Microbial Ecology

Dissolved Organic Matter Concentration and Quality Influences upon Structure and Function of Freshwater Microbial Communities

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Abstract

Past studies have suggested that the concentration and quality of dissolved organic matter (DOM) may influence microbial community structure. In this study, we cross-inoculated the bacterial communities from two streams and a dystrophic lake that varied in DOM concentration and chemistry, to yield nine fully crossed treatments. We measured dissolved organic carbon (DOC) concentration and heterotrophic microbial community productivity throughout a 72-h incubation period, characterized DOM quality by molecular weight, and determined microbial community structure at the initial and final time points. Our results indicate that all bacterial inoculate sources had similar effects upon DOC concentration and DOM quality, regardless of the DOM source. These effects included an overall decrease in DOM M_W and an initial period of DOC concentration variability between 0-24h. In contrast, microbial communities and their metabolic rates converged to profiles that reflected the DOM source upon which they were growing, regardless of the initial bacterial inoculation. The one exception was that the bacterial community from the low-concentration and low-molecular-weight DOM source exhibited a greater denaturing gradient gel electrophoresis (DGGE) band richness when grown in its own DOM source than when grown in the highest concentration and molecular weight DOM source. This treatment also exhibited a higher rate of productivity. In general, our data suggest that microbial communities are selected by the DOM sources to which they are exposed. A microbial community will utilize the low-molecular-weight (or labile) DOM sources as well as parts of the high-molecular-weight (refractory) DOM, until a community develops that can efficiently metabolize the more abundant high-molecular-weight source. This experiment examines some of the complex interactions between microbial community selection and the combined factors of DOM quality and concentration. Our data suggest that the roles of aerobic aquatic heterotrophic bacteria in carbon cycling, as well as the importance of high-molecular-weight DOM as a carbon source, may be more complex than is conventionally recognized.

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Introduction

Dissolved organic matter (DOM) is pervasive in aquatic ecosystems and is a necessary source of carbon and other nutrients for aquatic microorganisms. Although natural DOM is inherently heterogeneous and complex, for simplicity and functionality, it has often been divided into a smaller and lower molecular weight (MW) labile pool and a larger, higher MW refractory pool.

Studies in various aquatic environments support the generalization that the labile DOM pool is more important than the refractory DOM pool in sustaining bacterial secondary production. For example, the labile fraction of DOM from a lake supported fourfold higher secondary production than humic substances from the same environment [22]. Bacterial growth on blackwater river water was greatest in low MW DOM-enriched treatments, and production in high MW DOM treatments was supported only by the labile carbon fraction [21]. Ellis *et al.* [8] showed that microbial growth rate in a refractory humic acid treatment was lower than in more labile amino acid and carbohydrate treatments. Indirect evidence also

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supports the preferential use of the labile carbon pool by microorganisms. For example, surface-level DOM in a riverine wetland—which was presumably subject to increased photodegradation, and therefore more labile—was a more suitable bacterial substrate than DOM collected from lower depths [19]. Although autochthonous DOM in lakes, derived from lower MW algal sources, is lower in concentration than higher MW allochthonous DOM, the former is preferentially utilized for secondary production [18].

However, several recent studies that combined molecular microbial community analyses with DOM characterization have called into question the generalization that microorganisms predominantly metabolize the labile DOM fraction. On the contrary, DOM MW may have less to do with productivity than the metabolic capabilities of the microbial community. For example, bacterial growth upon humic lake water was limited mainly by concentration and not by molecular weight of the DOM [7]. Additionally, different microbial communities were present at high and low DOM concentrations. Members of the *β-Proteobacteria* and *Cytophaga–flavobacter* phyla were present at all DOM concentrations, whereas *α-Proteobacteria* dominated the lowest DOM concentrations [7]. Corresponding results were observed in a study investigating bacterial groups in estuarine and coastal waters. Members from *Cytophaga–flavobacter* were overrepresented in microbial communities consuming high MW chitin, *N*-acetylglucosamine, and protein, whereas *α-Proteobacteria* dominated communities consuming low MW amino acids [6]. Furthermore, hyporheic sediment microbial communities exposed to various MW DOM sources exhibited community shifts according to the carbon supply [9]. Sediment microbial communities also used high MW polysaccharides preferentially, regardless of molecular size [10]. Finally, the amount of high MW DOM can indirectly influence microbial communities. Enzyme activity, viral numbers, and nitrification rates are all affected by DOM quality, giving further evidence for the influence of DOM upon not only microbial community structure, but also function [2, 29, 32].

In addition to DOM affecting microbial community structure, bacterial activity can also influence DOM composition. Microbial reworking can be important in determining the proteinaceous component of DOM [37]. Within a short incubation period, combined bacterial metabolism and adsorption onto bacterial surfaces of stream DOM greatly decreased the molecular weight [36]. In coastal and oceanic systems, a recalcitrant and persistent fraction of high MW DOM is derived from bacterial activity [26, 38]. This evidence, combined with the effects that varying DOM sources exert upon microbial community structure, indicates a complex interaction between heterotrophic microbial production and community structure and DOM quality and concentration that has not been fully examined.

