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Source and supply of terrestrial organic matter affects aquatic microbial metabolism

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ABSTRACT: Aquatic ecosystems are connected to their surrounding watersheds through inputs of terrestrial-derived dissolved organic matter (DOM). The assimilation of this allochthonous resource by recipient bacterioplankton has consequences for food webs and the biogeochemistry of aquatic ecosystems. We used laboratory batch experiments to examine how variation in the source and supply (i.e. concentration) of DOM affects the productivity, respiration and growth efficiency of heterotrophic lake bacterioplankton. We created 6 different DOM sources from soils beneath near-monotypic tree stands in a temperate deciduous–coniferous forest. We then exposed freshwater microcosms containing a natural microbial community to a 1100 µM supply gradient of each DOM source. Bacterial productivity (BP) and bacterial respiration (BR) increased linearly over the broad gradient, on average consuming 7% of the standing pool of dissolved organic carbon (DOC). Bacterial metabolism was also influenced by the chemical composition of the DOM source. Carbon-specific productivity declined exponentially with an increase in the carbon:phosphorus (C:P) ratio of the different DOM sources, consistent with the predictions of ecological stoichiometry. Together, our short-term laboratory experiments quantitatively describe the metabolic responses of freshwater bacterioplankton to variation in the supply of terrestrial-derived DOM. Furthermore, our results suggest that dissolved organic phosphorus (DOP) content, which may be linked to the identity of terrestrial vegetation, is indicative of DOM quality and influences the productivity of freshwater bacterioplankton.

KEY WORDS: Allochthonous · Bacteria · DOC · DOM · Ecosystem · Plankton · Stoichiometry · Subsidy

INTRODUCTION

The metabolic activities of heterotrophic bacteria have important implications for the functioning of aquatic ecosystems. From a biogeochemical perspective, bacterial respiration (BR) constitutes a major carbon flux in freshwater and marine ecosystems (Biddanda et al. 2001, Gonzalez et al. 2003). From a food-web perspective, bacterial productivity (BP) converts reduced carbon into biomass that can be ingested by protists and macrozooplankton (Langenheder & Jürgens 2001, Sherr & Sherr 2002). BP and BR are metabolic activities that are fueled primarily through the consumption of dissolved organic matter (DOM). In aquatic ecosystems, DOM is supplied to bacteria both locally by primary producers (Bertilsson & Jones 2003) and externally by inputs from surrounding terrestrial ecosystems (Aitkenhead-Peterson et al. 2003).

Comparative studies have revealed that BP is positively correlated with net primary productivity (NPP) across fresh- and saltwater ecosystems (Cole et al. 1988), but is usually 70 to 90% less than NPP (Ducklow et al. 2002, Gaedke et al. 2002). This observation suggests that bacterial metabolism may be tightly coupled to the local production of photosynthetically derived DOM. Bacterial carbon demand, however, often exceeds NPP. For example, BR alone is greater than NPP in most oligotrophic waterbodies (del Giorgio et al. 1997) and BP:NPP ratios can sometimes exceed unity in freshwater ecosystems (Jansson et al. 2000, Karlsson et al. 2002, Waiser & Robarts 2004). These results demonstrate that bacterial metabolism can be

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decoupled from local primary productivity. One potential explanation for this decoupling is that bacterial carbon demand is subsidized by terrestrial-derived DOM.

Terrestrial ecosystems export large quantities of DOM to inland, estuarine and coastal marine waterbodies (Goni et al. 1997, Findlay et al. 1998, Neff & Asner 2001). However, terrestrial-derived DOM has traditionally been considered a poor-quality resource for aquatic bacteria because it is relatively old (Raymond & Bauer 2001) and comprises humic compounds with low nutritional content (McKnight & Aiken 1998). Nevertheless, a few lines of evidence indicate that bacterial metabolism may be supported to varying degrees by inputs of terrestrial DOM. First, numerous laboratory studies have directly demonstrated that aquatic bacteria grow on terrestrial fractions of DOM (Tranvik 1988, Bano et al. 1997, Moran & Hodson 1994). Second, as lake DOM concentrations increase, community respiration tends to exceed local primary productivity (Hanson et al. 2003), suggesting that allochthonous carbon may be consumed by heterotrophic bacteria. Third, experimental isotope-enrichment of Wisconsin lakes revealed that 35 to 70% of the bacterial biomass may originate from terrestrial-derived carbon (Kritzberg et al. 2004). Despite these studies, however, the relationship between aquatic bacterial metabolism and terrestrial carbon inputs remains unclear (Findlay 2003).

One potentially important factor that may influence aquatic bacterial metabolism is the bulk concentration of terrestrial DOM. In freshwater ecosystems, bulk DOM concentrations range over 2 orders of magnitude (Mulholland 2003) and can be explained in part by watershed attributes including wetland cover (Engstrom 1987, Gergel et al. 1999), soil properties (Nelson et al. 1993, McClain et al. 1997), drainage area (Rasmussen et al. 1989), forest cover (Goodale et al. 2000, Canham et al. 2004) and hydrology (Hinton et al. 1997, Boyer et al. 2000). Given the high demand for organic carbon by heterotrophic bacteria (Kirchman & Rich 1997), BP and BR may be expected to increase along increasing DOM concentration gradients.

