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Bacterial Degradation of Dissolved Organic Matter from Two Northern Michigan Streams

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This study used high-pressure size exclusion chromatography (HPSEC) to measure the changes in molecular weight distributions of dissolved organic matter (DOM) of two Northern Michigan streams following inoculation with bacterial concentrates from the same locations. During the initial 12 h of the experiment, weight average molecular weight (M_w) of DOM decreased, as high molecular weight components were lost from solution. After 12 h, the M_w of DOM increased, primarily because of a loss of intermediate to lower molecular weight components. Leucine incorporation showed little or no bacterial metabolism during the first 12 h, but metabolism increased substantially after 12 h. The initial loss of high molecular weight components during the period of little or no bacterial metabolism suggests preferential adsorption of these components to the bacterial surfaces, perhaps followed by metabolism. This suggested interpretation is consistent with previous observations of preferential adsorption of higher molecular weight components to viable but non-metabolizing *Bacillus subtilis* and to mineral surfaces. The latter loss of lower molecular weight components was most likely due to bacterial metabolism of the DOM, which is consistent with previous observations that lower molecular weight components are more biodegradable. The HPSEC technique uses 254 nm wavelength for detection and focuses primarily on humic- and fulvic-type components rather than low molecular weight organic molecules, such as carbohydrates. Thus, results confirmed that humic/fulvic components are biodegradable, but did not address other DOM components.

Keywords Dissolved organic matter, biodegradation, HPSEC

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INTRODUCTION

Dissolved organic matter (DOM) is ubiquitous in terrestrial and aquatic ecosystems and is an important source of carbon and other nutrients for aquatic microorganisms. DOM therefore has a substantial impact on aquatic food webs and nutrient dynamics (e.g., Thurman 1985; Wetzel et al. 1995; Azam 1998). DOM also plays an important role in a wide range of biogeochemical reactions, such as mineral growth and dissolution (e.g., Jardine et al. 1989; Zhou et al. 2001), and uptake and mobility of metals, radionuclides, and hydrophobic organic compounds (e.g., Sposito 1986; Chin et al. 1997; Tipping 1998). Despite its abundance and importance in aquatic systems, we do not yet understand quantitatively how the chemical composition of DOM affects its bioavailability, nor how bacterial degradation affects DOM properties and reactivity.

DOM is a heterogeneous mixture of organic compound, with molecular weights ranging from 100–100,000 Da (Thurman 1985). In surface-water ecosystems, DOM is composed of two broad fractions: the readily utilizable DOM or "labile pool" versus the residue that is not as easily utilized, termed the "refractory pool" (Ogura 1975; Geller 1986). The labile pool consists of the low molecular weight (LMW) components—sugars, amino acids, peptides and other simple compounds (Thurman 1985)—and generally accounts for <20% of the total DOM. Although the labile pool has been shown to support the majority of bacterial secondary production (Moran and Hodson 1990; Covert

and Moran 2001), it turns over rapidly—generally within hours to days. Bacterial utilization of labile DOM is restricted due to limitations of the cell membrane transport system, as this system can only accommodate uptake of substrates smaller than 600 amu (Weiss et al. 1991). Thus, DOM uptake is controlled by the availability of LMW substrates.

The refractory pool of relatively high molecular weight (HMW) humic and fulvic acids is more abundant, but turns over more slowly than the labile pool. Even though it has been considered relatively unimportant as a substrate for bacterial growth (Moran and Hodson 1990), at least some components of the refractory pool may be altered photochemically to produce more bioavailable compounds (e.g., Lindell et al. 1995; Wetzel et al. 1995; Moran et al. 2000). In addition, several studies have suggested that humic substances are more important components of the biodegradable DOM pool than previously thought (e.g., Meyer et al. 1987; Amon and Benner 1996; Volk et al. 1997; Young et al. in press).

Several studies have focused on how the quantity of DOM affects microbial communities (e.g., Tranvik 1990; Leff and Meyer 1991; Sabater et al. 1993; Eiler et al. 2003; Young et al. in press). However, relatively few studies have investigated how DOM quality may influence its bioavailability (e.g., Leff and Meyer 1991; Leff 2000; Young et al. in press). Research to date has focused on determining how DOM bioavailability varies with fundamental properties such as elemental composition (e.g., Kroer 1993; Hunt et al. 2000), and with processes such as photochemical reactions (e.g., Lindell et al. 1995; Bertilsson and Tranvik 1998). Several authors have used tangential-flow ultrafiltration to examine how molecular size of DOM affects bioavailability. Using 1000 Da cutoff membranes to separate LMW (<1,000 Da) and HMW (>1,000 Da) fractions of DOM, Meyer et al. (1987) showed that bacterial growth and DOM consumed were greatest in LMW enriched samples. Amon and Benner (1996) used ultrafiltration and also found that bacterial efficiencies were consistently higher in LMW fractions than HMW fractions. Covert and Moran (2001) used ultrafiltration to show that the bacterial communities living on LMW fractions of DOM were different than those communities living on HMW fractions of DOM. Young et al. (in press) showed that DOM biodegradation was influenced by source-specific microbial species and DOM physicochemical characteristics.

