Limnol. Oceanogr., 49(5), 2004, 1703–1712

Seasonal bioavailability of dissolved organic carbon and nitrogen from pristine and polluted freshwater wetlands

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Abstract

We examined the chemical composition and bioavailability of dissolved organic carbon (DOC) and nitrogen (DON) from two pristine and two polluted cedar bog wetlands across three seasons. Pristine and polluted wetlands differed in DOC and DON concentrations, chemical characteristics, and bioavailability. DOC and DON concentrations were higher in the polluted than in the pristine wetlands. In contrast, a higher percentage of the dissolved organic matter (DOM) was more aromatic in the pristine (54% ± 19) than in the polluted (27% ± 4) wetlands. A higher percentage of DOC was bioavailable in the pristine (22% ± 9) than the polluted (12% ± 4) wetlands. A similar percentage of DON was consumed in both the pristine (33% ± 25) and polluted (28% ± 25) wetlands. Seasonally, the bioavailability of DOC and DON varied and differed between the pristine and polluted wetlands. The availability of phosphate appeared to affect the amount of DOC incorporated into bacterial biomass, whereas inorganic nutrient availability did not affect the assimilation of DON. Bioavailable DOC primarily fueled bacterial respiration, whereas DON supported bacterial growth. Overall, our results demonstrate that anthropogenic activities and season affect the quantity and quality of wetland DOM exported to rivers and that different factors control the utilization and fate of DOC and DON within the bacterial community.

Carbon and nitrogen are important components of riverine dissolved organic matter (DOM). Dissolved organic carbon (DOC) is often the largest organic carbon pool in rivers (Schlesinger and Melack 1981). Likewise, dissolved organic nitrogen (DON) can comprise up to 90% of the total dissolved nitrogen (TDN) concentration and export from some rivers (e.g., Seitzinger and Sanders 1997). Recent studies have shown that DOM is metabolically important in rivers and estuaries; it supplies energy (carbon) and nutrients (nitrogen) to bacteria and some algae and potentially contributes to coastal eutrophication and hypoxia (e.g., Seitzinger and Sanders 1997; Stepanauskas et al. 1999; Glibert et al. 2001; Wiegner and Seitzinger 2001).

Riverine DOM is composed of organic matter from terrestrial, atmospheric, and autochthonous sources. The bioavailability of DOM from these sources differs from one another and varies seasonally (e.g., Wiegner and Seitzinger 2001; Seitzinger et al. 2002). Wetlands are an important source of DOM to rivers and estuaries (Mulholland 2003). Riverine DOC concentrations and fluxes are significantly correlated with the percentage of wetland area, peat cover, and stream channel slope, a parameter that varies inversely with wetland cover (Mulholland 2003). DOC export is also higher in rivers that drain lowlands dominated by wetlands than uplands dominated by forests (Mulholland 2003). Wetlands are a net source of DON to rivers in the few systems that have been examined (Devito et al. 1989; Leonardson et al. 1994). Despite the importance of wetlands as a DOM source to rivers, their importance as both a bioavailable DOC and DON source to rivers on an annual and seasonal scale is not well established.

Riparian wetlands are important transitional zones between the terrestrial and aquatic environment. They alter the forms of nitrogen in upland runoff and groundwater before it reaches the stream channel (e.g., Lowrance et al. 1984); riparian wetlands are often a net sink for dissolved inorganic nitrogen (DIN) and a net source for DON (e.g., Devito et al. 1989; Leonardson et al. 1994). These nitrogen transformations affect water quality in downstream systems (e.g., Lowrance et al. 1984). Anthropogenic activities either adjacent to or upland from riparian wetlands, such as agriculture and urban development, affect how inorganic nitrogen cycles in these ecosystems (e.g., Seitzinger 1994; Zhu and Ehrenfeld 2000). The effect of anthropogenic activities on the quantity and quality of DON and DOC exported from wetlands is not known.