Aquatic bacterial metabolism is also affected by the chemical attributes of different DOM sources, including its oxidation state (Vallino et al. 1996), molecular weight (Weiss & Simon 1999), humic content (Moran & Hodson 1990), percentage of aliphatic compounds (Sun et al. 1997) and the stoichiometric ratios of growth-limiting nutrients (Hunt et al. 2000, Castillo et al. 2003). Most of the DOM in freshwater and coastal ecosystems originates from DOM in the soils of nearby terrestrial ecosystems (Thurman 1985). Soil DOM, which tends to be in equilibrium with solid-phase soil organic matter (Zsolnay 1996, Strobel et al. 2001), is influenced by a number of factors including physical properties, sorption and biodegradation (Qualls & Haines 1992, Chantigny 2003). Soil DOM chemistry is also affected by the identity of the parent vegetation within the watershed (Grieve & Marsden 2001). The chemistry of plant material differs among taxonomic groups (Likens & Bormann 1970) and these differences are often reflected in the local soil organic matter (Preston & Trofymow 2000, Finzi et al. 2001, Quideau et al. 2001) because of litterfall, root exudation and local microbial interactions (Kalbitz et al. 2000). As such, variation in the chemical attributes of DOM due to the heterogeneity of terrestrial ecosystems may in turn affect microbial metabolism in aquatic ecosystems.

This study examined how aquatic bacteria respond to variation in the source and supply of terrestrial DOM. We created different DOM sources from soils under different types of terrestrial vegetation. Then, using batch cultures, we measured BP, BR, bacterial growth efficiency (BGE) and rates of carbon consumption of a lake microbial community on varying concentrations (i.e. supply) of the different DOM sources. In general, we predicted that bacterial metabolism would increase with DOM concentration. However, we also predicted that the relationship between bacterial metabolism and DOM concentration would be modified by the chemical attributes of the different DOM sources. Using a combination of univariate and multivariate statistical approaches, we identified DOM chemical attributes that had a strong influence on aquatic microbial metabolism.

MATERIALS AND METHODS

Soil collection and DOM preparation. Through experimental leaching, we created 6 different terrestrial DOM sources from the organic (Oa/A) soil horizons underneath near-monoculture stands of some of the most common trees in New England forests: pine *Pinus strobus*, hemlock *Tsuga canadensis*, beech *Fagus grandifolia*, maple *Acer saccharum*, birch *Betula alleghaniensis* and *B. populifolia*, and oak *Quercus rubra* and *Q. velutina*. We collected soils from a total of 6 different sites (≤200 m from a stream or lake) located within 100 km of Hanover, New Hampshire, USA. The soils represented a mixture of inceptisols and entisols and, based on texture, were classified as fine sandy loams and sandy loams.

To create each DOM source, we dried the soils at 60°C, pooled equal amounts of soil (100 g total) from multiple sites, and then extracted the organic matter for 48 h in 0.1 N NaOH (Schnitzer 1982). We removed particulate material (>0.7 µm) via serial filtration and then dialyzed (500 Da cellulose ester, Spectrum Labo-

ratories) the leachates in distilled water buffers for 24 h to reduce concentrations of salts and inorganic nutrients (Vinebrooke & Leavitt 1998, Lennon 2004a). We bottled the dialyzed leachates and had them gamma-irradiated (25 kGy dose, SteriGenics International) in order to kill soil-associated microorganisms while still maintaining the chemical integrity of the organic matter (McNamara et al. 2003).

Leachate characterization. We analyzed each DOM source for a suite of inorganic and organic attributes. We measured DOC with a Tekmar-Dohrmann TIC/TOC analyzer after H_2SO_4 digestion, and measured total nitrogen (TN) and total phosphorus (TP) spectrophotometrically after persulfate digestion (APHA 1998). After the filtration and dialysis procedures, we assumed that TN and TP represented dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP), respectively. In addition, we measured 15 major elements with inductively coupled plasma (ICP) atomic emission spectroscopy (AES) on Spectro Analytical Model FTM-08 ICPOES (see Table 1). We measured polyphenolic compounds using the Prussian Blue assay (Price & Bulter 1977) against a tannic acid standard (Herrera-Silveira & Ramirez-Ramirez 1996, Thoss et al. 2002) and humic acids as the amount of precipitated DOC after acidifying the leachate to pH 2 with H_3PO_4. We measured protein content using the bicinchoninic acid (BCA) method (Walker 1996) with a 'Compat-Able Protein Assay Preparation' kit (Pierce Biotechnology) and high molecular weight DOM (HMWDOM) as the amount of DOC retained in 12 000 to 14 000 Da dialysis tubing after 24 h dialysis.

We tested for differences in chemical attributes among DOM sources using both univariate and multivariate statistics. For individual chemical attributes, we used univariate 1-way ANOVA with Tukey's HSD test. We adjusted the α value from 0.05 to 0.0028, with a Bonferroni correction to account for multiple (18) comparisons. We also used principal component analysis (PCA) to describe the chemical characteristics of the DOM sources using 16 of the 18 chemical attributes (magnesium and manganese were excluded because of missing data). We used a correlation matrix of untransformed mean values in the PCA analysis because it standardizes data and thus does not overemphasize large values (Manly 1994).

Experimental design. We used batch cultures to quantify BP, BR, BGE and relative rates of carbon consumption on different sources and supplies of DOM. Each experimental unit consisted of a 1 l polycarbonate bottle filled with 450 ml of 0.22 µm-filtered lake water. We then inoculated each experimental unit with a 50 ml aliquot of 2.7 µm-filtered (Whatman GF/D) lake water containing a natural microbial assemblage. We obtained lake water and the microbial community from Norford Lake (Orange County, Vermont), which is an oligo-mesotrophic water body (chlorophyll *a* = 2.8 to 5.1 µg l^{-1}) with relatively low DOC (250 µM; Lennon 2004b). We manipulated DOM supply by exposing 11 experimental units to a concentration gradient ranging from 250 to 1400 µM DOC. Using an unreplicated regression design, we then crossed the DOM supply manipulation with 6 different DOM sources (pine, hemlock, beech, maple, birch and oak). We incubated all 66 experimental units in environmental chambers at 20°C without light. We estimated BP 36 h after the initiation of the experiment by measuring the uptake and incorporation of ^{3}H-leucine into bacterial protein (Kirchman 1993). We estimated BR as the change in dissolved oxygen concentrations between 24 and 48 h using spectrophotometric methods with corrections for color interference (Roland & Cole 1999) created by each of the different DOM sources. We used a respiratory quotient of 1 to convert oxygen consumption to carbon evolution. We calculated BGE as (BP)/(BP + BR). In addition to measuring the absolute rates of bacterial metabolism we also calculated the relative rates of carbon consumption as (BP + BR)/DOC.