The study reported herein used high-pressure size exclusion chromatography (HPSEC) to measure the changes in DOM molecular weight upon reaction with natural bacterial consortia collected from each of the same surface waters. The two study sites were chosen because they are located within 20 miles of each other, yet they have different DOM molecular weight characteristics as measured by HPSEC. HPSEC (high-pressure liquid chromatography) makes use of a size exclusion column to separate components according to molecular size, hence weight, and detects the DOM coming off the column with UV-Vis absorbance measurement. Here, absorbance was measured at a wavelength of 254 nm. This method is biased against low molecular weight components, particularly those with molecular weight <50 Da, and focuses primarily on light-absorbing compounds such as humic- and fulvic-type components. Nonetheless, our results shed light on ecosystem function and biogeochemical consequences because humic substances compose from 25% of the dissolved organic carbon concentration [DOC] in groundwaters to as much as 90% of [DOC] in wetlands (Thurman 1985). Previous studies (e.g., Chin et al. 1994; Hongve et al. 1996; Peuravuori and Philaja 1997) have used HPSEC to determine the weight average molecular weight (M_w) and number average molecular weight (M_n) of the humic components of DOM in surface and subsurface waters, and it is being used increasingly to study DOM reactivity (e.g., Chin et al. 1994; Chin et al. 1997; Namjesknik-Dejanovic et al. 2000; Chorover and Amistadi 2001; Zhou et al. 2001; Karthikeyan and Chorover 2002; Maurice et al. 2004).

In this study, HPSEC was used to observe changes in molecular weight distributions of DOM for 72 h after bacterial inoculation. Previous studies by our group showed that HMW components of DOM adsorbed preferentially to *Bacillus subtilis* (Frost et al. 2003; Maurice et al. 2004); this adsorption was reversible and came to equilibrium within 30 min (Maurice et al. 2004). Other studies have shown preferential adsorption of HMW components of DOM to Fe(III)(hydr)oxides and clays, although equilibrium was not attained until about 6 h (Meier et al. 1999; Namjesnik-Dejanovic et al. 2000; Zhou et al. 2001). Hence, we hypothesized that HMW DOM components would be removed preferentially from solution during the first few hours by adsorption to the bacterial surfaces and to any abiotic colloids that might be present. We further hypothesized that as the bacterial consortia began to undergo significant metabolism, at least some portion of the humic substances would be bioavailable—most likely, the LMW components.

MATERIALS AND METHODS

Site Description and Sampling

Surface water samples were collected from two streams located in northern Michigan (46° 13′ N, 89° 32′) (Figure 1). Two Mile Creek (TMC) and Nelson Creek (NLC) are located in the Ottawa National Forest. These sites have a fairly high [DOM]; TMC with 31.7 mg C L^{-1} and NLC with 35.3 mg C L^{-1} at the time of sampling. The geology of the study area consists of a thin layer of sedimentary rocks that are underlain by igneous and metamorphic Precambrian bedrock (Doonan and Hendrickson 1968). The surficial geology consists of young glacial deposits, and the drainage system is poorly developed. As much as 40% of the district is either open water or wetland.

NLC is a first-order stream and TMC a second-order stream. Both streams flow through and/or are surrounded by a number of wetlands, a likely source of their relatively high [DOC]. Their watersheds contain predominantly northern hardwood forest (e.g., *Acer saccharum* and*Fagus grandifolia*) in the uplands

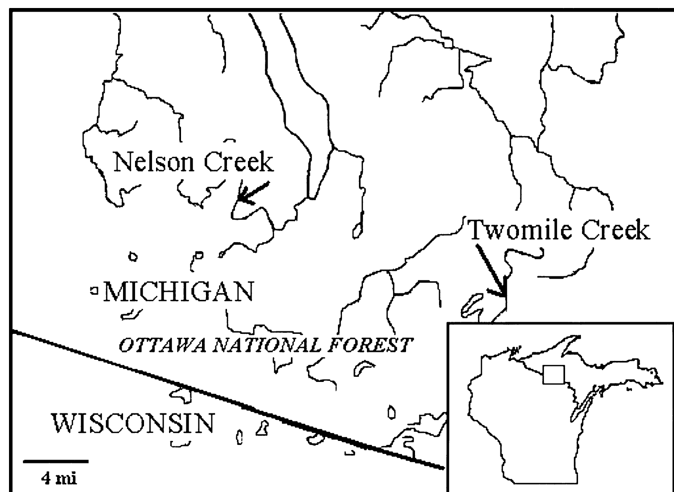

Figure 1. Location of the sampling sites.

with both deciduous and evergreen plant species in the extensive wetlands.

