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Terrestrial inputs of organic matter to coastal ecosystems: An intercomparison of chemical characteristics and bioavailability

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Abstract. Dissolved and particulate organic matter (DOM and POM) collected from rivers or groundwater feeding five estuaries along the east and west coasts of the USA were characterized with a variety of biogeochemical techniques and related to bioavailability to estuarine microbes. Surface water was sampled from the Columbia, Satilla, Susquehanna and Parker Rivers and groundwater was sampled from the Childs River. Several geochemical descriptors (percent organic matter of suspended particulate matter, C/N, lignin phenol content, ratio of vanillic acid to vanillin) suggested an ordering of the systems with respect to POM lability: Satilla < Parker < Columbia < Susquehanna.

DOC concentrations in these systems ranged from <100 μM for the Columbia River to >2000 μM for the Satilla River. Elemental analysis of DOM concentrates (>1000 D) was used to predict organic matter composition and to calculate degree of substrate reduction using two different modeling approaches. Models predicted aliphatic carbon ranging between 43 and 60% and aromatic carbon between 26 and 36%, with aliphatic content lowest in the Satilla and highest in the Columbia River. The degree of substrate reduction of the organic matter concentrates followed a pattern similar to that for aliphatic C, being lowest in the Satilla (3.5) and highest in the Columbia (4.0). Extracellular enzyme activity varied broadly across the systems, but again ordered sites in the same way as did aliphatic content and degree of substrate reduction. Bacterial growth rates ranged from 1.3 ug mg^{-1} d^{-1} DOC in the Satilla to 1.7 ug mg^{-1} d^{-1} DOC in the Parker River. Bioassays confirmed patterns of dissolved organic matter lability predicted by the chemical models. Between 67% to 75% of the variation in bacterial growth could be explained by differences in organic matter composition.

Introduction

The riverine export of organic matter from land to estuarine ecosystems is substantial, with estimates of C flux ranging from 0.2 to >1 x 10^{15} gC yr^{-1} (Kempe 1979; Schlesinger & Melack 1981; Meybeck 1982). Inputs of this magnitude are significant components of overall estuarine carbon budgets, being equivalent to about 25% of the global autochthonous organic matter production of estuaries (Woodwell et al. 1973). Specific transport rates of organic matter export from land range from 1 to >14 gC m^{-2} land yr^{-1}, depending on land cover and precipitation. As urban development and land use change are occurring rapidly throughout the world, but especially in coastal regions, it is likely that both the magnitude and nature of organic matter exported from the land surface to estuaries is changing dramatically as well.

The fate of this carbon and its significance to estuarine food webs in the coastal zone are not well understood (Moran & Hodson 1994; Smith & Hollibaugh 1995; Hopkinson & Vallino 1995; Kemp et al. 1996). It is generally thought that river-borne organic matter is relatively refractory and largely unavailable for metabolic breakdown in the time frame of estuarine mixing and burial (Ittekkot & Laane 1991). On the other hand, there is evidence of total system heterotrophy for a large number and variety of coastal systems (Hopkinson & Vallino 1995; Smith & Hollibaugh 1995) indicating a metabolic dependence on allochthonous inputs in many systems. These contrasting views are at least partially a function of inter-system variability in both the quantity and quality of allochthonous carbon. Here we use the term "organic matter quality" in the sense of its biodegradability and its ability to support microbial growth. To date, there have not been rigorous intercomparisons of organic matter quality and therefore no attempts to either explain or predict differences among systems. In order to resolve this paradox and to understand how the influx of terrestrial organic matter affects carbon budgets and food webs of coastal estuaries, we need to know how the quality of organic matter from various land covers differs, the extent to which quality changes during downstream transport and the quality of the organic matter when it reaches the estuary.

As a result of extensive organic matter transformations and the production of new organic matter during river transport, the quantity and quality of organic matter entering the coastal zone differ greatly from that entering the river from land. The concentration of organic matter in rivers varies regionally. While the median DOC concentration in North American rivers is 480 μM, concentrations range from <80 μM in alpine rivers to >1700 μM in river systems influenced by swamps and poorly drained soils, e.g., taiga and some southeastern U.S. blackwater rivers (Mulholland & Watts 1982).

During downstream transport organic matter is oxidized and transformed by microbes resulting in a decrease in aliphatic compounds and an increase in aromaticity (Sun et al. 1997). These compositional changes in the bulk organic matter reaching estuaries affect the ability of micro-organisms to process the organic matter and sustain growth (Sun et al. 1997).

Here we report the results of an intercomparison of chemical characteristics and quality of riverine organic matter entering a number of estuaries around the United States. The systems studied are part of the NSF-funded Land Margin Ecosystem Research network. The ultimate goal of the intercomparison is to develop a predictive understanding of the biological utilization and fate of terrestrial organic matter in the coastal zone so that we can evaluate the ecological effects of changes in land use in coastal watersheds. Our initial efforts focus on the identification of measurable characteristics of particulate and dissolved riverine organic matter that may provide indicators of the quality of organic material entering estuaries. Chemical characteristics of river-borne dissolved organic matter are related to preliminary measures of the ability of the organic matter to support microbial growth in an attempt to develop a predictive understanding of the rate at which estuarine microbes utilize allochthonous organic matter.

Description of sites

The five coastal systems (Figure 1) in the intercomparison represent a range of land-margin ecosystems with watersheds varying in size, climate, geographic province and land cover. Watershed size varies over four orders of magnitude, from 50 km^2 for the Childs River (component of Waquoit Bay) to over 600,000 km^2 for the Columbia River. Land use in the watersheds ranges from a large urban component in the small Parker and Childs River estuaries of the Northeast. to watersheds that are primarily forested and with large areas devoted to agriculture. As with size, there are large variations in discharge with mean flow ranging from 1 to almost 7,000 $m^3\ s^{-1}$. Four of the rivers exhibit pronounced seasonal cycles in discharge rates. The Childs River watershed is unique compared to the others because it relies almost exclusively on groundwater which has little seasonal fluctuarion. Freshwater residence time differs greatly among estuaries, as a result of differences in freshwater discharge, tidal range and estuarine mixing. The Columbia, Satilla and Childs River estuaries, while having very different flow rates, have extremely short residence times, on the order of 1 to several days. The Chesapeake Bay (Susquehanna River) has a residence time on the order of half a year or more.