We used multiple linear regression with indicator variables to evaluate the response of BP, BR, BGE and relative rates of carbon consumption to different sources and supplies of DOM. We used DOM concentration (measured as DOC) as a continuous predictor and DOM source as a qualitative predictor of bacterial metabolism (see indicator variables in Neter et al. 1996, p. 455–490). We examined residuals for assumptions of normality and homogeneity of variance, and used diagnostic tests (Studentized residuals, leverage, Cook's *D*, and Dffits) to identify potential outliers. We then calculated confidence limits (CL) around the differences between the slopes and intercepts for each possible pairing of DOM sources to determine whether DOM source affected bacterial metabolism along the supply gradient:

$$\mathrm{CL} = (b_i - b_j) \pm \left(t_{\alpha[n-1]} \cdot \sqrt{\sigma^2_{b_i} + \sigma^2_{b_j} - 2\left(\sigma_{b_i b_j}\right)} \right) \quad (1)$$

where b_i and b_j are parameter estimates for 2 of the 6 DOM sources, t is the critical value from the Student's t-distribution for a given α and sample size (n) used to create the parameters, $\sigma^2_{b_i}$ and $\sigma^2_{b_j}$ are the variances for each parameter estimate, and $\sigma_{b_i b_j}$ is the covariance between the 2 parameter estimates (Neter et al. 1996). We adjusted α with a Bonferroni correction to account for the 15 pairwise comparisons to determine whether different DOM sources affected the parameters of each metabolic response variable. We concluded that bacterial metabolism was not affected by DOM sources if adjusted confidence limits (99%) around the difference of parameter estimates ($b_i - b_j$) contained zero.

Table 1. Chemical attributes of 6 different DOM sources used in bacterioplankton regrowth experiments. Values are mean (±SE) of replicate samples from each DOM stock solution. Concentrations of all chemical attributes expressed as µmol $mmol^{-1}$ C for each respective DOM source. We used univariate 1-way ANOVA with Tukey's HSD test to examine chemical differences between DOM sources and adjusted the α value from 0.05 to 0.0028 with a Bonferroni correction to account for the 18 multiple comparisons. Shared letters within a row indicate DOM sources with similar chemical concentrations. *p-values that would be significant at an α of 0.05, but were not considered significant after Bonferroni correction; –: concentrations outside range of our standard curve; HMWDOM: high molecular weight dissolved organic matter

Element/ Compound	Beech	Oak	Hemlock	Birch	Maple	Pine	ANOVA results df	*F*	p
Aluminum	3.8 (0.08) c	7.1 (0.41) b	18.3 (1.01) a	6.9 (0.31) b	1.2 (0.04) d	5.4 (0.14) bc	5, 17	160.4	<0.0001
Cadmium	4.3E-04 (8.6E-05) ab	3.2E-04 (1.1E0-4) b	4.3E-04 (7.5E-05) ab	5.4E-04 (1.4E-04) ab	8.6E-04 (7.5E-05) a	4.3E-04 (7.5E-05) ab	5, 17	3.39	0.0386*
Calcium	9.4 (0.47) c	14.2 (0.90) b	9.7 (0.83) c	7.5 (0.68) c	36.3 (1.4) a	6.5 (0.62) c	5, 26	152.2	<0.0001
Chromium	7.0E-3 (9.3E-4) b	7.0E-03 (1.2E-03) b	1.8E-02 (8.3E-04) a	1.4E-02 (1.9E-03) a	9.3E-04 (2.3E-02) c	7.0E-03 (2.3E-04) b	5, 17	46.0	<0.0001
Copper	2.7E-02 (7.6E-04) ab	3.8E-02 (1.3E-03) a	3.0E-02 (3.8E-04) ab	1.5E-02 (5.7E-04) bc	3.6E-03 (1.3E-03) a	4.2E-02 (8.2E-03) a	5, 17	7.11	0.0026
HMWDOM	852 (15.3) a	877 (46.0) a	867 (62.5) a	683 (8.2) a	759 (33.7) a	759 (33.7) a	5, 16	3.23	0.049*
Humic acid	294 (7.4) c	367 (6.1) ab	399 (2.8) a	384 (7.3) ab	335 (13.1) bc	369 (19.1) ab	5, 17	12.6	0.0002
Iron	4.6 (0.07) c	3.8 (0.06) d	8.9 (0.01) a	8.1 (0.02) b	1.4 (0.03) f	3.4 (0.04) e	5, 19	2045	<0.0001
Lead	1.5E-02 (7.0E-04) b	3.5E-03 (1.2E-04) d	2.0E-02 (2.9E-04) a	1.9E-02 (9.9E-04) a	4.1E-03 (7.0E-04) d	1.1 E-02 (6.4E-02) c	5, 17	127.4	<0.0001
Magnesium	0.19 (0.006) d	1.29 (0.069) b	–	–	3.37 (0.079) a	1.04 (0.030) c	3, 11	594.5	<0.0001
Manganese	0.68 (0.02) b	0.90 (0.026) a	0.53 (0.009) c	0.86 (0.022) a	–	0.44 (0.020) c	4, 14	95.2	<0.0001
Total N	79.1 (1.09) a	92.4 (4.90) a	70.3 (2.88) b	77.7 (2.63) a	83.0 (4.52) a	79.6 (1.37) a	5, 23	6.88	0.0112*
Total P	7.3 (0.11) a	7.1 (0.12) ab	6.7 (0.13) b	5.7 (0.17) c	3.7 (0.11) e	4.2 (0.09) d	5, 17	276.9	<0.0001
Polyphenol	2.6 (0.03) ab	2.2 (0.02) d	2.6 (0.07) ab	2.7 (0.02) a	2.5 (0.03) bc	2.5 (0.09) bc	5, 17	29.3	<0.0001
Potassium	3.2 (0.09) b	4.0 (0.01) a	2.5 (0.03) c	2.0 (0.07) d	2.7 (0.04) c	2.7 (0.04) c	5, 17	162.2	<0.0001
Protein	3.2 (0.12) d	4.6 (0.14) a	3.4 (0.08) cd	3.5 (0.05) bcd	3.8 (0.07) bc	3.9 (0.13) b	5, 17	24.4	<0.0001
Sodium	169 (1.9) c	183 (2.1) b	233 (3.1) a	98 (1.3) f	107 (1.3) e	127 (1.5) d	5, 25	827.1	<0.0001
Zinc	0.07 (0.001) b	0.04 (0.002) d	0.04 (0.001) d	0.06 (0.001) c	0.06 (0.001) c	0.08 (0.001) a	5, 17	135.3	<0.0001