Sampling was conducted in mid-June of 2002. Water samples were collected using a polyethylene bottle attached to a pole and filtered on-site, using pre-combusted glass-fiber Whatman filters (GF/F) and a vacuum filtration system. The samples were stored in acid-washed and autoclaved glass bottles in the dark at 4°C for DOM preservation and to prevent photoreactions. Additional samples for the bacterial inoculate were first filtered through 1-μm Nuclepore filters to remove protozoa without removing all bacteria and were stored in the dark at ~22°C. The bacterial inoculate was later refiltered through 0.22-μm filters during inoculate preparation (see section below). Conductivity, pH, and water temperature were recorded in situ and inductively coupled plasma optical emissions spectroscopy (ICP-OES) was used to analyze inorganic constituent concentrations (Table 1). Within 48 h of collection, DOM samples were moved to a 4°C incubator and bacterial inoculates were prepared.

Bacterial Inoculates

Bacteria from each site were mixed with DOM from the same site. To do this, two bacterial inoculates were concentrated from sample water collected at TMC and NLC that had been filtered through 1-μm Nuclepore filters to remove protozoa. Because of this filtration procedure, this manuscript specifically deals with the <1 μm fraction of the microbial community. The inoculates were prepared by centrifuging 1.2 L of sample water using a Beckman J2-HS Centrifuge at 8,000 rpm for 30 min at room temperature (Figure 2). Pellets were resuspended in 0.22-μm filter-sterilized water from the native site, creating a 30-fold concentration of bacteria for each inoculum. Six replicate DOM samples from each site (386.64 mL) were inoculated with concentrated bacterial inoculum (13.36 mL) from its own native site (400 mL each) to yield approximately in situ population densities of bacteria. Previous data showed that a 100% concentration of inoculate would give optimal results for this procedure (data not shown). In order to prevent photochemical changes in the DOM, incubations occurred in amber glass bottles and were placed in room temperature on a shaker table at 50 rpm.

Table 1
Physical and chemical characteristics of TMC and NLC. Numbers in parentheses represent standard deviations

	TMC	NLC
DOM characteristics		
DOC (mg C L^{-1})	31.7	35.3
Abs. 280 nm	0.95	1.10
ε_{280} (L mol C^{-1} cm^{-1})	361	374
DOM molecular weight distributions		
M_w^a (Da)	1699	2034
M_n^b (Da)	764	886
ρ^c	2.28	2.43
Inorganic constituents		
Al (mg L^{-1})	0.015 (0.0003)	0.417 (0.0057)
Fe (mg L^{-1})	0.019 (0.0023)	1.46 (0.016)
Na (mg L^{-1})	2.76 (0.022)	3.53 (0.025)
K (mg L^{-1})	0.565 (0.0056)	0.304 (0.0018)
Ca (mg L^{-1})	18.8 (0.31)	5.72 (0.098)
Mg (mg L^{-1})	6.47 (0.044)	1.71 (0.025)
In situ measurements		
pH	6.9	5.8
Conductivity (μS/cm)	49.2	24.3
Temperature (°C)	11.34	17.5

[a] M_w is the weight-average molecular weight.
[b] M_n is the number-average molecular weight.
[c] ρ is the polydispersity, M_w/M_n.

Microbial Growth and Counts

Heterotrophic bacterial biomass production was determined using [2,3,4,5-^{3}H] L-leucine incorporation (Kirchman 1993), using ^{3}H of specific activity of either 73 Ci $mmol^{-1}$ or 171 Ci $mmol^{-1}$. Five-mL aliquots of inoculated sample were removed from each replicate at 0, 1, 3, 8, 12, 18, 24, 36, 48, 60, and 72 h and were used in analysis. A preliminary experiment indicated that a 1-hour incubation with ^{3}H-Leu yielded optimal results (data not shown). Counts per min (CPM) were measured using a Beckman LS 5000 TD and TA Liquid Scintillation System. Disintegrations per minute (DPM) were corrected for quench using an experimentally determined quench curve. DPMs were converted to microbial growth rates using a conversion factor of 3.1 kg C mol^{-1} (Kirchman 1993).