There are strong contrasts in the water chemistry of the five rivers. The Satilla, a blackwater river, has extremely high concentrations of dissolved

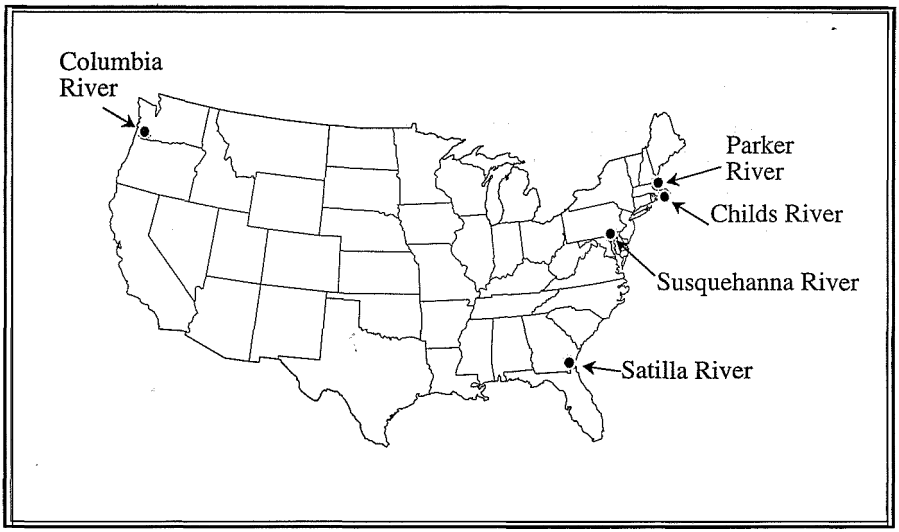

Figure 1. Location of rivers where dissolved organic matter composition and lability was studied.

organic carbon (DOC), averaging 1700 uM. In contrast, both the Columbia and the Susquehanna have low DOC concentrations, on the order of 100 uM. Childs River groundwater has no particulate load but has a relatively high DOC concentration. Concentrations of dissolved inorganic nitrogen (DIN) are extremely high in the Childs River system due to the suburban nature of the watershed that relies on septic tanks for sewage disposal. DIN is also very high in the Susquehanna, because of the heavy agricultural nature of its watershed. The potential importance of watershed inputs of organic matter relative to autochthonous production within the estuaries also varies considerably across the intercomparison sites, from less than 1% of the totel inputs for the Childs River to over 80% of total inputs to the Columbia River estuary.

Methods

Water sample collection.and treatment

River water was collected from each estuary during low flow conditions in late summer/early fall 1995. For particulate organic matter analysis, suspended solids were collected on pre-ashed 47 mm GF/F glass fiber filters. Upon drying, suspended solids were assayed by weight. Organic carbon and total nitrogen were assayed with a CHN analyzer, following carbonate removal via

acid fuming if necessary. Lignin phenol content was measured using cupric oxide oxidation (Hedges & Mann 1979; Hedges et al. 1986).

For dissolved organic matter analysis and bioassay work, 60 to 300-l of river water entering each estuary were collected in stainless steel vessels or Nalgene carboys. In the case of Childs River, groundwater was collected with wellpoint samplers adjacent to the estuary. Water samples were filtered (<7 psi) in two stages: 1) with acrylic filtration apparatus (acid washed and DI rinsed) using precombusted and rinsed Whatman GF/F glass fiber filters and 2) with thoroughly pre-rinsed Gelman 0.2 μm cartridge filters.

Following 0.2 μm filtration, dissolved organic matter was concentrated using an Amicon DC-10 or a Millipore Pellicon tangential flow ultrafiltration system equipped with a 1000-dalton MW-cutoff cartridge. Cartridges were pre-washed with dilute HCl and dilute NaOH, and pre-rinsed with ultrapure (18 Meg-ohm) deionized water until the retentate was of neutral pH and of conductivity approaching that of the ultrapure water. DOC in the water was then concentrated to a final volume of 3-4 liters of retentate (between 30X and 125X, depending on the initial DOC concentration and volume). At no time was the pH allowed to go below 4.5. The retentate was then diafiltered using 3–5 liters of ultrapure water to remove salts in the system. Following ultrafiltration the retentate solution was passed through a column of Dowex AG50 cation-exchange resin (40–60 mesh, H+ form, 1–3 meq/mL). Aliquots were then freeze dried.

Several chemical analyses were performed on the dissolved organic matter. Huffman Labs (Boulder, CO) analyzed all samples for C, H, O, N, S, ash and moisture content. At the Ecosystems Center (Marine Biological Laboratory, Woods Hole, MA) samples were redissolved and analyzed for cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}) and anions (Cl^{-}, SO_4^{2-}). Cations were determined by atomic absorption spectrometry and anions were determined with an ion chromatograph. Carboxylic acid concentration was calculated using a charge balance equation. DOC was analyzed following the procedure of Peltzer and Brewer (1993) and Peltzer and Hayward (1996) using high temperature combustion with Pt catalyst. Appropriate standardization with carbon free distilled water ensures appropriate assessment of instrument blank.

At a later time, a separate volume of water was collected from the oligohaline region of the Plum Island estuary (2 psu) for later use as a microbial inoculum for all bioassay experiments. In this case water was collected in a 20-l pressure vessel and then filtered through a 142 mm 0.6 μm Millipore filter to remove most bacterivores. Filtered water was refrigerated for subsequent bioassays (<1 day).

Dissolved organic matter composition

The method of analytical constraints computation and its validation with known chemical structures have been described previously (Perdue 1984; Wilson et al. 1987). Given a set of basic compositional parameters (elemental composition, carboxyl content, and estimated number-average molecular weight), the method yields values of sp^3-hybridized carbon (C_{al}, aliphatic carbon), sp^2-hybridized carbon in aromatic rings (C_{ar}, aromatic carbon), and all remaining sp^2-hybridized carbon (C_{xs}, excess carbon) by simultaneous solution of the following three equations:

$$C_{total} = C_{al} + C_{ar} + C_{xs} + COOH \quad (1)$$

$$H_{total} = (H/C)_{al}C_{al} + (H/C)_{ar}C_{ar} + (H/C)_{xs}C_{xs} + COOH + N_{total} \quad (2)$$

$$U_{total} = (U/C)_{al}C_{al} + (U/C)_{ar}C_{ar} + (U/C)_{xs}C_{xs} + COOH \quad (3)$$

All concentrations are in units of mmol g^{-1}. C_{total}, H_{total}, and N_{total} are calculated directly from elemental compositional data. Equation 3 is the conservation equation for unsaturation (rings and/or pi-bonds), and U_{total} can be calculated from the following equation

$$U_{total} = C_{total} + N_{total}/2 - H_{total}/2 + 1000/M_n \quad (4)$$

where the number-average molecular weight, M_n, is assumed to be approximately 1000 g mol^{-1} if a measured value is not available. If the average H/C and U/C ratios for the three forms of organic carbon were known, then Eq. 1–3 could be solved to obtain C_{al}, C_{ar}, and C_{xs} directly. None of these ratios were known, but a reasonable range for each ratio was readily estimated. For example, $(H/C)_{ar}$ almost certainly lies between zero and 516, and $(U/C)_{ar}$ is probably about 4/6 (the value for benzene rings).