Table 2. Weightings for the first 3 principal components (PC) generated from a matrix of 16 chemical attributes for 6 different DOM sources. Chemistry data represent untransformed concentrations scaled between leachates to µmol mmol^{-1} C. HMWDOM: high molecular weight dissolved organic matter

Chemical attributes	PC 1	PC 2	PC 3
Total nitrogen	−0.303	0.095	0.210
Copper	−0.265	0.163	0.054
Protein	−0.259	0.200	0.338
Calcium	−0.247	−0.137	0.068
Potassium	−0.218	0.351	−0.177
Cadmium	−0.102	−0.356	0.133
Zinc	−0.044	−0.311	−0.271
HMWDOM	−0.024	0.420	−0.223
Sodium	0.143	0.400	−0.088
Humic acids	0.161	0.085	0.572
Total phosphorus	0.179	0.221	−0.448
Aluminum	0.287	0.244	0.209
Polyphenolics	0.290	−0.297	−0.109
Chromium	0.352	0.095	0.229
Iron	0.369	0.069	0.128
Lead	0.381	−0.070	−0.042
% variance explained	42	29	15

We defined the slopes of BP and BR versus DOC as rates of carbon-specific metabolism, then attempted to explain variance in carbon-specific metabolism as a function of DOM chemistry. First, we used the output from the PCA of the DOM chemistry to explain carbon-specific metabolism. We used eigenvectors from the first 3 principal component (PC) axes as individual predictors of the BP–DOC and BR–DOC slopes. Second, we used individual chemical attributes as predictors of carbon-specific metabolism. We narrowed down the number of individual predictors by constructing a correlation matrix comparing all of the chemical attributes to the BP–DOC and BR–DOC slopes. We considered all individual chemical variables that had high correlation coefficients (>|0.7|, see Fowler et al. 1998) with BP–DOC and BR–DOC slopes as potential predictors of carbonspecific metabolism. We used curve-fitting (SigmaPlot Version 8.0, 2002) to describe the relationships between carbon-specific metabolism and the selected group of chemical predictor variables. We used SAS (SAS 1999) for all other statistical procedures.

RESULTS

Chemical composition of DOM

The DOM sources were chemically different from one another. All the individual chemical attributes were different at $\alpha = 0.05$ (1-way ANOVA), but cadmium, HMWDOM, and TN were not significantly different after applying a Bonferroni correction for multiple comparisons (Table 1). PCA also indicated that the 6 DOM sources had different chemical compositions. The first 3 PC axes explained 86% of the chemical variation in the different DOM sources (Table 2, Fig. 1). Hemlock DOM was positively correlated with PCA Axis 1 (iron and lead), while maple DOM was negatively correlated with PCA Axis 1 (TN). Oak DOM was positively correlated with PCA Axis 2 (HMWDOM and sodium), while birch and maple DOM were negatively correlated with PCA Axis 2 (cadmium and zinc). Finally, beech DOM was negatively correlated with PCA Axis 3 (TP).

Bacterial productivity

Bacterial productivity was affected by both DOC concentration and the different DOM sources. The