A 72-h time period was chosen because in a previous 308-hour pilot experiment, bacterial growth peaked at 24 h, declined at 72 hours, and began a second cycle of growth thereafter. The

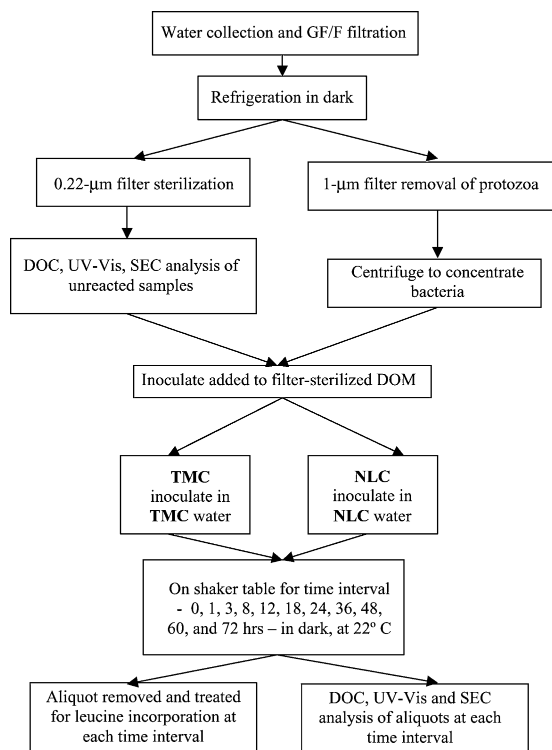

Figure 2. Schematic illustration of the experimental method.

second increase in growth was likely due to turnover of the microbial population, cell lysis, and/or production of labile DOM. Thus, to examine only the first growth cycle, the experiment was concluded at 72 h.

At each time interval, a second 5-mL aliquot was removed from each replicate and GF/F-filtered for DOC analysis. In addition, 5-mL samples were removed at 1 and 72 h and preserved in 37% formaldehyde for initial and final cell density enumeration by direct counts (Hobbie et al. 1977). Two mL of each replicate were incubated in the dark with 0.2 mL 0.1% acridine orange (AO) fluorescent stain for 2 min, and then filtered onto a 0.22-μm black Nucleopore filter for direct count analysis. At least 200 cells were counted per slide using an Olympus BH-2 epifluorescence microscope at 1,000X magnification.

DOC Analysis

Nonpurgeable dissolved organic carbon ([DOC]) was measured by combustion on a Shimadzu TOC5000 organic carbon analyzer with an autosampler. Prior to analysis, samples were acidified with distilled nitric acid and purged for 2 minutes with CO_2-free air to remove inorganic C. The samples were introduced via direct aqueous injection into a platinum-on-alumina catalyst heated to 680°C in a quartz reaction tube. Potassium hydrogen phthalate with a concentration range from 0–40 mg L^{-1} was used to prepare standards. Samples were run in replicate until the analytical uncertainty, as measured by relative standard deviation, was <2%.

High-Pressure Size Exclusion Chromatography (HPSEC)

The molecular weight distributions of DOM were measured on aliquots taken immediately following bacterial inoculation and at every sampled time interval. The HPSEC method of Chin et al. (1994), as revised by Zhou et al. (2000), was used. Briefly, the mobile phase consisted of degassed 0.1 M NaCl buffered with 0.002 M KH_2PO_4 and 0.002 M K_2HPO_4. A Waters Protein-Pak 125 modified silica column (30-cm long, 7.8-mm diameter) was used with the Waters HPSEC instrumentation (Waters Associates, Milford, MA). Sodium polystyrene sulfonate (PSS) standards (from Polysciences, Inc., PA) were used as molecular weight standards, with nominal weights of 18K, 8K, 4.6K, and 1.8K and manufacturer certified molecular weights (in Daltons) of 15,200, 6,530, 4,950, and 1,430, respectively. Acetone (58 daltons, HPLC grade) also was used as a low molecular weight standard. All standards were used with a concentration of 100 mg L^{-1}. All standards and samples were detected at 254 nm. It should be noted that small molecules are often invisible to the UV-Vis detector used in HPSEC determinations (e.g., O'Loughlin and Chin 2001); therefore, the average molecular weights reported herein represent primarily the humic and fulvic acids and are biased against the LMW components (particularly, <50 Da) of the DOM pool.

Molecular weight distributions were determined using the equations presented in Chin et al. (1994) and the chromatograph processing procedures described by Zhou et al. (2000). Weight-average molecular weight (M_w), number average molecular weight (M_n), and polydispersity (ρ) were determined using the following equations (Chin et al. 1994):

$$M_w = \frac{\sum_{i=1}^{N} w_i MW_i}{\sum_{i=1}^{N} w_i} = \frac{\sum_{i=1}^{N} f_i MW_i^2}{\sum_{i=1}^{N} f_i MW_i} \quad [1]$$

$$M_n = \frac{\sum_{i=1}^{N} f_i MW_i}{\sum_{i=1}^{N} f_i} \quad [2]$$

$$\rho = \frac{M_w}{M_n} \quad [3]$$

where f_i is the frequency of a characteristic molecular weight MW_i; w_i is the weight of a molecule with characteristic molecular weight of MW_i; MW_i is the characteristic molecular weight of i fraction; N is the number of fractions of molecules according to molecular weight. M_w is the weight of the molecule to which the average atom belongs, M_n is the weight of the average molecule in a mixture, and ρ is M_w/M_n. For a pure substance with a single molecular weight, M_n is equal to M_w. For a mixture of molecules, $M_n < M_w$ and $\rho > 1$.