Probabilistic estimates of C_{al}, C_{ar}, and C_{xs} were obtained by randomly generating H/C and U/C ratios within their allowed ranges and solving Eq. 1–3 for C_{al}, C_{ar} and C_{xs} until 1000 chemically valid solutions were obtained. From the population of chemically valid solutions, we calculated median values, mean values, and standard deviations for all parameters. The mean values of C_{al}, C_{ar}, and C_{xs} generally correspond well with the actual distribution of organic carbon (as measured by ^{13}C NMR or from known structures of organic compounds).

The elemental compositions were corrected for ash and moisture content prior to making calculations. Unfortunately, water samples from the Susquehanna River were contaminated during the ultrafiltration step, so it was not possible to use the measured elemental compositions from this site.

Degree of substrate reduction

The degree of substrate reduction has been used extensively in studies of bacterial growth (Roels 1983; Heijnen & van Dijken 1992), and represents the relative oxidation state of the compound. The degree of reduction of an organic compound is defined as the number of electrons that are transferred to oxygen when the substrate is oxidized to CO_2, H_2O, NH_3. and H_2SO_4 (Minkevich & Eroshin 1973). For a compound with elemental composition $C_\alpha H_\beta O_\gamma N_\delta S_\epsilon$ and charge ζ, the degree of reduction is defined by

$$\psi = 4\alpha + \beta - 2\gamma - 3\delta + 6\varepsilon - \zeta$$

or

$$\psi_C = 4 + (\beta - 2\gamma - 3\delta + 6\varepsilon - \zeta)/\alpha \quad (5)$$

which reflects the valence states of the five elements. Because ψ is based on a linear combination of elements and charge, it is a conserved quantity. Often, the degree of reduction is reported on a par-carbon-mole basis, ψ_C, (equation (5)) so that compounds of differing C content can be readily compared. For instance, the degree of reduction of methane is 8, while that of glucose is 4 per C mole.

The degree of reduction is one of several characteristic variables in a bioenergetic model developed to examine bacterial growth on DOM, NH_4^+, and NO_3^- (Vallino et al. 1996). This model has been used to examine bacterial growth on a spectrum of organic compounds. Although many factors affected the growth kinetics, it was found that the degree of reduction of DOM had a great impact on bacterial growth efficiency (i.e., yield) and growth rate. In particular, bacterial growth yield (Y: g Bacterial Carbon/g Carbon consumed) was found to be well described by the following linear relationship,

$$Y = 0.17\psi_C - 0.038 \quad (6)$$

and the specific growth rate (d^{-1}) followed the following parabolic relationship,

$$\mu = 0.18\psi_C^2 \quad (7)$$

Potential extracellular enzymatic activity as a measure of organic composition

Bacteria produce a broad array of extracellular enzymes that release smaller assimilable compounds from larger complex molecules of DOM. The activity of these enzymes can be used to provide information on what classes of compounds are being degraded in situ. As the composition of DOM shifts

over time or space, the suite of enzymes synthesized by bacteria should co-vary with changes in the availability of various components. Extracellular snzyme activity (EEA) is fairly well constrained in that the metabolic and material costs of synthesizing these enzymes are controlled by the nature and abundance of high and low molecular weight DOM available in the environment (Chrost & Rai 1993).

Unfiltered water samples were collected contemporaneously with samples for elemental analysis and concentration, and mailed frozen to the Institute of Ecosystem Studies in Millbrook, NY, where activity of eight extracellular enzymes was assayed using previously described techniques (Sinsabaugh et al. 1997). The enzymes assayed included (enzyme:substrate): esterase: 4-MUF-acetate; phosphatase: 4-MUF-phosphate; leucine aminopeptidase: L-leucine 7-amido-4-methyl-coumarin; β-glucosidase: 4-MUF-β-D-glucoside; a-glucosidase: 4-MUF-a-D-glucoside; -xylosidase: 4-MUF-xyloside; -N-acetyl-β-D-glucosaminidase: 4-MUF-N-acetyl-P-glucosaminide; and endo-peptidase: MUF-p-guanadinobenzoate. Briefly, 150 μl of sample water plus phosphate buffer (pH 8) were placed in 96-well plates containing 400 μM MUF-labeled substrate. Release of MUF was monitored as an increase in fluorescence (excitation = 365 nm, emission = 450 nm) over time using a plate reader attached to a spectrofluorometer. Blanks (substrate plus buffer or substrate plus DI water) were run in each plate. Estimates of variability were derived by analyzing water from replicate bottles from each site.

Bioassay procedure

Microbial growth bioassays were prepared with freeze-dried organic matter from each river by combining: 1) ultrapure deionized water, 2) sufficient freeze-dried organic matter to produce about 500 μM final DOC concentration, adjusted to pH 5.0 with NaOH, 3) salts media to give a final concentration of 9 mM NaCl, 200 mM $KHCO_3$, 17 mM $MgSO_4$, and 4.5 mM $CaCl_2$, 4) nutrients-NH_4Cl (1.34 mg/l), KH_2PO_4 (0.34 mg/l), and 5) the 0.6 pm-filtered innoculum water (1:20 ratio). Salts were necessary to replace inorganic ions that had been removed previously during the organic matter concentration procedure. Bioassays were conducted in triplicate in thoroughly washed and rinsed 50 ml glass ampoules. $HgCl_2$ killed controls were run in parallel.

The inoculated water was immediately sampled for initial DOC concentration and bacterial numbers. Bacterial growth was calculated from the increase in bacterial numbers following a 2-day incubation at room temperature (—17 °C). Preliminary studies indicated that bacterial numbers in the bioassays peak at 2 days and that this point coincides with the onset of grazing pressure exerted by reestablished protozoan populations. To determine bacterial numbers, duplicate 19 ml subsamples were fixed in formaldehyde

(2% final concentration) and stored at 4 °C. Subsamples stained with acridine orange were filtered through blackened 0.2 μm pore-size polycarbonate filters, and bacterial cells were counted in 10 microscope fields on each filter via epifluorescence microscopy (Hobbie et al. 1977).

The effect of concentration and freeze-drying on bioavailability was determined in separate experiments by comparing rates of respiration between 1) unconcentrated and concentrated DOC and 2) concentrated DOC and DOC reconstituted from freeze-dried, concentrated DOC. In both cases, effects were minimal: rates of oxygen consumption over 14 days were not significantly different ($P > 0.05$) between either set of treatments (results not shown).