The bioavailability of the bulk and humic fraction of wetland DOC has been examined in a number of studies (e.g., Satoh and Abe 1987; Moran and Hodson 1990); fewer studies have examined the bioavailability of wetland DON (Stepanauskas et al. 1999), and no work to date has studied the bioavailability of wetland DOC and DON simultaneously. Furthermore, the effects of season and anthropogenic activ-

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Acknowledgments

R. Lauck, R. Styles, M. Calisto, J. Pierce, T. Minegishi, T. Komada, and P. Bergmann assisted with field and laboratory work. R. Zampella graciously supplied watershed basin information for the wetland sites. C. Dow kindly constructed a map of the research site locations. P. Glibert, C. Reimers, O. Schofield, K. Rowley, and three anonymous reviewers provided comments that helped strengthen this manuscript.

This research was conducted under a graduate research fellow award (NA97OR0150) from the Estuarine Reserves Division, Office of Ocean and Coastal Resource Management, National Ocean Service, National Oceanic and Atmospheric Administration. Financial support while writing this manuscript was provided by the Institute of Marine and Coastal Sciences of Rutgers University, the Natural Science Foundation (grant DEB-0109122), and the Stroud Water Research Center.

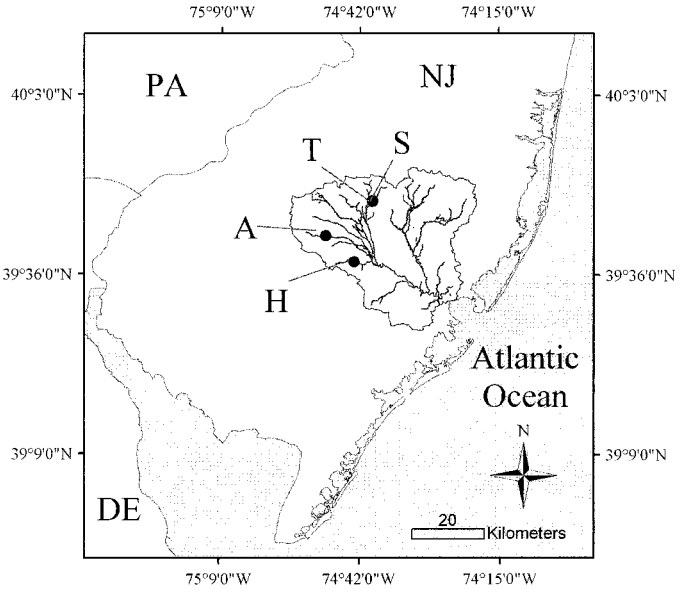

Fig. 1. Map of cedar bog sites in the Mullica River watershed in the Pine Barrens region of New Jersey. S: the pristine cedar bog located next to Skit Branch (Pristine-1), T: the pristine cedar bog located next to Tom Roberts Branch (Pristine-2), A: the polluted cedar bog site located next to Albertson Brook (Polluted-1), H: the polluted cedar bog site adjacent to Hammonton Creek (Polluted-2). The map was generated using data from New Jersey Department of Environmental Protection (1991/1997 land use/cover update 2001) and Arc View 3.X software, Environmental Systems Research Center Institute.

ities on the bioavailability of wetland DOM and its contribution to the bioavailable riverine DOM pool are unknown. Understanding how DOC and DON cycle relative to one another in rivers is important, because this process affects the quality of DOM exported downstream and its subsequent role in riverine and estuarine metabolism and food webs. We examined the bioavailability of DOC and DON from two pristine and two polluted freshwater wetlands to riverine bacteria across three seasons.