Further understanding of the connection between microbial community structure and DOM requires knowledge about their reciprocal influences in a controlled environment. Our group has previously investigated the influences of different microbial community inoculates upon dissolved organic carbon (DOC) concentration and molecular weight. Generally, bacterial inoculates decreased the molecular weight of a sample of DOM, but either slightly decreased or had no significant effect on the DOC concentration [35, 36]. In this work, we provide a further connection between DOM source and microbial community by investigating previously unexamined changes in bacterial profiles using molecular methods. To provide a direct test of how DOM concentration and quality and microbial community structure covary, we isolated microbial communities from two streams and a dystrophic lake located in northern Wisconsin and the Upper Peninsula of Michigan. Each microbial community was reciprocally inoculated into DOM from each source. Our objectives were to determine: (1) if microbial community profiles were altered by DOM source quality and concentration and (2) how DOM concentration and quality were affected by the respiration and reworking by different microbial communities. Our results demonstrate that DOM source has a significant influence on microbial community structure, regardless of the initial bacterial inoculate, and that microbial reworking of high molecular weight DOM may be an important factor in freshwater wetland-associated systems.

Methods

Site Description. Three aquatic sites (two streams and one bog-lake) located in northern Wisconsin and northern Michigan were selected based on previous studies [35, 36]. Nelson Creek (NLC) and Two Mile Creek (TMC) are located in Ottawa National Forest, whereas Forest Service Bog (FSB) is located at the University of Notre Dame Environmental Research Center. This area has streams and lakes that are closely associated with wetlands and have high DOC concentrations. The selection of these sites represented a rough gradient of DOM concentration and quality for the experimental DOM sources (Table 1).

Forest Service Bog is a small "bog" lake with an open-water area of ~0.21 ha and a maximum depth of 4.9 m. The larger portion of the lake has been filled by an encroaching peat mat of similar maximum depth to the current open-water zone. The peat mat is vegetated by *Sphagnum* spp. mosses, sedges, ericaceous shrubs, black spruce, and tamarack, which are all typical of peatlands in this region. The relatively low DOC concentration and pH in the open-water zone suggest substantial ground-

Table 1. Initial abiotic DOM source water characteristics

	FSB	*TMC*[a]	*NLC*[a]
In situ measurements			
pH	5.1	6.9	5.8
Conductivity (μS cm^{-1})	9.2	49.2	24.3
Temperature (°C)	17.5	11.3	17.5
Inorganic constituents			
Al (mg L^{-1})	0.02	0.02	0.42
Fe (mg L^{-1})	0.03	0.02	1.46
Na (mg L^{-1})	1.41	2.76	3.53
K (mg L^{-1})	0.29	0.57	0.30
Ca (mg L^{-1})	0.07	18.80	5.72
Mg (mg L^{-1})	NA	6.47	1.71
Total N (mg L^{-1})	0.22	0.78	1.24
Total P (mg L^{-1})	28.21	27.00	38.41

[a]Data found in this column have been previously published, except for total N and P concentrations [36].

water input from mineral soil and surrounding uplands [35].

Nelson Creek is a first-order stream and TMC a second-order stream. Both streams are associated with neighboring wetlands, a likely source of their high DOC concentration. Their watersheds are predominantly northern hardwood forest containing both deciduous and evergreen species [35].

Sample Collection and Preparation. Surface water grab samples were collected in June 2002 and filtered (Whatman GF/F) on site into sterile 20-L carboys. Bacterial inoculate water samples were filtered (1 μm Nucleopore) to remove protozoan grazers, and stored in the dark at 22°C. Samples for DOC concentration and molecular weight analysis were collected and filtered through Whatman GF/F filters. During travel, DOM samples were stored on ice, whereas bacterial inoculate samples were kept at ambient temperatures. Within 48 h of collection, we moved the DOM samples to a 4°C incubator, and prepared bacterial inoculates by centrifuging 1.2 L of 1-μm filtered water at 8000 rpm for 30 min at room temperature. Final concentrated bacterial inoculate pellets were resuspended in 0.22-μm filter-sterilized water from each of the DOM sites. We inoculated six replicate DOM samples from each site (386 mL) with a concentrated bacterial inoculum (13 mL) from each site, to yield nine treatments with approximately *in situ* population densities of bacteria. The samples were stored in acid-washed, ashed at 500°C and sterilized glass bottles at room temperature and shaken at 50 rpm. We covered the bottles with a black sheet to prevent any possible photodegradation of DOM and to inhibit phototrophic microbial activity. Bottles were plugged with glass wool to prevent contamination, but allow for passage of air to prevent the system from becoming anaerobic. Samples were incubated over a period of 72 h.

Chemical Characterization and Analysis. We collected samples for DOC concentration measurement at 0, 1, 3, 8, 12, 18, 24, 36, 48, 60, and 72 h from each of the six replicates. Before sampling, each treatment bottle was inverted three times to prevent biofilm formation on the glass. Samples were passed through Whatman GF/F filters, acidified with 2 N HCl, and refrigerated until analysis. Nonpurgeable organic carbon concentrations (DOC concentrations) were measured within a day of collecting the samples by combustion on a Shimadzu TOC5000 with an autosampler.

We measured weight average molecular distributions (M_W) of DOM on aliquots taken immediately after bacterial inoculation and at every sampled time interval, by using high-pressure size exclusion chromatography (HPSEC; Waters Associates, Milford, MA, USA). The HPSEC method of Chin *et al.* [5], revised by Zou *et al.* [38], was used, as previously described by Young *et al.* [36].