Results

Particulates

At the time of sampling, suspended particulate matter (SMP) in all four rivers was low, ranging from 3.3 mg l^{-1} in the Parker to 7.6 mg l^{-1} in the Satilla River (Table 2). As only groundwater was analyzed in Childs River, it is not included in this comparison. The low SPM values are consistent with the relatively low discharge observed in the fall. Particulate carbon concentration was similar in three of the rivers (around 1 mg l^{-1}), while in the Columbia it was exceptionally low (0.2 mg l^{-1}).

POC made up from 3.5 to 32% of SPM. Particulate organic matter (assuming POC:POM of 1:2) was a major component of SPM in all the rivers except the Columbia, making up about 65% in the Susquehanna, 50% in the Parker, 30% in the Satilla, but only about 7% in the Columbia. The percentage POC decreases exponentially for world rivers as SPM increases (Meybeck 1982; Ittekkot & Laane 1991) suggesting a background population of organic particles that are progressively diluted by mineral particles. With the exception of the Columbia River sample, our data (Figure 2) align closely with the Meybeck curve, suggesting a typical relationship between organic and inorganic particles. The Columbia sample plots well below the curve, suggesting a background particle population that was anomalously rich in inorganic material, probably detrital particles.

The nitrogen content of the POM was very high in three of the rivers (Table 2), with POCPN values close to the Redfield ratio ($\sim$7), while in the Satilla POM was nitrogen-poor (POCPN = 11.1). These values compare with a global mean POCPN of about 10 (Meybeck 1982). The N-poor material is indicative of soil organic matter or vascular plant detritus while the N-rich material may be indicative of algal derived material. High chlorophyll

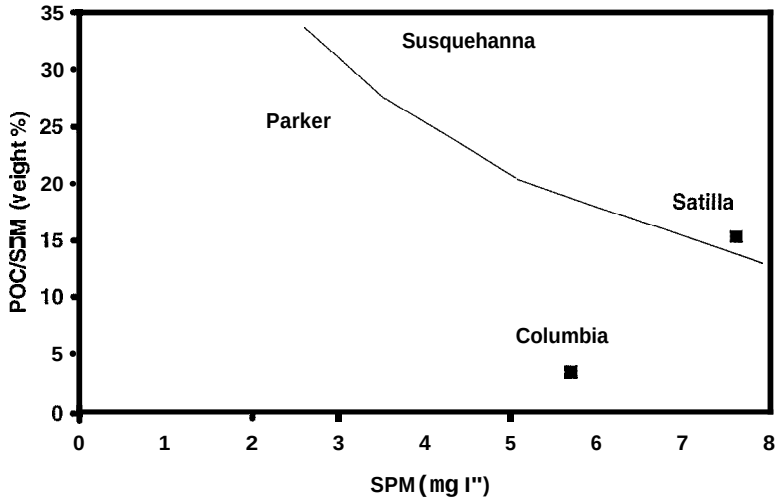

Figure 2. Variation in particulate organic carbon content with turbidity as measured by suspended particulate matter (spm). Included is the trend for rivers of the world as determined by Meybeck (1982).

concentrations, which are common for these 3 systems at this time of year, support the contention that phytodetritus comprises a large fraction of POM (see Table 1).

Lignin content of the particles provides clues to the nature of the allochthonous component. Total lignin phenols normalized to organic carbon varied over an order of magnitude (Table 2), from very low in the Susquehanna to very high in the Satilla River. This is generally consistent with the nitrogen data, supporting a dominance of autochthonous material such as phytoplankton in the Susquehanna and Parker Rivers and of allochthonous material in the Satilla River (Figure 3). Though nitrogen-rich, the Columbia River sample showed a moderate amount of lignin. If the lignin data indicate a significant nitrogen-depleted component, it must be balanced by an autochthonous component which has an unusually low POC/PN ratio.

The ratios of phenolic families comprising lignin can be used to infer the provenance of the vascular plant derived organic matter. The ratios of syringyl to vanillyl phenols (S/V) and of cinnamyl to vanillyl phenols (C N) generally indicate the relative contributions of angiosperms versus gymnosperms and of woody versus non-woody tissues respectively (Ertel & Hedges 1985; Hedges et al. 1988). A C/V versus S/V plot (Figure 4) expresses a trend leading away from gymnosperm wood (G) toward an endmember rich in angiosperm wood (A) with some dilution from a non-woody source. The sample from the Columbia River plots closest to G, with the others becoming

Table 1. Physical and chemical characteristics of rivers and their watersheds involved in the intercomparison of organic matter lability.

Parameter	Columbia		Susquehanna		Satilla		Parker		Waquoit	
	Annual	Study	Annual	Study	Annhual	Study	Annual	Study	Annual	Study
Watershed area (km^2)	660,500		164,200		9,143		609		46	
% Forest	49.5%		59.0%		56.4%		50.0%		46.0%	
% Urban	1.6%		8.0%		1.1%		25.0%		51.0%	
% Agriculture	12.0%		32.0%		26.1%		13.0%		2.0%	
% Wetland	3.1%		1.0%		16.4%		12.0%		1.0%	
Discharge (m^3/s)	6944.0	3500.0	1064.0	359.0	70.0	12.0	11.0	0.1	1.0	1.0
Residence time (d)	2	3	210	NA	66	190	17	55	1	1
River loading (% of total C inputs)	79%		4%		14%		9%		1%	
DOC (μM)	160.0	114.1	213.3	240.0	1700.0	2391.7	594.0	477.0	630.0	620.0
DON (μM)	NA	NA	23.0	22.0	59.0	208.0	26.0	25.0	40.0	40.0
DIN (μM)	10.0	NA	93.0	77.0	3.6	NA	11.0	1.1	100.0	100.0
POC (μM)	50.0	16.7	81.7	67.5	61.0	91.0	37.0	68.0	NA	NA
PON (μM)	6.3	2.3	13.0	14.0	3.7	7.9	4.0	8.8	NA	NA
DOC:N (atom ratio)	NA	NA	9.3	10.9	28.8	11.5	17.0	19.0	15.5	15.5
POC:N (atom ratio)	8.0	7.3	6.3	4.8	16.5	11.5	11.0	7.8	NA	NA
Chl (μg/l)	7.5	NA	9.6	6.0	2.0	NA	2.8	NA	NA	NA

Notes: NA – not applicable or data not available; Columbia River land areas do not sum to 100%, remaining 33.8% comprised of grazing/grass land; residence time refers to the estuary; river loading refers tocontribution of organic C from watershed sources relative to autochthonous sources such as tidal wetlands and benthic and pelagic algae.

Table 2. Particulate geochemical data for riverine sites. Childs River groundwater sample did not contain particulates. See text for explanation and key to symbols.