Ultraviolet Visible Absorption Analysis (UV-Vis)

The absorbance spectra of the samples were analyzed at 280 nm using a double-beam Varian Cary III scanning spectrophotometer. One-cm quartz cells were used with Milli-Q water as a reference. Absorptivites were normalized per mol C (ε_{280}), which were calculated based on absorbance at 280 nm

and DOM measurements ($\varepsilon_{280} = A_{280}$ $[DOM]^{-1}$, with units L mol C^{-1} cm^{-1}) of organic matter in solution (e.g., Traina et al. 1990; Chin et al. 1994). This technique is useful because 280 nm is the region of π to π^* transitions, so that absorbance at this wavelength is sensitive to the aromatic nature of the DOM.

RESULTS AND DISCUSSION

Site Chemical Characteristics

Both sites were organic-rich, with more than 30 mg C L^{-1}; NLC had slightly greater [DOC] than did TMC (Table 1). The pH values of the sites were circumneutral to slightly acidic. Conductivity, pH, and the concentrations of dissolved Ca, Mg, and K were higher in TMC, though NLC had significantly higher Fe and Al concentrations. The high Fe and Al concentrations were most likely due to metal complexation by DOM. The average molecular weight (M_w) of the DOM in raw surface water samples was several hundred Daltons lower in TMC (1,699 Da) than in NLC (2,034 Da). NLC, which had the higher [DOC], also had higher M_w, M_n, and ε_{280} values. The ε_{280} values of the two sites were comparable, suggesting similar DOM aromaticities, although TMC was slightly less aromatic than NLC DOM.

The DOM properties at these two sites can be compared with the DOM properties at the Suwannee River (SR) (GA), which is the collection site for the International Humic Substances Society (IHSS) humic and fulvic and surface water standards. Chin et al. (1994) reported that SR whole-water sample DOM had M_w of 2190 Da, M_n of 1330 Da, and ε_{280} of 509 L mol C^{-1} cm^{-1}. The [DOC] values at NLC and TMC are similar to those commonly observed at SR (Meier et al. 1999). NLC M_w is similar to SR, although M_n is considerably lower, indicating a greater polydispersity at NLC. The M_w and M_n at TMC are both considerably lower than at SR. The ε_{280} values at both NLC and TMC are considerably lower than at SR, indicating that the sites used in our study have considerably less aromatic DOM than the SR. There have been relatively few studies of whole-water DOM properties from other sites. Meier et al. (1999) reported values for the Great Dismal Swamp (VA). The [DOC] was 41.5 mg C L^{-1}, M_w was 1,890 Da, and ε_{280} was 320 L mol C^{-1} cm^{-1}. Maurice et al. (2002) reported values for McDonalds Branch (NJ), a small wetland stream. The [DOC] was 23.8 mg C L^{-1}, M_w was 2056 Da, M_n was 1,184 Da, and ε_{280} was 410 L mol C^{-1} cm^{-1}. Hence, NLC and TMC have DOM M_w within the range of other DOM-rich surface waters. The ε_{280}values are comparable to other sites with the exception of the SR, which appears to be particularly rich in aromatic components.

Bacterial Productivity

Bacterial productivity increased slowly to Hour 12, quickly increased to a peak at Hour 24, and then declined throughout the rest of the experiment (Figure 3). Although bacterial metabolism in both TMC and NLC were essentially the same throughout the first 8 h, after Hour 8, TMC metabolism increased at a greater rate and peaked at a higher level of metabolism than did NLC.

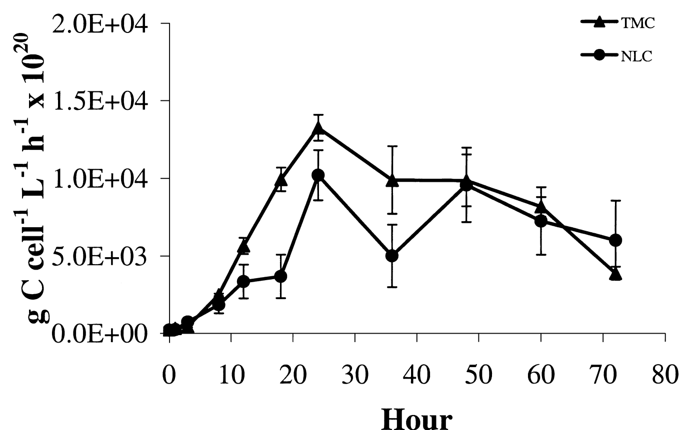

Figure 3. Mean productivity, as determined by leucine incorporation. TMC inoculate in TMC DOM (▲) and NLC inoculate in NLC DOM (●). Error bars represent +/− 1 SE (n = 3).