We analyzed the absorbance spectra of the samples at 280 nm by using a double-beam Varian Cary III scanning spectrophotometer. Quartz cells (1 cm) were used with sterile Milli-Q water as a reference. Absorptivities were normalized per mol C (ε_{280}), which were calculated based on absorbance at 280 nm and DOC concentrations ($\varepsilon_{280} = A_{280}$ $[DOC]^{-1}$, expressed in L mol C^{-1} cm^{-1}) [5, 31, 36]. Absorbance at this wavelength is sensitive to the aromatic characteristics of DOM.

Conductivity, pH, and water temperature were recorded in the field from surface water samples. We used inductively coupled plasma optical emissions spectroscopy (ICP-OES) to analyze preincubation inorganic constituent concentrations of each DOM source (Table 1). We collected and froze samples for total phosphorus and total nitrogen analysis at the initial and final time points of the incubation period. Total phosphorus (TP) samples were prepared using a persulfate autoclave digest method and measured by using a 10-cm cell on a Spectronic Genesys 2 spectrophotometer at 885 nm [3]. We measured total nitrogen (TN) with a standard in-line digestion Lachat QuickChem 8000 autoanalyzer method (Hach Corporation, Loveland, CO, USA).

Microbial Activity. We determined the heterotrophic bacterial productivity by using 5-mL aliquots from each sample at all 11 time points during the incubation. A [2,3,4,5-^{3}H]leucine uptake assay (171 mCi $mmol^{-1}$ specific activity) was used with a 1-h incubation time. Leucine uptake at each time point was determined by using a Beckman LS 5000 TD and TA Liquid Scintillation System, and biomass conversion was calculated according to Kirchman [15]. We removed 5-mL samples at the start of the incubation and preserved them in 37% formaldehyde for initial cell density enumeration by direct counts

[14]. Two milliliters of each replicate was incubated in the dark with 0.2 mL of 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. We counted ≥200 cells per slide using an Olympus BH-2 epifluorescence microscope at 1000× magnification.

DNA Extraction. Fifty milliliters of the sample was removed from three of the six replicates for each treatment at the initial and final time points, and stored at –20°C. After thawing, each sample was filtered (0.22 μm Nucleopore). We used the seston trapped by these filters for DNA extraction with an UltraClean Soil DNA Kit (MoBio Laboratories, Solana Beach, CA, USA). When necessary, a Wizard DNA Clean-up system (Promega) was used to remove humic substances from extracted DNA and facilitate PCR. Five microliters of extracted DNA per sample was PCR-amplified for 40 cycles of 95°C denaturing/55°C annealing/72°C extension using universal Eubacterial primers (Eub GC-341f; Eub 534r), which target a 193-bp portion of the 16S rDNA. The forward primer contains a GC-clamp to improve band separation (5-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGGG-3) [23].

Microbial Community Characterization. We used denaturing gradient gel electrophoresis (DGGE) to characterize the changes in microbial community structure from the initial and final time points of the experiment. DGGE gels were run for 14 h at 60 V, with a 20 min/20 V start-up period, in a DCode (Bio-Rad, Hercules, CA, USA) apparatus using 8% acrylamide with a urea-formamide denaturing gradient of 40–60%. Gradient gels were poured by using a minipump variable flow + gradient maker (CBS Scientific Co. Inc., Del Mar, CA, USA). The gels were stained in 6.5 μL ethidium bromide and 100 mL 1× TAE buffer for 1 h, and viewed via a Kodak EDAS 290 imaging system. We used Kodak 1D Image Analysis Software Version 3.6 (Eastman Kodak Co., Rochester, NY, USA) to quantify the percent lane intensities of all bands accounting for 10% or more of total lane intensity from digital images of the DGGE gel. Three replicate gels were run and analyzed for each set of fully crossed inoculate DOC treatments to yield replicated band richness and intensity data. We excised bands that accounted for 50% or more of the total lane intensity and froze them in 50 μL sterile water. These bands were reamplified under the same PCR conditions as previously described, and PCR products were cleaned by using a Wizard PCR Prep Kit (Promega). Three clones of each excised band were created and isolated via Topo TA Cloning for Sequencing Kit (Invitrogen) and LB + ampicillin media. Plasmids were isolated and insert size was checked by EcoR1 restriction enzyme digest. Total DNA template quantity was determined by using a *Hin*dIII ladder. Templates were prepared for sequencing by using the T7 priming site and the Dye Terminator Cycle Sequencing Quick Start Master Mix (Beckman Coulter), and amplified for 96°C, 20 s/50°C, 20 s/60°C, 4 min for 30 cycles. PCR products were then ethanol-precipitated and vacuum-dried, and resuspended in 40 μL of the sample loading solution. Next, we analyzed the sequences with a Beckman Coulter 8000 capillary sequencer and compared them to homologous sequences in GenBank.

Statistical Analyses. We analyzed all data with SYSTAT version 10 software (SPSS, Inc., 2000, Chicago, IL, USA). Repeated-measures analysis of variance (ANOVA) was performed upon the DOC concentration and bacterial metabolism data sets. When incubation time had a significant interaction with the main factors (inoculate and DOM sources), individual ANOVAs were performed at each time point. Pairwise comparisons were performed with Tukey's HSD. Average M_W and direct count estimates and band richness data obtained from the DGGE gels were analyzed via one-way ANOVAs and paired t tests.

