and by supplying a pulse of water high in DOM.

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