Bacterial cell counts increased by 400% for TMC and by 660% for NLC during the course of the experiment (Table 2).

Changes in DOM Molecular Weight Distributions

Immediately after inoculation (Hour = 0), the M_w was slightly higher than its initial, noninoculated value in both sites (1,739 vs. 1,699, 2,157 vs. 2,034 for TMC and NLC, respectively). The M_w of NLC slightly increased at Hour 1, though TMC began a sharp decrease at that hour (Figure 4). The M_w of both treatments decreased until Hour 12, where they both reached a minimum. The M_w in TMC declined 20% from its initial value of 1,699 Da to its Hour 12 value of 1,353 Da; M_w in NLC declined 19% from 2034 Da to 1,640 Da. The M_w of both treatments began to increase after Hour 12. M_w in TMC increased until Hour 24, when it reached 1,593 Da. From there, it hovered between 1,480 and 1,530 Da. M_w in NLC showed the same pattern, with a low of 1,521 at Hour 12, and then a slow increase to Hour 36, after which it decreased slowly to 1,700 Da.

Potential Controls of DOM Changes upon Interaction with Bacteria

The initial increase in M_w was likely the result of release of some high molecular-weight components upon mixing of the particles with the DOM solution. We believe that the decrease in

Table 2
Initial and final cell counts of inoculated TMC and NLC samples

	Average cells L^{-1}	
	TMC	NLC
Hour 1	2.97E + 10	1.58E + 10
Hour 72	1.49E + 11	1.20E + 11

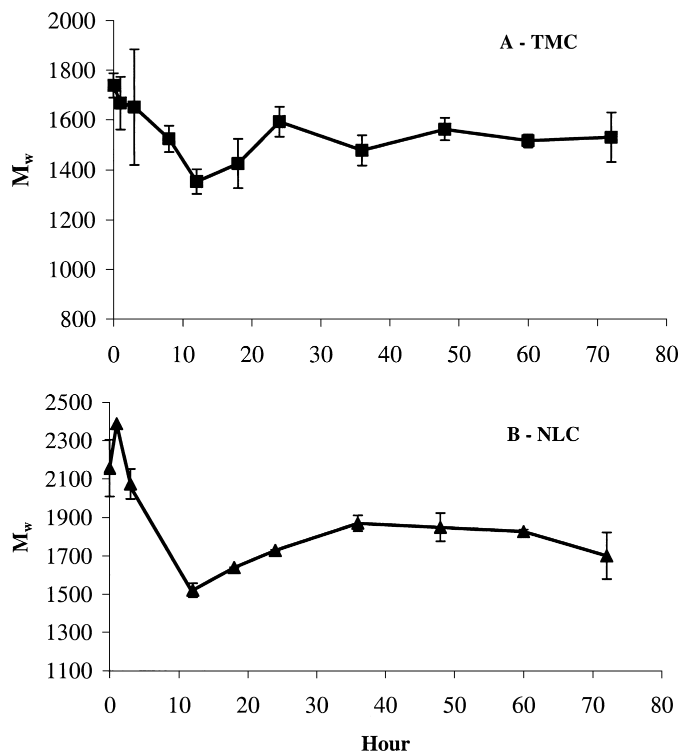

Figure 4. Changes in weight average molecular weight (M_w) of DOM in solution throughout 72 hours after inoculation: (A) TMC inoculate in TMC DOM (■); (B) NLC inoculate in NLC DOM (▲). Note difference in scales.