Results

Initial Treatment Differences. The three DOM sources used in this experiment differed in general chemical properties (Table 1) [36]. Conductivity, pH, and concentrations of dissolved Ca, Mg, and K were higher in TMC, although NLC had significantly higher Fe and Al concentrations. The high Fe and Al concentrations were most likely attributable to metal complexation by DOM [36]. An inherent limitation of a cross-inoculation study using natural waters is that important chemical factors other than those relating to DOM may vary among water sources (Table 1). Many of these factors, such as Al, Fe, total N, and pH, are intimately associated with the concentration and chemistry of DOM, so even concentrating and isolating the DOM from the source waters would not eliminate this problem.

The initial (0-h) weight average molecular weight (M_W), absorptivity (Abs 280 nm), aromaticity (ε_{280}), and DOC concentrations reflected a general duality among the DOM sources ($p < 0.001$) (Table 2). Initial ε_{280}, M_W, and DOC concentration were lowest in FSB DOM source water ($p < 0.001$), but did not differ between the TMC and NLC treatments ($p \geq 0.27$).

Our inoculate preparation procedure may have increased the concentration of abiotic and inorganic components of the 0.22-μm filtered DOM. However, we did not observe any significant variations in initial 0-h treatments according to the bacterial inoculate source (Table 2). Rather, all initial parameters varied by DOM source only, indicating that there was little input of car-

Table 2. Initial (0 h) and final (72 h) treatment parameters ± 1 standard error

Bacterial inoculate source	*DOM source*	*UV–vis Abs 280 nm*		*[DOC] (mg L^{-1})*		*ε_{280} (L mol C^{-1} cm^{-1})*		*M_W (Da)*		*Bacterial cell count (cells L^{-1})*		*DGGE band richness (bands lane^{-1})*	
		0 h	*72 h*	*0 h*	*72 h*	*0 h*	*72 h*	*0 h*	*72 h*	*0 h*	*72 h*	*0 h*	*72 h*
FSB	FSB	0.1954	0.0945	12.48	7.05	188.06	160.99	779	1229	$1.66 \times 10^{10} \pm 4.27 \times 10^{6}$	$4.20 \times 10^{10} \pm 1.18 \times 10^{7}$	3.3 ± 0.9	8.6 ± 1.2
TMC	FSB	0.1543	0.1249	7.38	8.12	251.12	184.75	1043	1187			4.3 ± 1.3	7.0 ± 2.5
NLC	FSB	0.1528	0.1524	7.55	8.77	243.08	211.19	1054	1107			4.0 ± 0.6	10.3 ± 2.2
FSB	TMC	0.9406	0.9243	33.62	21.79	336.04	509.49	1583	1626			8.6 ± 2.3	8.6 ± 3.2
TMC[a]	TMC[a]	0.9535	0.9097	31.67	30.57	361.62	357.42	1739	1530	$2.97 \times 10^{10} \pm 4.86 \times 10^{6}$	$1.49 \times 10^{11} \pm 4.42 \times 10^{7}$	8.0 ± 2.1	12.6 ± 1.3
NLC	TMC	0.9266	0.9122	32.21	32.62	345.50	335.88	1712	1645			6.0 ± 1.5	14.0 ± 0.6
FSB	NLC	0.9433	1.0735	38.13	33.55	297.14	384.32	1987	1756			3.3 ± 0.9	7.0 ± 1.5
TMC	NLC	1.0060	1.0583	32.89	34.68	367.38	366.53	1810	1817			3.6 ± 0.3	6.0 ± 2.5
NLC[a]	NLC[a]	1.0991	1.0643	35.27	33.86	374.29	379.77	2157	1699	$1.58 \times 10^{10} \pm 2.59 \times 10^{6}$	$1.20 \times 10.^{11} \pm 1.85 \times 10^{6}$	5.0 ± 0.6	5.3 ± 2.3

Dissolved organic matter was characterized by UV–vis absorbance, DOC concentration, ε_{280}, and M_W; bacterial inoculates are characterized by total bacterial cell counts (n = 3), and DGGE community profile band richness (n = 3).
[a]DOM characterization data that have been previously published [36].