Variable	Parker	Satilla	Susquehanna	Columbia
SPM (mg/l)	3.3	7.6	3.5	5.7
C total (mg/l)	0.82	1.26		0.20
C/N total (atoms)	7.8	13.3		7.3
C fumed (mg/l)		1.09	1.13	
C/N fumed (atoms)		11.1	6.9	
C/SPM (%)	25.0	16.6		3.5
OC/SPM (%)		14.3	32.1	
Lignin (mg/gC)	4.6	26.9	1.8	15.4
Lignin (mg/gSPM)	1.15	3.85	0.59	0.53
C N	0.200	0.074	0.210	0.080
S N	1.320	0.795	2.067	0.418
Vac/Val	0.297	0.465	0.291	0.292

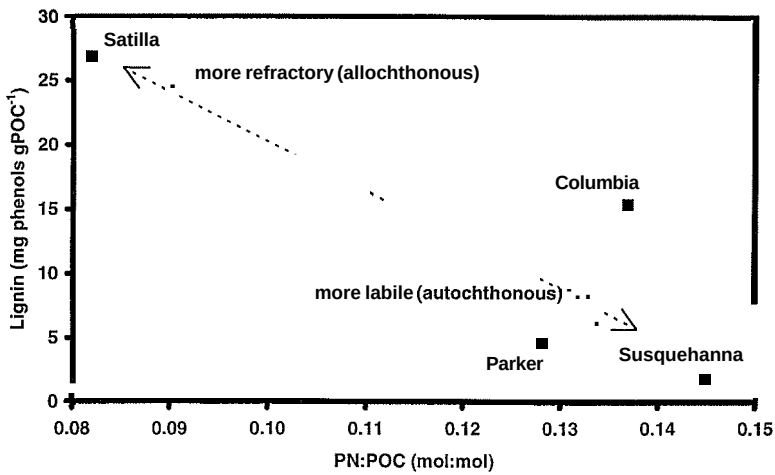

Figure 3. General trend in lability and provenance of particulate organic matter as assessed by lignin and nitrogen content.

increasingly enriched in angiosperm tissue in the sequence Satilla, Parker, and Susquehanna Rivers. This is a reasonable sequence, given the relative abundance of forest types within the various watersheds. The Parker and Susquehanna appear to be relatively enriched in non-woody material, possibly due to the extensive areas of freshwater marsh in the watershed.

Another phenolic parameter can be used to gauge the diagenetic state of the vascular-plant derived POM. Fungal degradatioh is known to enhance the relative abundance of vanillic acid (Vac) to vanillin (Val) in decaying wood

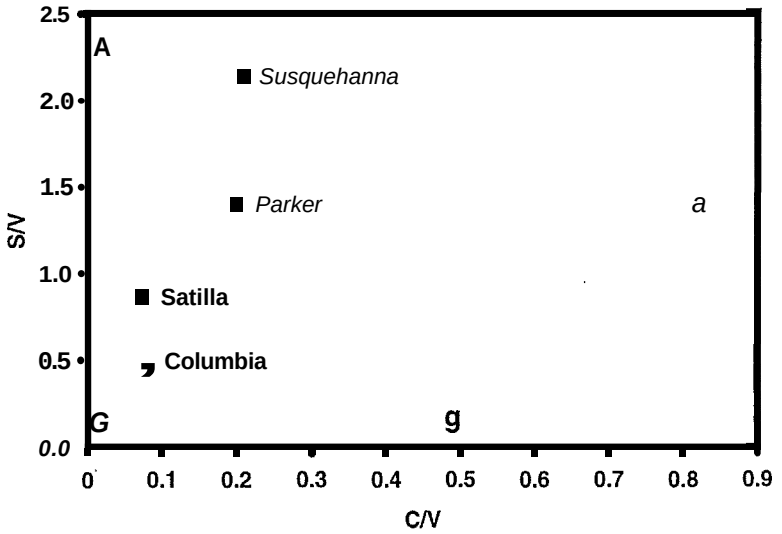

Figure 4. Ratios of lignin phenols: V – vanillyl phenols, S – syringyl phenols, C – cinnamyl phenols. Also shown are approximate end member compositions from Ertel and Hedges (1985) of A – angiosperm wood, a – angiosperm non-woody tissue, G – gymnosperm wood, and g – gymnosperm non-woody tissue.

(Hedges et al. 1988). The ratio of these two phenols (Table 2) in the river samples indicates that the Parker, Susquehanna, and Columbia contain relatively fresh plant material, while the plant debris in the Satilla is substantially more degraded.

Ultrafiltration

Field DOC concentrations ranged from 114 μM to 1884 μM between the five freshwater input sites. The concentration factor during the ultrafiltration procedure varied from 30X for high-DOC, Satilla River water to 125X for low-DOC, Columbia River water (Table 3). The amount of the initial DOC retained in the >1000 dalton fraction ranged from 39–90% being highest in the Satilla and lowest in the Susquehanna River. Low recovery in the Susquehanna is likely due to destruction of DOC by SO_4^{2-} that was concentrated during improper ultrafiltration. The pH of the concentrated Susquehanna water was extremely low. We therefore exclude the Susquehanna from further analysis of DOC composition.

Dissolved organic matter composition

The molar H/C ratios of the Columbia, Parker, Childs, and Satilla River samples are 1.21, 1.12, 1.07, and 0.97, respectively. Similarly, their respective

Table 3. Ultrafiltration results for DOC in river water from the five sites. Ultrafiltration was performed using 1000 D cartridges in Amicon or Millipore systems.

Site	Initial DOC (μM)	Initial volume (l)	Concentration factor	%DOC retained
Columbia River	114	292	125	62
Susquehanna River	240	95	53	39
Parker River	542	90	36	75
Childs Groundwater	623	68	30	76
Satilla River	1884	60	30	90

Table 4. Most probable structural distribution of organic matter isolated from intercomparison sites expressed as a percentage of total carbon mass.

Site	Aliphatic	Aromatic	Excess
Parker	54	30	16
Childs	49	33	18
Columbia	60.5	26	13.5
Susquehanna	–	–	–
Satilla	42.4	36	21.6

molar N/C ratios are 0.037, 0.032, 0.029, and 0.020. Thus both the molar WC and N/C ratios decrease systematically in the order Columbia > Parker > Childs > Satilla. The molar O/C ratios of these four samples are not as accurately known, because direct measurements of oxygen were not in exact agreement with the oxygen content as calculated by difference. Using the average of these two estimates of oxygen, the respective molar O/C ratios of the four samples are 0.60, 0.71, 1.04, and 0.76.