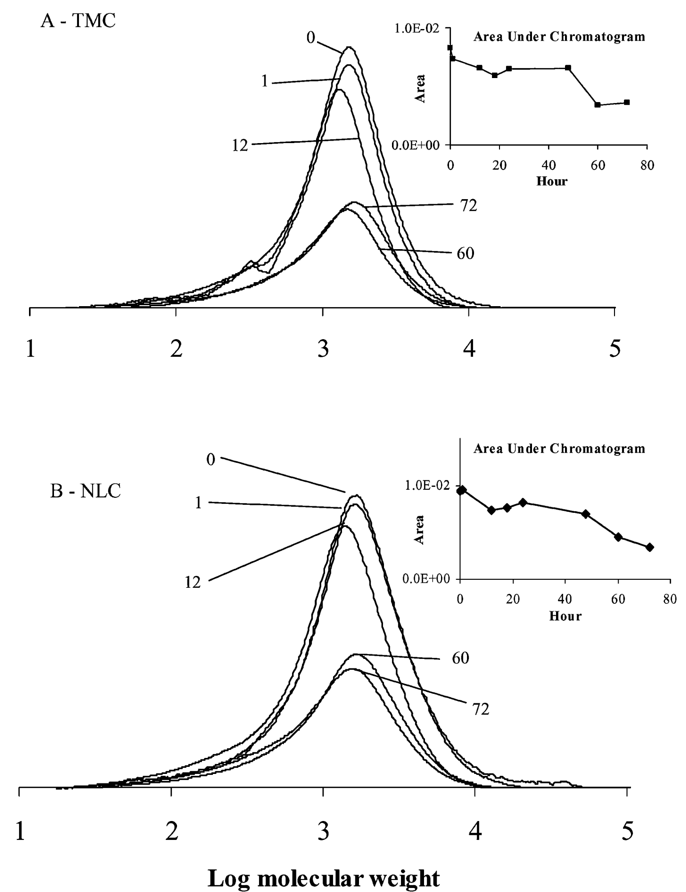

Figure 5. HPSEC chromatograms of DOM at 0, 1, 12, 60, and 72 h after inoculation: (A) TMC inoculate in TMC DOM; (B) NLC inoculate in NLC DOM. Inset graphs show changes in [DOC] over 72 h, as estimated by changes in area under chromatographs.

M_w until ~12 h was likely the result primarily of preferential adsorption of high-to-intermediate molecular weight components of the DOM to bacterial surfaces and other abiotic sorbates that might have been concentrated along with the bacteria because it is unlikely that bacterial metabolism accounted for the change in M_w, as microbial activity was low during this period. The best test of this hypothesis might have been to conduct reversibility experiments as described by Maurice et al. (2004) for DOM adsorption to *Bacillus subtilis*. However, the use of natural consortia mandated quick experiments and precluded the complex experiments needed to test for reversibility. Moreover, if the DOM uptake were not reversible, then it would be difficult to determine whether this was the result of biodegradation, lack of full equilibration, or some other process such as cell lysis upon changing pH as required for reversibility experiments.

Maurice et al. (2004) showed that relatively high molecular weight components of fulvic acid (FA) (~1,500–8,000 Da) adsorbed to *Bacillus subtilis* first, followed by the highest molecular weight compounds (>8,000 Da). The *B. subtilis* were viable but nonmetabolizing, so that the change in molecular weight could be ascribed to adsorption rather than biodegradation. A fraction of low molecular weight FA components failed to adsorb, even after 4 h. Adsorption came to equilibrium rapidly, within the first 30 min of the experiment. In Figure 5, we illustrate changes in molecular weight distributions with DOM in natural community assemblages, rather than a bacterial monoculture, over time. The chromatograms demonstrate that for samples from both sites, as DOM concentrations decrease (indicated by a decrease in area under each chromatogram) there is preferential removal of high molecular weight compounds from solution. This causes the M_w to decrease throughout the first 12 h.

Presumably, the high molecular weight DOM adsorbed to bacterial surfaces. Although centrifugation concentrated the bacteria from the filtered natural water samples, inorganic colloids likely passed through the filters and may have been present in the inoculate, as well. These particles may have provided additional sorbent surfaces. Previous research has shown that higher molecular weight components also adsorb preferentially to iron oxides and clays (Meier et al. 1999; Namjesnik-Dejanovic et al. 2000; Zhou et al. 2001).

A sharp increase in bacterial metabolism after 12 h (Figure 3) suggests that after the initial 12 h, changes in M_w of the DOM remaining in solution were due primarily to biodegradation or consumption. As bacterial productivity (determined by measuring the incorporation of leucine) noticeably increased after Hour

12, the M_w of the DOM also increased. This suggests that bacteria began to consume the lower molecular weight components of the DOM.

At most time periods, bacterial growth was greater in DOM from TMC than from NLC, despite the higher [DOC] in NLC. Because NLC DOM generally had higher M_w and the bacteria showed lower metabolism than TMC, we hypothesized that DOM from NLC was less labile than from TMC. Previous researchers have shown that the labile component of DOM is low molecular weight, and is more bioavailable, as it is usually degraded within hours to days. Although Amon and Benner (1996) showed that high rates of bacterial metabolism were supported by high molecular weight DOM components, they also showed that low molecular weight DOM supported higher bacterial growth efficiencies (Amon and Benner 1996). Also, Meyer et al. (1987) showed that the lower molecular weight fraction of DOM supported more bacterial growth than the higher molecular weight component in several river systems. In our experiment, after Hour 12, bacterial consumption depleted the solution of lower molecular weight DOM components, resulting in an increase of the M_w of DOM left in solution. Thus, the M_w of the DOM from each site increased once rapid bacterial growth and metabolism ensued (Figure 3).