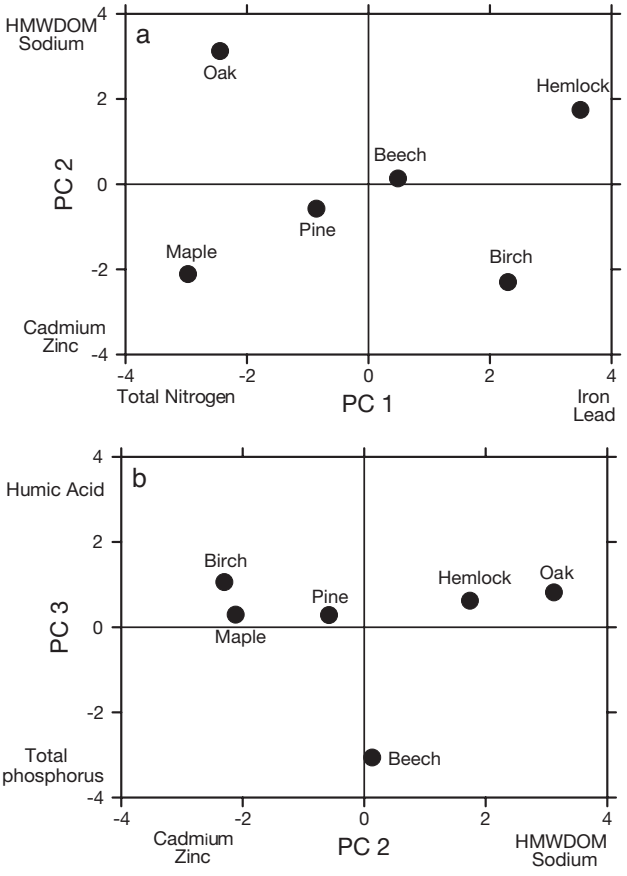

Fig. 1. Principal component analysis (PCA) of chemical attributes of 6 terrestrial DOM sources used in the bacterioplankton batch culture experiments. Data points shown for first 3 principal component axes. (a) PC2 versus PC1, (b) PC3 versus PC2. HMWDOM: high molecular weight dissolved organic matter

Table 3. Parameter estimates for bacterial productivity and bacterial respiration (mean ± SE) along a DOC concentration gradient for 6 different DOM sources. Slope (carbon-specific metabolism) is in µmol C l^{-1} h^{-1} $mmol^{-1}$ DOC and intercepts in µmol C l^{-1} h^{-1}. Shared letters within a column identify parameters not statistically different from each other as determined by pairwise comparisons with 99% confidence limits

DOM source	Bacterial productivity						Bacterial respiration					
	Intercept			Slope			Intercept			Slope		
Beech	0.17	(0.136)	ab	1.8	(0.24)	a	−0.29	(0.358)	a	2.2	(0.55)	a
Oak	−0.01	(0.121)	a	1.6	(0.20)	ab	−0.23	(0.366)	a	1.8	(0.60)	a
Hemlock	0.49	(0.120)	b	1.1	(0.19)	abc	0.16	(0.363)	a	1.9	(0.59)	a
Birch	0.30	(0.082)	ab	0.8	(0.12)	bc	−0.20	(0.247)	a	1.5	(0.36)	a
Maple	0.27	(0.127)	ab	0.6	(0.22)	c	0.33	(0.384)	a	1	(0.67)	a
Pine	0.42	(0.082)	b	0.6	(0.17)	c	−0.12	(0.354)	a	1.8	(0.53)	a

indicator variables multiple regression model explained 88% of the variation in BP ($F_{11,71}$ = 37.8, p < 0.0001). Carbon-specific productivity was approximately 3× higher on beech and oak DOM sources than on maple and pine DOM sources (Table 3). Carbon-specific productivity was intermediate on hemlock and birch DOM sources and could not be statistically distinguished from carbon-specific productivity on the other DOM sources (Table 3).

We removed one BP data point from the multiple regression model. BP in the beech treatment measured 1.5 µmol l^{-1} h^{-1} at 1250 µm DOC, creating a humped-shape relationship (see Fig. 2). Diagnostic checks from the multiple regression (Studentized residuals, leverage, Cook's *D*, and Dffits) indicated that this data point weighed heavily in the analysis. When this point was included, however, we could fit the data well for this DOM source by including a polynomial term in a regression analysis:

$$BP_{(beech)} = -0.4 + 0.005\,(DOC) - 0.000005\,(DOC)^2,\quad R^2 = 0.93,\ p < 0.0001 \qquad (2)$$

Eigenvectors from the PCA were poor predictors of carbon-specific productivity (p > 0.05). Instead, our correlation analyses identified 3 chemical attributes as potential predictors of carbon-specific productivity: TP (r = 0.90), HMWDOM (r = 0.73), and potassium (r = 0.72). We did not identify models where HMWDOM or potassium was a significant predictor of carbon-specific productivity (p > 0.05), but carbon-specific productivity was explained by phosphorus content. We represent this relationship as an exponential function of the carbon:phosphorus ratio (C:P) of the DOM sources (see Fig. 3):

$$\text{Carbon-specific productivity} = 0.05 + 329e^{(-0.05 \cdot C:P)},\quad R^2 = 0.99,\ p < 0.0003 \qquad (3)$$

Phosphorus content was not correlated with any of the other chemical attributes measured on the 6 DOM sources (p > 0.05).

Bacterial respiration

BR increased significantly over the DOC concentration gradient, but was not affected by DOM source (Fig. 2, Table 3). Multiple regression using DOC concentration and DOM source as predictors explained 67% of the variation in BR ($F_{11,70}$ = 10.9, p < 0.0001). We did not attempt to use DOM chemical attributes to explain variance in carbon-specific respiration because BR was not significantly affected by the different DOM sources (Table 3).

Bacterial growth efficiency

BP and BR were significantly correlated with one another in all treatments except maple DOM (Table 4). As such, BGE was not affected by DOM concentration or DOM source (R^2 = 0.23, $F_{11,63}$ = 1.4, p = 0.20). BGE averaged 0.54 ± 0.022 (SE) for all samples.