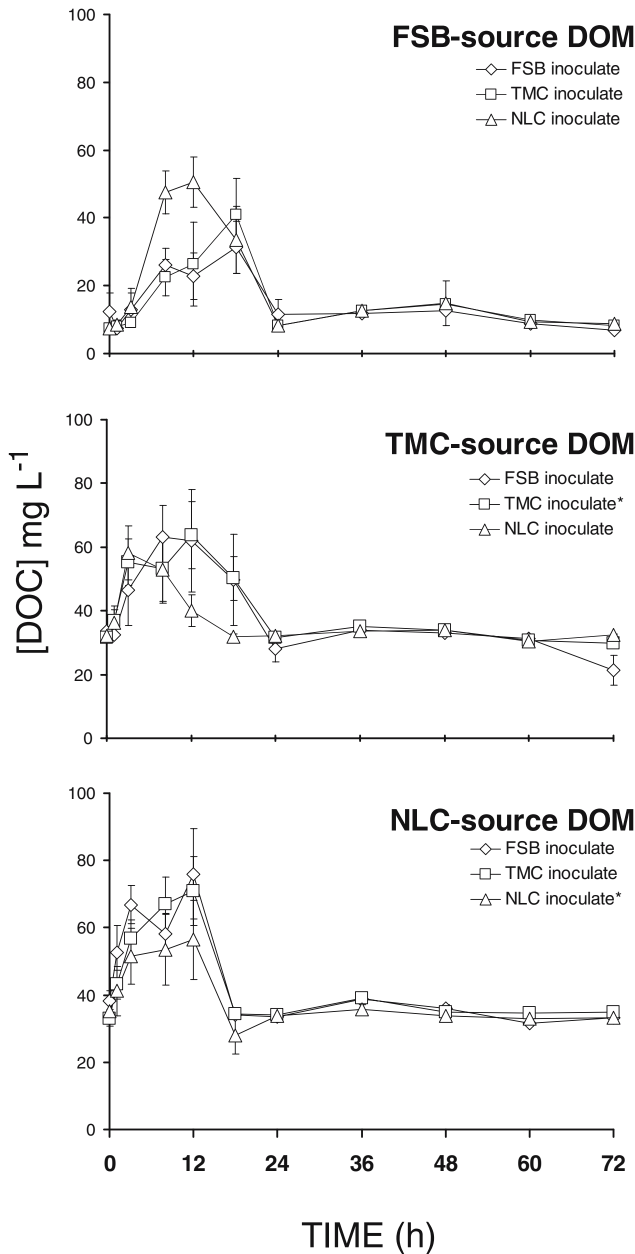

Figure 1. Dissolved organic carbon (DOC) concentrations ± 1 standard error through 72 h of incubation with three bacterial inoculates, grouped by DOM source. For all treatments, $n = 6$.

bon with the addition of each inoculate. Direct count estimates of bacterial biomass were higher in TMC bacterial inocula-in-TMC DOM treatments ($p = 0.032$) than in FSB-in-FSB and NLC-in-NLC treatments, which did not vary significantly ($p = 0.980$, Table 2). However, all bacterial counts were within 10^{10}–10^{11} cells L^{-1}, indicating that the bacterial densities in each inoculate were fairly well standardized among the nine treatments. Statistical differences calculated using DOC metabolism values on a per cell basis were not different from the results uncorrected for biomass. This low variation among bacterial densities also indicates that our DOM filtration procedure removed most bacterial cells from the DOM, and that bacterial sources in our treatments were derived from the inoculation.

Changes in Dissolved Organic Matter Concentration and Quality. Trends in DOC concentrations were similar throughout the 72-h incubation period in all treatments (Fig. 1). DOC concentrations in all nine treatments rapidly increased within the first 12 h of incubation, and then settled down to about initial levels by 24 h, where they remained throughout the 72-h final time point. Bacterial inoculate source had no effect on

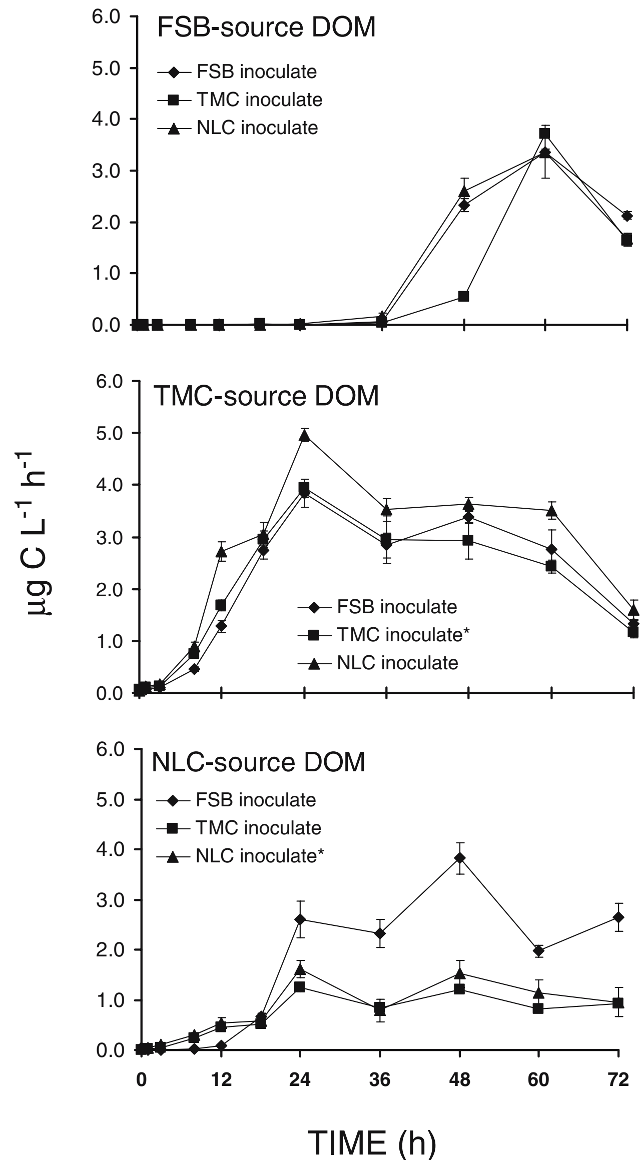

Figure 2. Heterotrophic microbial community productivity ± 1 standard error of three bacterial inoculates in three DOM sources, grouped by DOM source. For all treatments, $n = 6$.