Materials and Methods

Site description—Water for the DOM bioavailability experiments was collected from two pristine and two polluted Atlantic white cedar bogs (*Chamaecyparis thyoides*) located in the Mullica River watershed, in the Pine Barrens region of New Jersey, during the spring (March 2001), summer (July 2001), and fall (November 2000; Fig. 1). Previous work from another blackwater stream from the Coastal Plains Physiographic Province demonstrated that wetland soil leaching accounts for 85% of the annual stream DOC export (Dosskey and Bertsch 1994). Sites Pristine-1 and Pristine-2 were located adjacent to Skit Branch and Tom Roberts Branch (Fig. 1). Forests and wetlands make up >87% of the land cover in these two watersheds (Table 1). Site Polluted-1 was adjacent to Albertson Brook, whose watershed is affected by agriculture and urban development (Fig. 1, Table 1). Blueberry and turf grass farms directly border Polluted-1. Site Polluted-2 was adjacent to Hammonton Creek, whose watershed is predominantly agricultural (Fig. 1, Table 1). Polluted-2 also directly borders a blueberry farm and is affected by sewage discharged into Hammonton Creek. Soils in cedar bogs are classified as shallow muck and consist of <1 m of muck or peat over gray or gravelly sand with slopes generally <1%.

Subsurface waters were sampled for these experiments because shallow groundwater flow through cedar bogs is an important source of water to streams in the Pine Barrens and is affected by upland activities (Rhodelhamel 1970; Laidig and Zampella 1999). On average, shallow groundwater discharge from the cedar bogs accounts for 89% of the annual stream discharge in the Pine Barrens (Rhodelhamel 1970). Upland groundwaters also feed the cedar bogs and thus should affect their biogeochemistry (Laidig and Zampella 1999).

Sample collection—At each site, subsurface wetland water was collected in three wells that were constructed of PVC pipe with drainage holes wrapped in a Geo Sieve (Drainage Products) sleeve. The wells were buried ~50 cm below the soil surface and installed at random locations in the wetlands within ~5 m of the stream edge. Wells were pumped dry ~24 h before sampling. During the summer, wetland water for Polluted-2 was sampled from alcoves along the wetland/stream interface.

Subsurface wetland waters were collected in acid-cleaned, deionized water (DIW)–soaked 1-liter plastic bottles that were rinsed several times with sample water, filtered on site

Table 1. Cedar bog locations and the adjacent river's drainage basin land cover.*

Wetland site (adjacent river)	Latitude	Longitude	Drainage area (km^2)	% agriculture†	% forest	% wetland	% urban	% barren	% water
Pristine-1 (Skit Branch)	39°47′09″N	74°39′31″W	13.0	0.2	80.3	19.4	0.1	0	0
Pristine-2 (Tom Roberts Branch)	39°47′17″N	74°39′33″W	11.1	8.7	54.9	32.9	0.6	0.2	2.8
Polluted-1 (Albertson Brook)	39°41′59″N	74°48′38″W	44.3	25	43	6.7	22	1.2	2.1
Polluted-2 (Hammonton Creek)	39°38′04″N	74°43′06″W	24.9	40.5	24.7	9.1	21.4	2.4	1.9

* R. Zampella pers. comm. Land use/land cover profiles were prepared using Arc View 3.X software, Environmental Systems Research Institute. 1988–1992 digital land use/land cover data were obtained from New Jersey Department of Environmental Protection (NJDEP) (1991/1997 land use/land cover update 2001). Drainage basin boundaries were prepared using Arc View software, and digital hydrography data were obtained from NJDEP (1996 NJ GIS CD-ROM series 1, volume 1–4).

† Includes areas of active cultivation (row and field crops), pasture and grazing lands, vineyards, orchards, wetland agriculture (cranberry and blueberry cultivation), nurseries, confined feeding operations, and other lands used in support of agricultural activities (i.e., farmsteads, barns, stables, and corrals).

through 0.5-μm string-wound polypropylene canister filters (Cole-Parmer), and stored on ice during transport and overnight in the laboratory. At the laboratory, samples were filtered through glass-fiber (GF/F Whatman) and 0.2-μm polycarbonate membrane (Poretics) filters. During this 2-d filtering process, samples were kept at 4°C. Subsamples for chemical analyses (NH_4^+, $NO_3^- + NO_2^-$, TDN, DOC, soluble reactive phosphorus [referred to as PO_4^{3-}], absorbance, and pH) were taken just before the bioavailability experiments. Samples for inorganic nutrients were immediately frozen until analysis, and TDN, DOC, absorbance, and pH samples were stored at 4°C in the dark until analysis the following day. The glassware used in these experiments were acid-cleaned and combusted at 500°C. All filters were rinsed with DIW, and GF/F filters were combusted at 500°C.