Analytical constraints calculations yielded C_{al}, $C_{}$ and C_{xs} values for the four samples in this study (Table 4). The "excess" carbon was assumed to consist mainly of carbonyl-containing moieties (aldehydes, ketones, esters, amides, etc.). When carbon distribution is expressed as the mole fraction of non-carboxyl carbon, the Columbia, Parker, Childs, and Satilla samples are predicted to contain 60.5, 54, 49, and 42.4 percent C_{al}, 26, 30, 33, and 36 percent C_{ar}, and 13.5, 16, 18, and 21.6 percent C_{xs}, respectively. These predicted carbon distributions lie generally in the predicted range for freshwater fulvic acids.

Sun et al. (1997) have recently shown that the bioavailability of dissolved organic matter (mg of bacterial biomass per mg DOC in a three-day growth experiment) is correlated strongly with its elemental composition. Elemental composition accounted for 93% of the variance in bacterial growth in 20

Table 5. Degree of reduction of DOM based on elemental composition and equation (5). Equations (6) and (7) were used to estimate bacterial yield and growth rate (d^{-1}) from the degree of reduction, respectively.

Site	H/C	O/C	N/C	S/C	Deg. reduction	Proj. yield	Proj. growth
Parker	1.11	0.71	0.032	0.028	3.77	0.60	2.56
Childs	1.06	1.04	0.029	0.132	3.69	0.59	2.45
Columbia	1.20	0.60	0.037 .	0.020	4.00	0.64	2.88
Susquehanna	2.33	2.13	0.055	0.430	4.492	0.73	3.63
Satilla	0.97	0.76	0.020	0.015	3.48	0.55	2.18

samples. Significantly, growth was positively correlated with WC and with N/C, but was negatively correlated with O/C. In the present study, it might thus be anticipated that bioavailability will decrease in the sequence Columbia > Parker > Childs > Satilla. Sun et al. (1997) further observed that variations in bioavailability along a river continuum correlate strongly with the C_{al} content of the dissolved organic matter. If their approach is applied to the four independent samples in the present study, the relative bioavailability of the samples are once again predicted to be in the order Columbia > Parker > Childs > Satilla.

Degree of substrate reduction

The degree of reduction of the DOM ranged from about 3.5 to 4 with the following trend: Satilla < Childs < Parker < Columbia River. Projected bacterial C yields (Table 5) varied from 55% to 64%. Projected specific growth rates (Table 5) exhibited the same trend as for yield, being highest in the Columbia River and lowest in the Satilla River.

Potential extracellular enzymatic activity

Potential enzyme activity varied broadly across the estuarine systems. Enzyme activity was found to be significantly correlated with DOC concentration for several enzymes (positive correlation with DOC: xylosidase, $p < 0.01$; negative correlations with DOC: leucineaminopeptidase, $p < 0.05$; esterase, $p < 0.05$; guanidinobenzoate, $p < 0.01$). Most of these correlations were driven by the high DOC concentration of the Satilla River.

Because measurement of a suite of covarying enzymes represents a multivariate approach, the EEA data were analyzed with a principal component analysis (PCA) approach. Normalized activities yielded 8 PCs, with the first two accounting for 62% of the total variability. A two-dimensional plot of

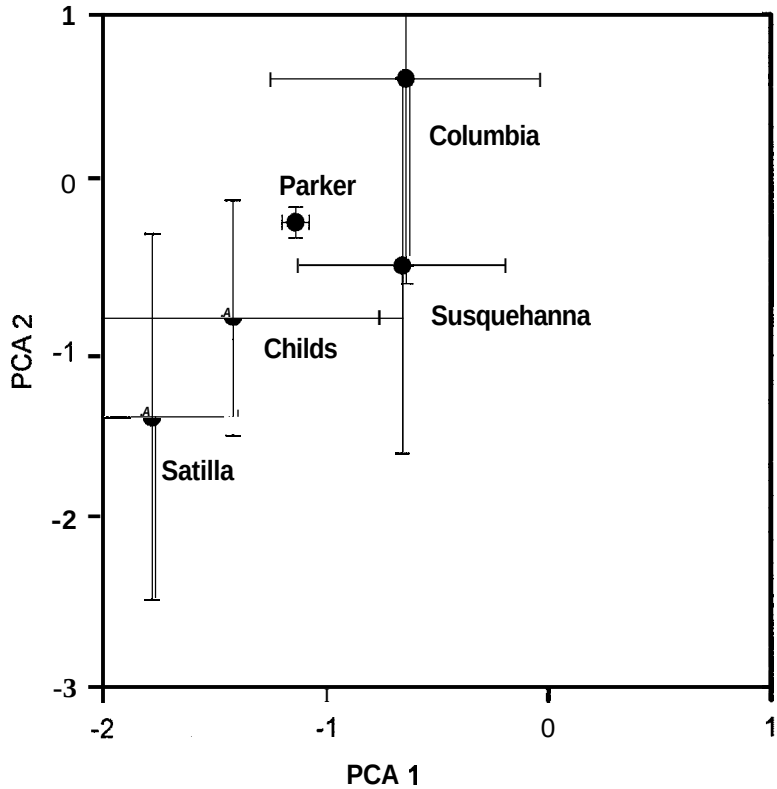

Figure 5. Results of a principle components analysis of extracellular enzymatic activity for the study sites.

the first two PCs resulted in a linear arrangement of the five sites (Figure 5). Samples in the upper right appear to have enzyme activities indicative of relatively higher standing stocks of microbial substrates susceptible to degradation by peptidases and esterases (high leu-aminopeptidase and esterase activity). Samples near the lower left have enzyme activities consistent with a relatively greater dependence on plant complex polysaccharides and amino sugars such as chitin (high xylosidase and N-acetyl-β-D-glucosaminidase). To simplify comparisons with other variables describing DOC composition, a univariate "enzyme index" was calculated. This index is simply the scalar distance of each sample from the Satilla (arbitrarily 0,0).

Bioassays

Bacterial numbers at the outset of the experiment ranged from 0.04 x 10^6 to 0.05 x 10^6 ml^{-1}. At the end of the incubation cells reached densities between

0.67 x 10^6 and 1.14 x 10^6 ml^{-1}. Specific growth rates were lowest for the Childs River (1.35 d^{-1}) and highest in Parker River water (1.66 d^{-1}). These* rates are similar to those observed for estuarine bacteria in the Parker River (Wright & Coffin 1987).