To further test the hypothesis that the decrease in intermediate to low molecular weight components was due primarily to biodegradation rather than to adsorption on increasing numbers of bacterial cells, we calculated DOM removal from solution as a function of molecular weight, normalized to cell count. Figure 6 shows the normalized distribution of the molecular weight of the DOM removed from solution (calculated by subtracting the chromatograms of the original from the remaining DOM in solution) at 1 h and at 72 h. The M_w of the original, unreacted DOM is indicated by a vertical line on each graph. Peak height represents the amount of material adsorbed or consumed, whereas peak morphology shows changes in relative adsorption or consumption of different molecular weight material. In NLC, there clearly was a much greater amount of DOM removed from solution per cell at Hour 72 than at Hour 1; this suggests that the increased removal was not due simply to the increased surface area for adsorption provided by more cells, but rather to biodegradation. At 72 h, material was removed from the full spectrum of molecular weights, but particularly the dominant intermediate and the low molecular weight components. The shift from more high- to intermediate-molecular weight components to more intermediate-to-low-molecular weight components removed from solution per cell was fairly subtle for this sample. For TMC, the amount per cell did not increase substantially overall, but there was a clear shift from removal primarily of the high molecular weight components to removal primarily of the intermediate to lower molecular weight components (Figure 6b). Initially in TMC, the most adsorbed DOM component had a log molecular weight around 3.5, whereas at Hour 72, the most removed DOM component had a log molecular weight around 3.1. The adsorbed DOM of the initial TMC had more of the larger components (greater than log $M_w = 3.5$) than that of the final TMC.

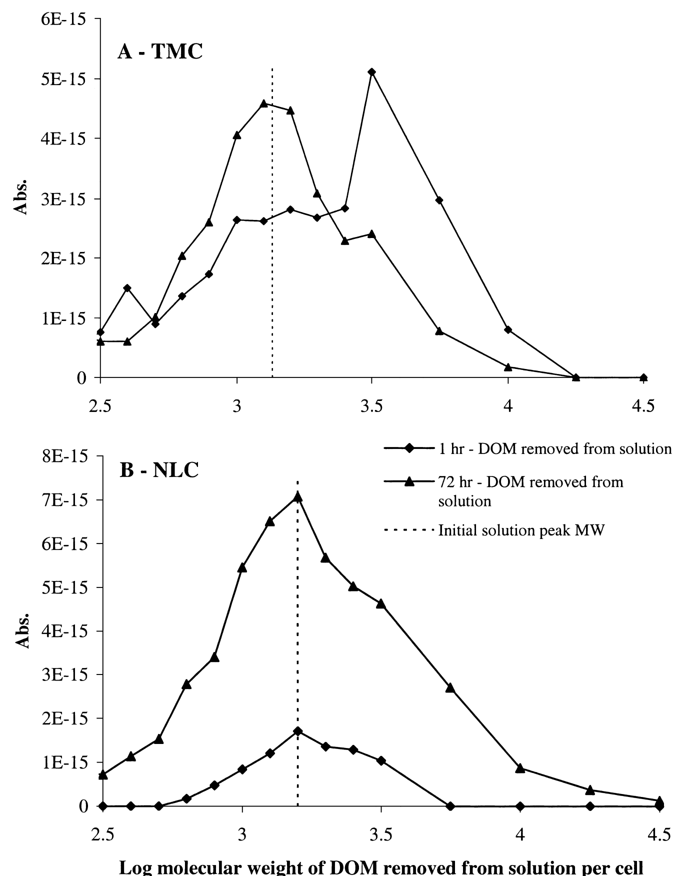

Figure 6. Molecular weight distributions of DOM adsorbed per bacterium. Initial (♦) and final (▲) distributions are shown for TMC (A) and NLC (B) sites.

The shift in peak molecular weight component removed from solution per bacterium reinforces our hypothesis that the high molecular weight DOM in solution adsorbs first. Figure 6 shows that DOM was removed from solution throughout the 72 h, yet because there was a shift in the distribution of DOM molecular weight adsorbed per bacterium, the adsorption and consumption rates did not balance and were not constant throughout the 72 h. Our data do not allow us to determine whether the high molecular weight material that first apparently adsorbed subsequently was degraded.

SUMMARY

Results of this study showed that after inoculation, the average molecular weight of DOM remaining in solution initially decreased, indicating preferential loss of the HMW components. Because previous studies have shown preferential adsorption of HMW DOM components onto bacterial cell walls and obtaining adsorption equilibrium within one hour, we suggest that the initial loss of HMW components in our experiments was likely

due primarily to adsorption of HMW components to the bacteria along with other dissolved solids or inorganic colloids. This hypothesis is consistent with the low bacterial productivity observed by leucine during the initial 12 h. After 12 h, M_w of DOM in solution increased, corresponding to a distinct increase in leucine incorporation. We suggest that bacterial consumption of intermediate to low molecular weight components from the DOM caused the M_w of DOM in solution to increase. This study indicated that the bacteria were able to consume the humic/fulvic-type components of the DOM, which are generally considered to be refractory.

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