Relative rates of carbon consumption

The relative rates of carbon consumption were not affected by the concentration of DOC, but were apparently influenced by the source of DOM (R^2 = 0.39;

Table 4. Correlation between bacterial respiration (BR) and bacterial productivity (BP) for 6 different sources of terrestrial-derived DOM

DOM source	Correlation coefficient (r)	p-value
Pine	0.62	0.0404
Birch	0.80	0.0032
Hemlock	0.68	0.0203
Beech	0.90	0.0004
Oak	0.78	0.0045
Maple	0.07	0.8473

$F_{11,64} = 3.05$, $p < 0.003$). Relative rates of carbon consumption were significantly higher on hemlock DOM when compared to the rates of the other pooled DOM sources ($p = 0.004$). However, pairwise comparisons revealed that there were no significant differences in the relative rates of carbon consumption among the different DOM sources. Relative rates of carbon consumption ranged from 1 to 18% d^{-1}; the average rate for all samples was 7 ± 0.3% d^{-1} (SEM).

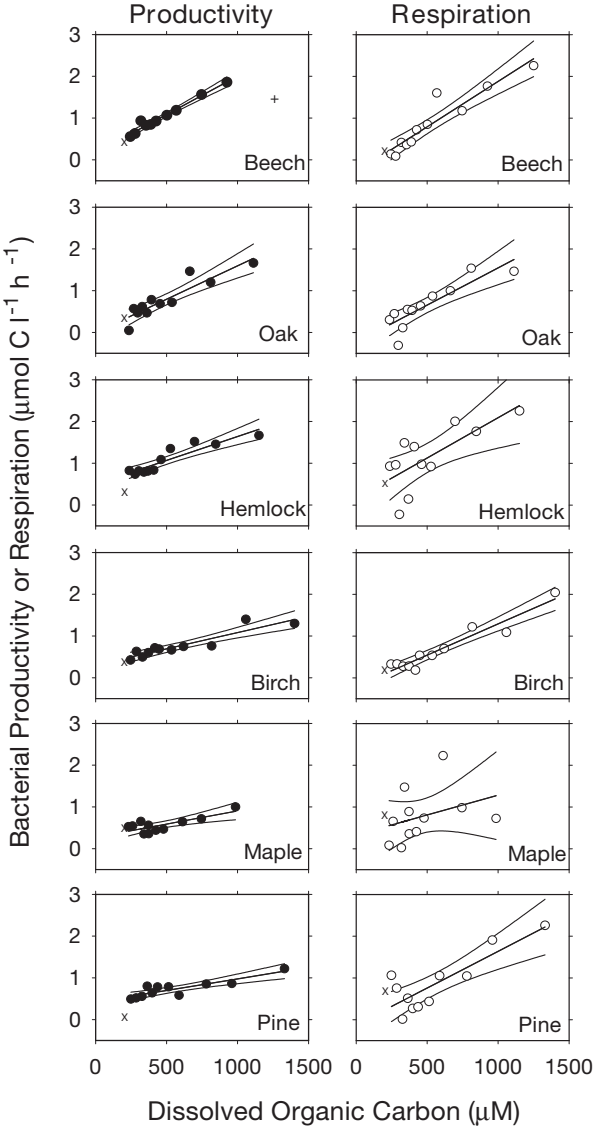

Fig. 2. Bacterial productivity and bacterial respiration in response to different concentrations and sources of terrestrial-derived DOM. Graphs presented in descending order of carbon-specific bacterial productivity (i.e. the slopes). Lines: predicted values and 95% confidence intervals from multiple regression models; +: productivity outlier detected and removed from multiple regression analysis, but included in a separate polynomial regression (see second subsection of 'Results'); ×: bacterial productivity and bacterial respiration measurements in ambient lake water not included in multiple regression analyses

DISCUSSION

Terrestrial-derived DOM can serve as carbon and nutrient subsidies for bacterioplankton in both freshwater and marine ecosystems. The consumption of this allochthonous resource should influence bacterial metabolism and may in turn have consequences for food webs and the biogeochemistry of aquatic ecosystems. This study examined how variation in the source and supply of DOM affects the metabolism of lake bacterioplankton. We estimated that bacteria consumed approximately 7% of the bulk DOC pool, which is less than the consumption reported for some lakes (14%), rivers (19%), and marine environments (19%) (Søndergaard & Middelboe 1995). Nevertheless, both BP and BR increased linearly with increasing supply of terrestrial DOM. In addition, BP was strongly affected by the DOM source. In particular, DOP content was a good predictor of DOM quality for BP.

Bacterial metabolism and DOM supply

We predicted that BP and BR would generally increase with additions of DOM because heterotrophic bacterioplankton are often carbon-limited (Kirchman & Rich 1997). However, we did not assume that metabolic rates would be constant along the DOM gradient. For example, radiotracer studies (i.e. ^{14}C-labeled glucose, leucine, acetate) commonly reveal that that carbon uptake rates of microbial communities saturate at high substrate concentrations according to Michaelis-Menten dynamics (Wright & Hobbie 1965, Garnier & Benest 1991, Münster 1993). Such kinetic constraints could affect resource uptake and subsequent biomass production. In addition, the functional relationship between metabolism and DOM could be shaped by the relative concentrations of co-limiting nutrients (e.g. nitrogen, phosphorus, etc.). However, we found little evidence that bacterial metabolism saturated with increasing DOM supply. Instead, both BP and BR increased linearly along the experimental supply gradient (Table 3, Fig. 2).

Only in the beech treatment did it appear that metabolic rates might have changed along the DOM supply gradient (Fig. 2). At the highest DOC concentration, BP in the beech treatment was 63% lower than predicted based on the linear equation in Table 3. The inclusion of the outlier data point resulted in a hump-shaped relationship so that BP actually decreased at the highest DOC concentration instead of saturating at a maximum level of productivity. In contrast, BR remained linear along the DOC gradient, suggesting that BP and BR were decoupled at high levels of metabolism in this treatment.