Microbial growth rates normalized for the initial DOC concentration (357–552 μM) ranged from 1.31 ug mg^{-1} d^{-1} DOC for the Satilla River bioassay to 1.71 ug mg^{-1} d^{-1} DOC for the Parker River. They were intermediate for the Childs River (1.50) and the Columbia River (1.64 ug mg^{-1} d^{-1} DOC). These growth rates are within the lower range of rates observed for the Ogeechee River in coastal Georgia (Leff & Meyer 1991; Sun et al. 1997). Lower growth rates in our experiment may reflect a prolonged lag period during which the microbial community adapted to the altered salinity regime of the bioassay. Leff and Meyer used a freshwater stock innoculum for river bioassays as opposed to the natural assemblage of estuarine microbes used in our bioassays.

Using bacterial growth rate as a measure of the bioavailability of organic matter used in the bioassays, these results suggest a range in bioavailability of the organic matter in the river systems we ingestigated. Bioavailability is highest in the Parker and Columbia Rivers, intermediate in the groundwater of the Childs River system and lowest in the Satilla River.

Discussion

There were major differences in the concentration and composition of particulate organic matter in river water entering four of the estuarine systems. Quantitatively, the Columbia was low in POM, while the other sites were relatively rich in POM. The Satilla appears to contain relatively refractory DOM that is high in lignin and low in N, while the particulate material at the other three sites appears to be more labile (lower lignin and higher N) (Figure 3). The Susquehanna, Columbia and Parker Rivers are highly dammed or geomorphologically ponded and much of the particulate matter entering the estuaries likely consists of phytodetritus originating from phytoplankton production in the reservoirs. In the Parker River, phytodetritus (measured as chlorophyll content) comprises up to 50% of the POC load during low flow months of the year (unpublished LMER database).

The composition, magnitude and presumably quality of riverborne POM is highly variable on spatial as well as seasonal and interannual scales (Findlay et al. 1991; Pocklington & Tan 1987). Here we present comparative data for only a single low-flow sample. In the Columbia River, for example, POC concentrations vary over an order of magnitude (Tables 1 and 2). Our study is but a snapshot of the nature of DOM in the various systems. Thus we express

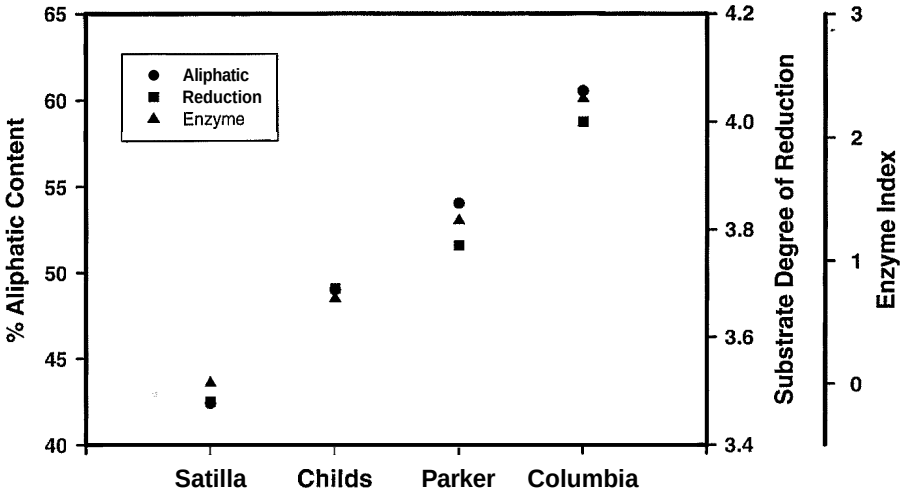

Figure 6. The relations between organic matter aliphatic content, the degree of substrate reduction and enzyme index (see text for explanation).

caution in interpreting these results too broadly. Nevertheless, we assume that relations between composition and bioavailability will hold for all conditions.

The three descriptors of dissolved organic matter composition (aliphatic1 aromatic content, degree of substrate reduction and enzyme activity) portray similar patterns of organic matter quality (Figure 6). Each model predicts a range of dissolved organic matter lability with the Satilla and Columbia Rivers as high and low quality end-members, respectively (Figure 6). The Satilla River has a high aromatic content but low aliphatic content and low degree of substrate reduction. The enzyme assays indicate that complex carbohydrates (likely originating in plant structural materials) represent a greater fraction of the DOC pool being degraded by bacteria in that system. In contrast, the compounds present in the Columbia River samples have a high aliphatic contribution and high degree of reduction inducing high levels of esterase and peptidase activity. The positive correlation between aliphatic content, the degree of substrate reduction and the enzyme index (scalar distance Figure 5) suggests that DOC pools relatively enriched with proteinaceous compounds are of higher quality than pools dominated by dissolved carbohydrates derived from complex plant polysaccharides or amino sugars.

Bioassays confirmed patterns of dissolved organic matter lability predicted by the chemical models. Regression analyses between chemical descriptors and bacterial growth normalized to initial DOC concentration indicated that 67% to 75% (all $p \leq 0.05$) of the variation in growth could be explained by differences in organic matter composition (Figure 7). Bacterial growth

was positively correlated with aliphatic content, enzyme index and degree of substrate reduction. Growth was negatively correlated with aromatic content: Bacterial growth for the Parker River organic matter was high relative to the predicted lability for all 3 indices. This may be due to a shorter lag period prior to growth for the microbial community in the Parker River medium than in the other river media, as a Parker River inoculum was used for all the bioassays. We did not test this hypothesis however.

These results also agree with patterns of lability observed in other studies. In the Ogeechee River, a system similar to the Satilla River, Sun et al. (1997) observed a strong relationship between chemical composition and bioavailability of DOM. Both organic matter composition and bioavailability changed with distance downstream, becoming progressively less aliphatic, more aromatic and less labile. Likewise, Moran and Hodson (1994) found vascular plant influence (which is related to aromaticity) to be negatively correlated with biological availability for DOM from three coastal environments. In our study, Columbia River DOM, which has a high aliphatic content, likely contains a large fraction of "fresh" DOM, presumably due to phytoplankton production in upstream reservoirs. Satilla River DOM, however, has the highest aromatic content, is the most depleted in aliphatic carbon and has the lowest bioavailability. Old, diagenetically altered compounds, including humic and fulvic acids, are known to be major components of the DOM in blackwater systems like the Satilla (Beck et al. 1974). Fresh, aliphatic carbon sources are apparently masked by a high background of resistant, vascular plant-derived organic matter. Enzyme assays likewise indicate that the DOM from the Satilla is dominated by dissolved carbohydrates derived from complex plant polysaccharides.