DOC concentration within the three DOM sources (Fig. 1, $p \geq 0.542$), and the effects of the inoculate source did not vary through time (inoculate source × time interaction, $p \geq 0.82$).

After 72 h, DOM treatments from FSB source water had lower absorptivity (UV–vis), aromaticity (ε_{280}), and average molecular weight distribution (M_W) than treatments with TMC and NLC source water ($p < 0.001$, Table 2). Nelson Creek treatments, which had the highest DOC concentrations, also had highest the M_W values (Table 2). The UV–vis and ε_{280} values of DOM from TMC and NLC source water were comparable, suggesting similar DOM aromaticity among those six treatments (Table 2). Generally, M_W decreased through time in treatments containing DOM from TMC and NLC source water, but remained the same in treatments with FSB DOM source water (Table 2). However, aromaticity (ε_{280}) and absorptivity (UV–vis) decreased in treatments with FSB DOM source water, and did not differ over time for treatments with TMC and NLC DOM (Table 2).

Bacterial Growth. Within each DOM source, microbial activity peaked at different times (Fig. 2). In treatments with FSB as the DOM source, maximum productivity occurred in all inoculates between 48 and 60 h. In treatments with DOM from TMC and NLC, maximum productivity was reached closer to 24 h after inoculation. The greatest amount of bacterial productivity (by all inoculates) occurred in the TMC DOM-source treatments, and peak productivity in both FSB and NLC DOM sources were lower ($p < 0.001$).

In general, the bacterial inoculate source had little effect on secondary productivity, which was similar within a DOM-source treatment, regardless of the bacterial inoculate source. However, the FSB bacterial inoculate grown in NLC DOM source water exhibited a higher productivity rate than the other inoculates grown in the NLC DOM source at 24 and 72 h ($p < 0.001$), and incubation time was a significant factor in this particular treatment (time × inoculate source interaction; $p < 0.001$).

Microbial Community Analysis. We conducted molecular microbial community analyses by using DNA-PCR-DGGE at the initial and final time points of the incubation for each of the nine treatments. Initially, microbial communities consisted of many low-intensity bands, indicating the low densities of a variety of bacterial genotypes (data not shown). After 72 h, however, microbial community banding patterns for each treatment varied primarily based on the DOM source in which they were incubated (Fig. 3). Initial inoculate sources did not appear to have an impact on the microbial community structure, and all communities converged according to the DOM source. However, we observed one exception to this trend. The FSB inoculate-in-NLC source DOM treatment did not exhibit the same microbial community structure as the TMC-in-NLC and NLC-in-NLC treatments; it also had higher overall productivity (Figs. 2 and 3). We saw the same trends in all three replicate DGGE gels (data not shown).

Using Kodak 1D Image Analysis software, we analyzed initial and final DGGE band number per lane to estimate microbial community richness (Table 2). Richness increased over the 72-h incubation period ($p < 0.001$). At both time points, treatments with DOM from the TMC water source had higher band richness than

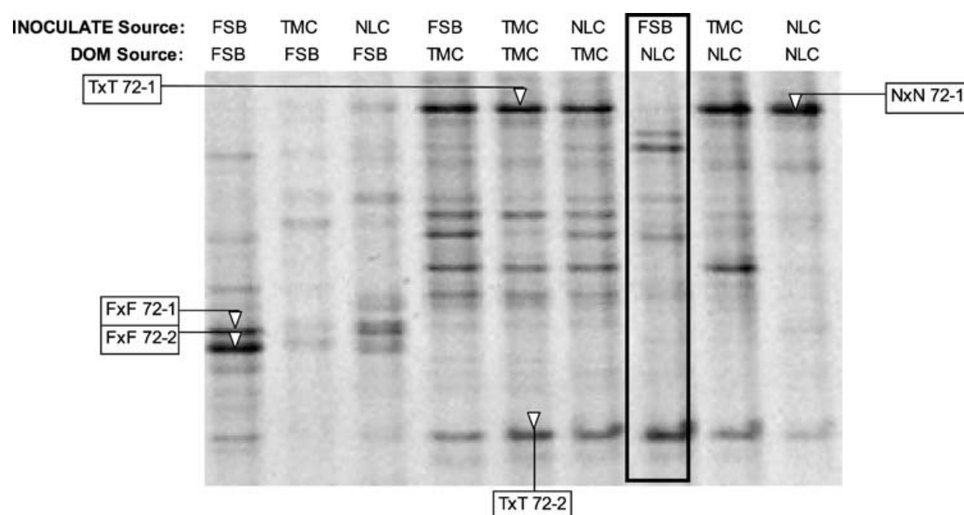

Figure 3. DGGE profile of nine microbial communities amplified by universal Eubacterial primers after 72 h of incubation in three DOM source treatments. Bands indicated by *arrows* were excised and sequenced. All microbial communities were visually similar within a DOM source, regardless of the initial bacterial inoculate community, with one exception. The FSB–in–NLC treatment, indicated in the box, did not follow this trend.

treatments with FSB and NLC DOM sources, which were not different ($p \leq 0.013$). Inoculate source did not have a significant effect on band richness at either time point ($p \geq 0.714$).