The positive relationship between bacterial metabolism and DOM supply in our study is consistent with some trends found in freshwater and marine ecosystems. For example, both BP and BR of coastal microbial communities increased near linearly when exposed to 3 levels of high molecular weight carbon (>1 kDa) ranging from 116 to 302 µM DOC (Amon & Benner 1996). Similarly, BR in the Bay of Biscay increased over a relatively narrow DOC gradient (72 to 109 µM) that was associated with a coastal upwelling event (Gonzalez et al. 2003). In lake communities, bacterial biomass production increased with experimental additions of aged humic material (Eiler et al. 2003). Finally, cross-system analyses of batch culture experiments have shown that the proportion of labile DOC (Søndergaard & Middelboe 1995) and the rate of DOC consumption (del Giorgio & Davis 2003) increase with increasing bulk DOC concentration.

However, an equally large number of studies have failed to detect patterns between bacterioplankton metabolism and bulk DOM concentration. For example, there was no relationship between either bacterial growth or BP along a DOC gradient (208 to 758 µM) in 20 Quebec lakes (Smith & Prairie 2004). Likewise, BP was unaffected by DOC concentrations in tropical coastal lagoons (Farjalla et al. 2002). In tidal freshwater stretches of the Hudson River, 10 years of data have revealed that there is no relationship between BP and DOC (125 to 583 µM) (Findlay 2003). The null relationships found in this latter group of studies suggest that factors besides bulk DOM content, such as DOM chemistry, may be important in driving the metabolism of heterotrophic bacterioplankton.

Contrary to our initial predictions, BGE did not increase over our DOM concentration gradient. Under low DOM supplies, we anticipated that most assimilated carbon would be devoted to maintenance costs, resulting in low BGE (Russell & Cook 1995). Occasionally, BGE does not increase with substrate concentration because resources are not always sufficiently energy-rich for bacteria to reduce all of the available organic matter into cellular carbon (del Giorgio & Cole 1998). However, this explanation cannot account for the lack of pattern between BGE and DOM since BP always responded positively to increased DOM concentrations (Fig. 2, Table 3). Instead, the lack of a BGE response in this study most probably reflects our experimental approach. Bacterial growth is often unconstrained on a given resource in batch cultures that are conducted over relatively short time periods. Under such conditions, BP and BR will often be correlated (e.g. Table 4), leading to a relatively constant BGE. Additionally, a lack of a relationship between DOC and BGE may have arisen if bacterial growth rates were decoupled from ^{3}H-leucine incorporation rates. In general, our results are consistent with the empirical trend that BGE plateaus at approximately 0.5 when BP is >0.4 µmol C l^{-1} d^{-1} (del Giorgio & Cole 1998).

Chemical attributes of different DOM sources

Our leaching approach created DOM sources with different chemical characteristics (Tables 1 & 2, Fig. 1). It is important to note, however, that it was not our explicit objective to test hypotheses about how the identity of terrestrial vegetation influences DOM chemistry. Nevertheless, attributes of our DOM sources are consistent with some vegetation–soil chemistry patterns reported in the literature. For example, our hemlock leachate had high concentrations of aluminum, iron and lead (Table 1, Fig. 1), consistent with soils in coniferous forests (David & Driscoll 1984, LaZerte & Scott 1996) and probably reflecting the fact that metals are mobilized by the production of organic acids in hemlock stands (Dijkstra et al. 2001). Conifers also have low foliar nitrogen relative to most deciduous species and consequently produce litter with high C:N ratios (Templer et al. 2003). This pattern was reflected in our hemlock leachate (lowest nitrogen concentration), but not in the pine leachate (intermediate nitrogen concentration). Finally, our maple DOM source had relatively high concentrations of nitrogen, calcium and magnesium, but low concentrations of aluminum, consistent with the chemical attributes commonly associated with soils in sugar maple stands (Minocha et al. 2000, Dijkstra & Smits 2002, Lovett & Mitchell 2004).

Despite similarities between our DOM chemistry and the soil chemistry patterns in the literature, we must be cautious about using our data to link specific forest compositions to the metabolism of terrestrial DOM by aquatic microorganisms. First, there were most likely some biases in our extraction process, although the percentages of HMWDOM and polyphenolic compounds are generally consistent with other reports (e.g. Thurman 1985, Engelhaupt & Bianchi 2001). Second, our approach does not address whether the chemical signal of different DOM sources would be preserved along land–water flowpaths of DOM (see Sommer et al. 1997). For example, soil microorganisms may modify chemical attributes of DOM (Smolander & Kitunen 2002, Marschner & Kalbitz 2003) before it reaches aquatic ecosystems.

Bacterial metabolism on different DOM sources

Variation in the chemical composition of DOM can modify aquatic microbial metabolism (e.g. Vallino et al. 1996, Sun et al. 1997). Despite variation in a suite of

chemical attributes, our results suggest that the phosphorus content of DOM is an important determinant of BP. We observed a 3× exponential decline in carbon-specific productivity with decreasing phosphorus content when expressed as a C:P ratio (Fig. 3). Since we did not manipulate phosphorus directly, it is possible that this trend represents a correlation with other unmeasured attributes of our DOM sources. However, multiple lines of evidence suggest that phosphorus content should be an important factor influencing DOM quality for aquatic bacteria.

Phosphorus often limits or co-limits bacterial metabolism in freshwater (Currie & Kalff 1984, Pace & Cole 1996, Drakare 2002) and marine ecosystems (Rivkin & Anderson 1997, Sala et al. 2002). The high phosphorus demand of aquatic bacteria is reflected by their relatively low biomass C:P ratios (8 to 173:1 by moles; Chrzanowski et al. 1996, Hochstadter 2000, Vrede et al. 2002). In contrast, water-column ratios of DOC:DOP are typically much higher (400 to 800:1 by mol; Hopkinson et al. 1997, McKnight et al. 1997). The C:P ratios of our DOM sources were slightly lower (137 to 275:1, by mol), but were still higher than Redfield values (106:1 by mol).