Experimental design—The experimental design for examining the bioavailability of wetland DOM consisted of adding riverine bacteria to sterile filtered sample waters and DIW (control) and then monitoring nutrient concentrations and bacterial production over time (Seitzinger and Sanders 1997; Wiegner and Seitzinger 2001). The bioavailability of DOM was quantified through measured decreases in DOC and DON concentrations over the course of the experiments and is expressed as the percentage of DOM utilization (amount DOM consumed/amount DOM initially present × 100).

The bacteria inoculum consisted of an equal volume of water from the four rivers adjacent to the cedar bog sites for the wetland DOM bioavailability experiments. River water for the bacteria inoculum was collected on the same day as the wetland waters, under base flow conditions, in a pre-rinsed bucket from the rivers' thalweg, filtered on site through a 0.5-μm string-wound polypropylene canister filter (Cole-Parmer), and stored on ice in 1-liter plastic bottles. At the laboratory, the inoculum was prepared by filtering river waters through a glass-fiber filter (GF/F Whatman) then pulse sonicating them to remove remaining protists (Seitzinger and Sanders 1997).

Before the addition of the bacteria to the wetland waters, 200 ml of water from each well at each site was mixed, for a total of 600 ml of wetland water per site. A mixture of wetland water from the individual wells was used to measure the average bioavailability of DOM from a wetland site. Sixty milliliters of bacteria inoculum were added to 600 ml of the sterile filtered wetland and control waters to give an approximate bacterial abundance of 10^5 cells ml^{-1} (4′,6′-diamidino-2-phenyl-indole staining method; Porter and Feig 1980). Wetland waters were then mixed and divided evenly into two 250-ml Erlenmeyer flasks or brown glass bottles, covered with aluminum foil (dark conditions), gently stirred with Teflon-coated stir bars or slowly shaken on a shaker table (25 rpm), and incubated at 22–25°C for 4 d. Initial and time-series NH_4^+, $NO_3^- + NO_2^-$, PO_4^{3-}, TDN, DOC, absorbance, and pH samples were collected over the course of the experiment. Water for these analyses was filtered through 0.2-μm polycarbonate membrane filters (Poretics). Samples for inorganic nutrients were immediately frozen, and TDN and DOC samples were analyzed on the day the samples were collected. Absorbance and pH samples were stored in the dark at 4°C and analyzed within 1 week of the experiment. Changes in the concentrations of DOC and DON in the control samples were negligible and within the analytical variance of the instruments (data not shown).

Analytical methods—NH_4^+ (Lachat QuickChem 31-107-06-1-A), $NO_3^- + NO_2^-$ (Lachat QuickChem Method 31-107-04-1-A), and PO_4^{3-} (Lachat QuickChem Method 31-115-01-3-A) were measured using standard wet chemistry methods. TDN was analyzed by high-temperature combustion followed by chemiluminescent detection of nitric oxide using an Antek Model 7000 Total N Analyzer (Antek) equipped with a quartz combustion tube (1,000 ± 10°C) and a ceramic insert (Seitzinger and Sanders 1997). Acid was not added to the TDN samples because it precipitated the colored DOM (Wiegner unpubl. data). TDN samples were analyzed within 2 h of collection. DON was determined by the difference between TDN and DIN concentrations. DOC was measured by high-temperature combustion (Shimadzu TOC-5000A; Sharp et al. 1993). Absorbance scans (200–750 nm) were performed on a Perkin Elmer UV/VIS Spectrometer Lambda 12 using a 1-cm quartz cuvette. Molar absorptivity at 280 nm (ε_{280nm}) was calculated using Beer-Lambert law; this wavelength was chosen because π-π^* electron transitions occur for a number of aromatic substances in this region (Traina et al. 1990). pH was measured using an Orion pH meter (model 290A) with an Orion low-maintenance triode pH electrode (model 9107) at room temperature.