We attempted to sequence some of the major bands present, which represent ≥50% of the total lane intensity, to characterize these microbial communities. We were able to gain some phylogenetic information indicating that bands T × T72-2, N × N72-1, T × T72-1, F × F72-1, and F × F72-2 are all part of the Proteobacterium phylum (Fig. 3). However, because of the small universal Eubacterial PCR product size used for DGGE (<200 bp), we were unable to obtain enough phylogenetic information to construct a tree or infer any differences within or between each treatment, so no further bands were sequenced. We chose a primer set that would yield a small PCR product because previous studies have shown that the upper size limit for adequate DGGE band separation is less than 500 bp [24, 30].

Discussion

Based on a previous study, we chose the three DOM source sites as illustrative of a gradient of DOM concentration and recalcitrance, represented by molecular weight distribution, ε_{280}, and absorbance [35]. However, DOM concentration and average M_W in TMC and NLC sources were fairly similar. Thus, the true DOM gradient that existed in this experiment was represented by low MW and DOM concentration (labile) in FSB, and by high MW and concentration in both TMC and NLC (Table 2). We recognize a limitation inherent in a cross-inoculation study design in that several important chemical parameters, such as ion concentrations and pH, may vary depending on the DOC source. However, our results suggest large interactive effects among DOM concentration and chemistry and microbial community structure and activity.

Dissolved organic carbon concentrations varied through time in all nine treatments, but did so most drastically during the first 24 h (Fig. 1). These variations occurred regardless of the DOM source or the bacterial inoculate. After 24 h, DOC concentrations returned to approximately their initial source levels, and remained there until the final time point of the experiment (Fig. 1). We also observed general changes in the quality of DOM throughout the incubation time, indicating an overall decrease in M_W in TMC and NLC DOM source treatments, and an increase in M_W in FSB DOM source treatments (Table 2). Previous studies indicate that these changes in DOM quality occur during the first 24 h of the incubation period, at the same time when DOC concentration was most variable [36].

However, the greatest changes in bacterial productivity occurred after these DOM variations had leveled out (Fig. 2). The 0–24 h changes in DOM concentration and quality were most likely attributable to abiotic desorption from bacterial surfaces and other abiotic particles that might have been concentrated along with the bacteria, rather than by bacterial metabolism [36]. For example, relatively high molecular weight components of fulvic acid (1500–8000 Da) adsorbed to viable but nonmetabolizing *Bacillus subtilis*, followed by the highest molecular weight compounds (>8000 Da) [12, 20]. A fraction of low-molecular-weight fulvic acids did not adsorb to bacterial surfaces, even after 4 h of incubation. A similar process likely occurred in our experimental treatments, accounting for the initial changes in DOM concentration and quality after bacteria were added, without a corresponding link to bacterial productivity. The lower initial M_W of the FSB source DOM may explain its lack of change through time compared to the higher MW TMC and NLC sources of DOM. Additionally, initial DOM release may be attributable to lysing of microbial cells and immediate uptake of labile carbon by actively metabolizing organisms.

From our initial DOM characterization results, it would be expected that bacterial growth upon FSB DOM would have been both the fastest and the highest, and that metabolism upon the more refractory TMC and NLC sources would have required a longer time for breakdown. We did not observe this. Rather, microbial metabolism peaked in both TMC and NLC DOM source treatments during the first 24 h of incubation with all three inoculates, and then reached an upper limit or began to decrease (Fig. 2). However, bacteria growing on FSB DOM source water did not begin to measurably take up ^{3}H-leucine until 36–48 h of the incubation. Again, there were few significant differences among the bacterial inoculate trends of activity, and any changes were driven by the DOM source only (with the exception of the FSB inoculate-in-NLC-DOM treatment). This lag in growth time upon FSB DOM may have been attributable to the lower concentration of carbon present in these treatments (Fig. 1, Table 2). Although DOM from TMC and NLC was more refractory, they had higher initial DOC concentrations than FSB. As such, they may have contained more labile organic carbon present initially than the FSB DOM source water, just by virtue of higher initial concentrations.

If *in situ* microbial community structure is determined by the concentration and molecular weight distribution of DOM, then one would expect that microbial community structure would vary with incubation in a particular DOM source. This observation was reflected in the microbial community profiles shown in Fig. 3. At 0 h, microbial communities consisted of many low intensity bands, either as a result of a low concentration of DNA template for PCR, or because the initial communities were very high in bacterial richness. As a result, little

profile information on the initial microbial communities could be obtained. After 72 h, however, banding patterns of the microbial communities varied based solely on the DOM source (Fig. 3). The initial inoculate source did not appear to have any impact on the microbial community structure, and all communities converged according to the DOM source over only 72 h. In addition, microbial communities grown in TMC and NLC DOM source water, which had similar average molecular weight and DOC concentration, also had more visually similar banding patterns than those communities grown in FSB DOM, with lower M_W and DOC concentration. However, although they are similar, there were differences in microbial community profiles grown in TMC and NLC DOM, indicating that although concentration and quality are important, other DOM or environmental parameters could be selecting agents as well. For example, the presence and abundance of specific chemical groups of DOM may be the deciding factor in determining bacterial abundance and activity [10].