As a result, carbon-specific productivity was relatively high on DOM sources with low C:P ratios. In contrast, carbon-specific productivity dropped when substrate C:P ratios moved outside the reported stoichiometric range required for optimum bacterial biomass production (8 to 173:1 by mol; Fig. 3). Together, this information suggests that phosphorus demands of rapidly growing bacteria may be alleviated by the natural variation found among different sources of terrestrial DOM. In addition, higher DOP may allow bacteria to consume more recalcitrant carbon molecules (Benner et al. 1988), possibly through increased extracellular enzyme production (Sinsabaugh et al. 1997).

In contrast with BP, we found no effect of different DOM sources on carbon-specific respiration. There are 2 potential explanations for this lack of pattern. First, it is possible that BR might not be affected by different DOM sources because of a slight decoupling of catabolic and anabolic respiratory reactions (del Giorgio & Cole 1998). BP and BR were not perfectly correlated (Table 4), and thus anabolic respiratory costs could be masked by the independent effect of different DOM sources on catabolic respiratory costs. Second, we may not have been able to detect an underlying effect of different DOM sources because of the inherent variability associated with BR measurements (see del Giorgio & Cole 1998). For example, the coefficient of variation (CV) for replicate BP measurements on an experimental unit was only 3%, whereas the CV for replicate BR measurements on an experimental unit was 35% (data not shown). This variability should not influence our parameter estimates (Miller 1986), but would increase the confidence interval around the parameter estimates and thus reduce our ability to detect significant effects of different DOM sources on BR. Obviously this variability in BR also affects our estimates of BGE.

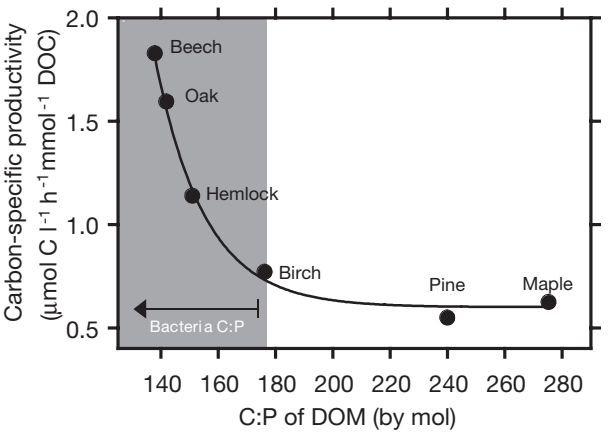

Fig. 3. Carbon-specific bacterial productivity (slopes of the bacterial productivity versus DOC relationship in Fig. 2 & Table 3) declined exponentially with C:P ratio of different DOM sources. Shaded area: range of C:P ratio of aquatic bacterial biomass reported in the literature (Chrzanowski et al. 1996, Hochstadter 2000, Vrede et al. 2002)

In contrast with a number of studies, BGE was not affected by DOM source with varying chemical attributes. For example, BGE can be influenced by DOM characteristics such as mineral nutrient content (Benner et al. 1988), molecular weight (Tranvik 1990), amino acid content (Carlson & Ducklow 1996) and carbon:nutrient ratios (Goldman & Dennett 2000). However, these patterns are not always consistent between studies (del Giorgio & Cole 1998).

Often, BGE is correlated with factors that drive BP. For example, BP was more sensitive to environmental conditions than BR in the Hudson River, and thus BP was largely responsible for the observed changes in BGE (Roland & Cole 1999). However, in the present study, the magnitudes of response for BP and BR to DOM manipulations were roughly equal (Table 4). As such, BGE remained relatively constant, despite substantial variation in key chemical attributes of DOM that sometimes affect bacterial metabolism.

In theory, if BP changed with DOM quality and BR remained constant (e.g. Fig. 2, Table 3), then one would predict that BGE must have also changed with DOM quality. We were unable to detect this pattern with our data. As mentioned above, the variability of our BR measurements may have prevented us from detecting an underlying effect of DOM quality on BGE. However, the predicted values generated from our multiple regression analyses represent unbiased esti-

mates of BP and BR. A slightly different pattern emerged when we estimated BGE from predicted instead of observed BP and BR. Predicted BGE ranged from 0.25 to 0.47 and qualitatively declined with increasing C:P of the DOM sources, although this relationship was not statistically significant ($p = 0.19$, $n = 6$). An analysis with more power may have revealed that there was a true relationship between BGE and the phosphorus content of DOM.

CONCLUSION

Our results contribute to a growing body of literature which documents the significance of terrestrial DOM as a carbon subsidy for microorganisms in aquatic ecosystems. More importantly, our findings indicate that inputs of certain DOM sources alleviate phosphorus-limited bacterial biomass production. DOM sources from beech, oak and hemlock soils had elevated concentrations of phosphorus that resulted in relatively high rates of carbon-specific productivity. These results suggest there may be strong links between the terrestrial vegetation, soil chemistry, and aquatic microbial metabolism. However, there is currently a lack of data linking vegetation type and phosphorus content of soil DOM (Chantigny 2003). In addition, more detailed studies are needed to explore the dynamics of DOM chemistry along watershed flowpaths. For example, it is well known that soils have a high phosphorus sorption capacity (Frossard et al. 1995) relative to other nutrients and that soil microorganisms modify DOM chemistry prior to being exported to nearby waterbodies (Marschner & Kalbitz 2003). Comparative surveys of aquatic bacterial metabolism in watersheds with contrasting forest composition would be one useful approach to test the results of this laboratory study.

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