Bacterial production was measured daily using ^{3}H-leucine incorporation (modified from Smith and Azam 1992; as detailed in Wiegner and Seitzinger 2001). Sample water (1.7 ml in triplicate) was incubated with 5 μl of L-4, 5 ^{3}H-leucine (TRK 510, Amersham, 146 Ci $mmol^{-1}$ ^{3}H-leucine, final leucine concentration 20 μmol L^{-1}) at 25°C for 30 min. Blanks for each site consisted of 90 μl of 100% trichloroacetic acid, 1.7 ml sample water, and 5 μl ^{3}H-leucine. The centrifugation, vortex, and wash sequence described in Smith and Azam (1992) was used, with the addition of a final 80% ethanol wash. Theoretical conversion factors determined for natural aquatic environments were used to convert measured, blank-corrected ^{3}H-leucine incorporation rates into bacterial carbon biomass production (Kirchman 1993). The bacterial biomass production was integrated over the course of the experiments and normalized to initial DOC concentrations (e.g., Hopkinson et al. 1998). Bacterial growth efficiencies (BGEs) were estimated by dividing the integrated bacterial production by the amount of DOC consumed during the experiments (Mann and Wetzel 1995).

Statistical analyses—Wetland water composition and DOM bioavailability data were analyzed by analysis of variance (ANOVA; Systat 10.0 software) to examine overall and seasonal differences between pristine and polluted wetlands. NH_4^+, DON, and DOC concentration data and DOC : DON ratio data were log transformed to satisfy the normal distribution requirement for ANOVA. The concentration of $NO_3^- + NO_2^-$, ε_{280nm}, and BGE estimates were rank transformed to satisfy the equality of variance requirement for ANOVA (Potvin and Roff 1993). Post hoc analyses were performed using Tukey's Studentized Range test.

Table 2. Average ± SD ($n = 3$) chemical composition of subsurface wetland waters.

Wetland site	Season	Date collected	Temp (°C)	pH	$NO_3^- + NO_2^-$ (μmol N L^{-1})	NH_4^+ (μmol N L^{-1})	DON (μmol N L^{-1})	TDN (μmol N L^{-1})	% TDN as DON	DOC (μmol C L^{-1})	DOC : DON	PO_4^{3-} (μmol P L^{-1})
Pristine-1	Spring	16 Mar 2001	10.2±0.3	5.1±0.8	0.9±0.4	20.1±17.2	19±10	40±27	53±16	599±185	36±17	0.0±0.0
	Summer	7 Jul 2001	22.3±0.6	6.2±0.5	0.3±0.1	43.7±29.6	21±9	65±39	34±9	544±175	27±5	0.0±0.0
	Fall	25 Nov 2000	8.0±0.0	5.5±0.3	0.3±0.0	29.2±21.1	13±7	43±28	34±8	467±231	39±13	0.1±0.1
Pristine-2	Spring	16 Mar 2001	9.0±0.0	4.4±0.4	0.5±0.0	5.7±3.8	11±2	17±5	67±12	371±19	34±6	0.0±0.0
	Summer	7 Jul 2001	19.3±0.6	5.4±0.6	0.4±0.1	54.1±33.7	22±10	76±44	30±5	466±161	22±4	0.0±0.0
	Fall	25 Nov 2000	6.7±0.6	5.3±0.5	0.3±0.2	39.9±38.5	21±14	61±53	43±21	452±210	24±6	0.1±0.1
Pristine average			12.6±6.2	5.3±0.7	0.5±0.3	32.1±27.8	18±9	50±36	44±17	482±167	31±10	0.0±0.1
Polluted-1	Spring	16 Mar 2001	9.7±0.6	3.9±0.1	14.4±6.8	6.3±3.2	19±1	40±4	48±5	1,179±202	63±10	0.1±0.0
	Summer	7 Jul 2001	19.7±0.6	5.1±1.0	6.7±7.9	23.9±9.6	41±12	72±14	56±7	1,272±590	30±6	0.1±0.2
	Fall	25 Nov 2000	6.3±0.6	4.4±0.9	24.4±31.2	9.7±6.5	25±17	59±9	45±33	2,200±1,349	109±45	0.3±0.2
Polluted-2	Spring	16 Mar 2001	10.0±0.0	5.1±0.3	30.9±41.2	3.5±2.7	13±5	47±39	48±49	1,046±62	86±26	2.4±0.6
	Summer	7 Jul 2001	22.2±0.3	6.7±0.0	46.6±0.9	3.0±0.4	24±4	74±3	33±4	369±29	15±1	6.4±0.2
	Fall	25 Nov 2000	6.7±0.6	5.5±0.8	42.3±55.7	5.8±1.9	50±25	98±36	59±36	1,442±837	29±12	5.1±0.7
Polluted average			12.6±6.6	5.1±1.2	27.4±29.2	9.0±8.6	30±18	65±26	48±23	1,263±832	53±39	2.4±2.7