Although sequencing did not provide us with species-level information, we did find that all sequenced major bands fell within the *Proteobacterium* phylum. This observation coincided with other investigations on freshwater ecosystems, which reported that the cosmopolitan β-*Proteobacterium* group seasonally constituted about half of microorganism densities in a humic lake water column, and that members of β-*Proteobacterium* and *Cytophaga–flavobacterium* dominate organisms by using natural DOM [4, 6, 7, 16, 34].

There was one exception to the otherwise ubiquitous observation that the bacterial inoculate source appeared to have no effect on microbial metabolism or microbial community development. This treatment was FSB bacteria grown in the NLC DOM source water. In this treatment, we placed a bacterial community accustomed to a low-concentration- and low-molecular-weight (labile) carbon source into a high-concentration- and high-molecular-weight (refractory) carbon source. The result was both a higher heterotrophic productivity from 24-72 h, as well as a different microbial community structure from the TMC and NLC concentrated bacterial inoculates in NLC DOM source water treatments (Figs. 2 and 3). Mixing bacteria and organic matter from two different systems can lead to novel nutrient and bacterial dynamics [13]. Also, when highly divergent sources of bacterial biomass and organic carbon are paired, more time than 72 h may be required for the community to change and equilibrate to a microbial community capable of efficiently metabolizing the more refractory carbon source [35]. Sudden and increased amounts of different carbon sources may lead to higher microbial productivity by the heterotrophic community in general, because a greater diversity of microorganisms may be supported for a time. In addition, we observed the greatest change in average M_W in the three NLC DOM source water treatments, suggesting that proportionally NLC was a more heterogeneous DOM source. It is reasonable to assume that the FSB-derived microbial community, from a low MW DOM environment, should initially and preferentially have been able to use the low MW compounds present in the NLC DOM source water. However, as time progressed, and high MW aromatic fractions possibly adsorbed to bacterial surfaces, the NLC DOM would eventually select for a community capable of using the inherently refractory remaining DOM.

We analyzed the 0 and 72 h DGGE gels for the number of bands or an indication of microbial community richness in each treatment. During the incubation, the number of bands present in each microbial community significantly increased in all treatments (Table 2). This is not surprising, because environmental bacteria provided with ambient temperature conditions and fresh utilizable carbon sources should inevitably grow. However, within a particular DOM source, different microbial inoculates had no effect on community richness, even in the FSB-in-NLC treatment, which exhibited a different microbial community banding pattern and higher metabolism. Visually, this treatment appeared to have greater band evenness than the TMC and NLC-in-NLC treatments, which were mainly dominated by the most intense N × N72-1-type band, which comprises >50% of the total lane intensity in those treatments (Fig. 3). The FSB-in-NLC banding pattern appeared more evenly distributed, with several lower intensity bands represented in the community. Similar results were seen in all three replicate DGGE gels (data not shown), indicating that this difference is a function of the treatment, and not of PCR amplification. Unfortunately, with the limitations of both PCR bias and DGGE band separation, and the lack of possible sequence information (given the <200-bp size of the PCR products used), we were unable to conclusively determine the true diversity parameters.

In this experiment, we attempted to address how microbial community composition and DOM quantity and quality interact in aquatic environments. Many other factors, such as other limiting nutrients, may be as important as DOM concentration and quality in determining microbial community structure. For example, although microbial communities may group according to carbon sources, the major factor that drove community equilibration in oligotrophic lakes was the addition of nitrogen and phosphorus [11]. In a riverine study, dissolved nitrogen was the limiting nutrient for microbial growth, even though DOC supported the majority of heterotrophic productivity [33]. Some studies showed that under extreme oligotrophy bacterial metabolism was not correlated to DOC concentration, but was directly related to phosphorus concentrations, which may influence microbial community structure as well as autotrophic versus heterotrophic competition for phosphorus

[27, 28]. Viral lysis and protozoan grazing, which are important selection factors to bacteria, can also be affected by DOM quality [2, 34]. Conversely, photomineralization and biomineralization are known to compete strongly for complete mineralization of overlapping fractions of DOM [25]. Sunlight has also been shown to decrease concentrations of dissolved humic substances and DOM average molecular weight in aquatic ecosystems, indicating that biological activity is by no means exclusively important in DOM formation and quality characterization [1, 17].

Our results suggest that removing external factors such as photodegradation, protozoan grazing, and viral lysis can provide valuable insight into the connection between microbial communities grown on variable DOM sources. Despite multiple potential confounding factors in this cross-inoculation experiment, our results demonstrate strong interactions among DOM concentration and chemistry and microbial community composition and activity. After inoculation, the average-molecular-weight DOM remaining in solution decreased, and high-molecular-weight components were removed, presumably through adsorption to bacterial surfaces or other abiotic particles. We further found that even divergent microbial communities appeared to converge, based on combined factors of DOM quality and concentration, to a uniform microbial community capable of using the DOM source at similar levels of heterotrophic production. This suggests an interesting versatility of microbial communities to rapidly adjust to new carbon sources present in the environment. The one exception we observed was when the low concentration and molecular weight bacterial source (FSB) was inoculated into the highest concentration and molecular weight DOM source water (NLC). This study indicated that bacteria were able to consume high MW components of DOM that are generally considered to be refractory, and that microbial reworking of high MW DOM may be in important factor in freshwater wetland-associated systems.

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