A strong dependence between bacterial yield and the degree of substrate reduction has also been demonstrated using a simulation modeling approach for a range of specific substrates (Vallino et al. 1996). Substrate reduction incorporates information on the elemental content of the organic matter and helps explain the microbial response to variations in the degree of oxidation of the organic matter and whether exogenous N is required (at cost) to maximize growth. Simulations demonstrated that growth can not always be explained by single descriptors, such as C:N ratio of the substrate, since several constraints are often active simultaneously. While applicable for specific compounds, the relationship between bacterial yield and substrate reduction had not been tested previously for mixed bacterial populations growing on bulk DOM. This intercomparison study suggests that the relationship holds in these circumstances. In addition, our study suggests that it is not necessary to measure substrate reduction of the "labile pool" in isolation from the bulk DOM, as the degree of substrate reduction of bulk DOM was a good predictor

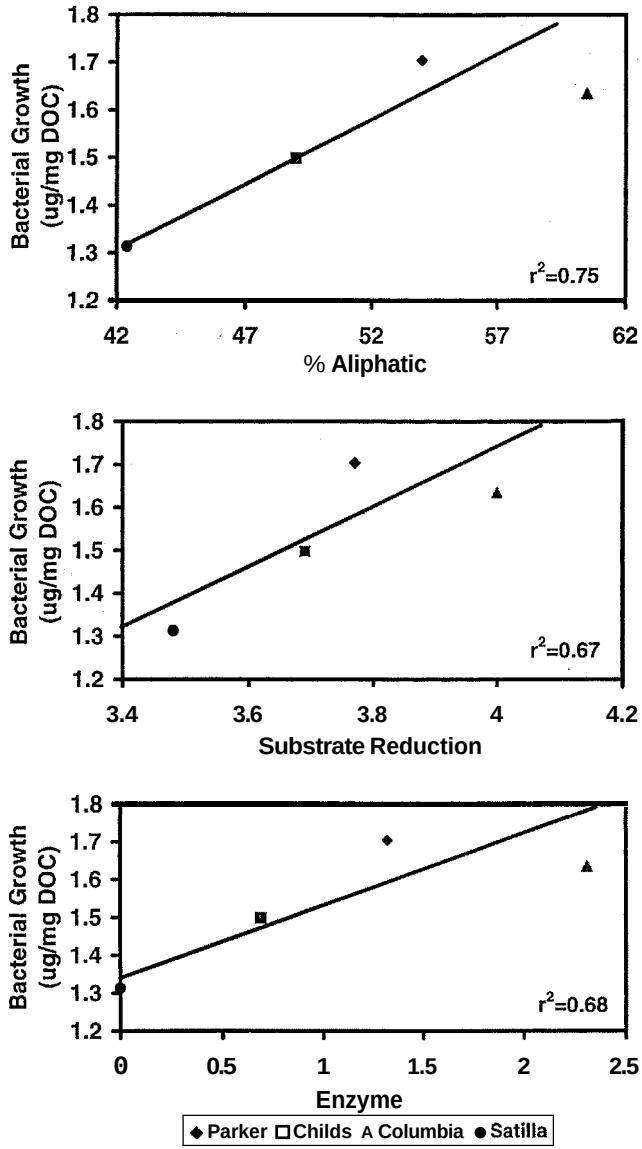

Figure 7. Relations between bacterial growth and organic matter composition as measured by aliphatic content, degree of substrate reduction and enzyme index. Results of linear regression analyses of the relation between variables are shown in each panel.

of bioavailability (r^2 = 0.67). We note substantial differences between the magnitude of observed and predicted growth rates however, with observed rates being about 35 to 45% lower than predicted for all sites.

Ranking of sites based on organic matter quality was similar for both particulate and dissolved components, with sites ordered as: Satilla < Parker < Columbia < Susquehanna for the particulate compounds and Satilla < Childs < Parker < Columbia for dissolved compounds. Lignin content, C/N ratios and levels of inducible enzyme activity indicate a primary importance of allochthonous, terrestrial sources of organic matter in the Satilla but autochthonous sources for the Columbia and Susquehanna Rivers. A range in bioavailability is suggested by acid/aldehyde ratios, aliphaticlaromatic composition and degree of substrate reduction, with the Satilla and Susquehanna or Columbia Rivers again being end-members of the gradient. There is a positive correlation between C/N ratios in the dissolved and particulate fractions, although C/N ratios for the dissolved component are substantially elevated relative to those for the particulates. While the explanation for this is not clear, nitrogen depleted DOM relative to POM has also been observed in the marine environment (Hopkinson et al. 1997).

The similarity in patterns for dissolved and particulate matter suggests that the two components share common sources and that the two pools are dynamically linked. This is as expected for any aquatic system, whether it be driven by allochthonous or autochthonous organic matter sources or dominated by grazing or detrital food webs. Dissolved and particulate components are produced directly (e.g., phytoplankton excretion, leaf litter leaching, and organism death) and indirectly (e.g., solubilization of POM by microbial exoenzymes and flocculation of DOM into particulates) from the same sources. While similarities in characteristics exist for the dissolved and particulate pools, it remains to be seen whether the rates of degradation and incorporation into microbial biomass are also similar.

Conclusions

We have identified several easily measured characteristics of the bulk dissolved organic matter pool that give an indication of the ability of the organic matter to support microbial growth in coastal estuaries. We found major differences in the nature of the organic matter between river systems ranging from aliphatic-rich, highly reduced organic matter in the Columbia River to aromatic-rich, more oxidized organic matter in the Satilla River. As seen by differences in bacterial growth between river systems, these organic matter characteristics can be directly related to rates of microbial processing.

Our ultimate goal is to develop a predictive understanding of the rates and extent of biological utilization and the fate of terrestrial organic matter in the coastal zone, so that we can evaluate the ecological effect in estuaries of changes in land cover and use in coastal watersheds. Our initial efforts have

focused on the ability of various types of organic matter to support microbial growth. The next logical step is to measure substrate degradation directly over a range of DOM source types. We still need to determine respiration rates and loss of DOC to estimate microbial growth efficiency, and ultimately gauge organic matter export to the continental shelf. By comparing degradation times to estuarine water transit times, we can determine the fractional utilization of riverine organic matter in estuaries.

We have also found patterns of quality for the particulate organic matter component entering estuaries from rivers that are similar to those of DOM. In the future we will want to develop a predictive understanding of the fate of this material when it enters estuaries. There are fundamental differences in the behavior of POM relative to DOM however, because the vast majority of particulate inputs settle out and are metabolized in the benthic environment where the residence time is orders of magnitude longer than in the water column.

While we have found basic differences in organic matter quality and bioavailability for the small subset of temperate rivers studied in this intercomparison, we are uncertain as to the generality of these patterns. Patterns will have to be confirmed through more inclusive analysis of other temperate zone rivers in not only North America but in other continents of the world as well. Lastly, we will be interested in comparing temperate zone patterns to patterns for polar and tropical regions, as these are the regions where land use change and global climate change are most likely to influence production and decomposition of terrestrial systems.

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