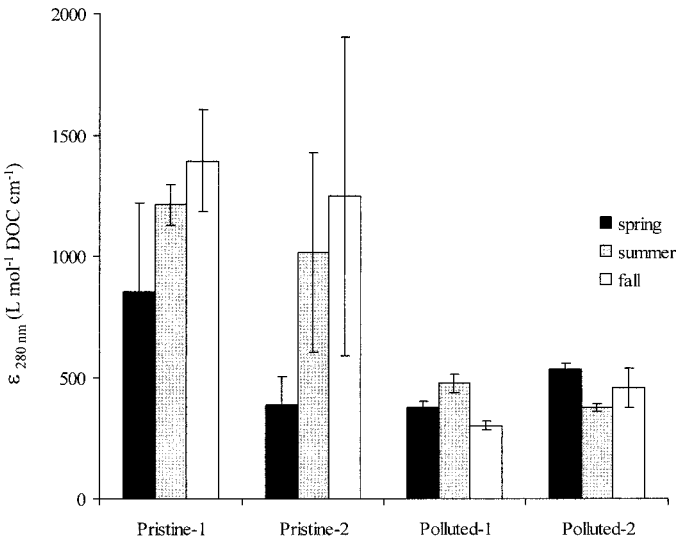

Fig. 2. Seasonal molar absorptivity at 280 nm ($\varepsilon_{280\ nm}$) of subsurface waters from pristine and polluted cedar bogs. Average (±SD, $n = 3$) values for cedar bog sites are shown.

Results

Wetland water chemical composition—NH_4^+ concentrations ($p = 0.001$) and ε_{280nm} ($p < 0.001$) were significantly higher in the pristine than in the polluted wetlands (Table 2, Fig. 2). In contrast, DOC ($p < 0.001$), DON ($p = 0.028$), and $NO_3^- + NO_2^-$ ($p < 0.001$) concentrations and the DOC : DON ratio ($p = 0.015$) were significantly higher in the polluted than in the pristine wetlands (Table 2). The concentration of TDN was similar in the pristine and polluted wetlands ($p = 0.42$; Table 2).

Wetland DOM bioavailability—A similar ($p = 0.16$) absolute amount of DOC was consumed in the pristine and polluted wetlands; however, this amount made up a significantly ($p = 0.001$) greater percentage of the DOC from the pristine than from the polluted wetlands (Table 3). In contrast, a significantly greater absolute amount of DON was consumed in the polluted than in the pristine wetlands ($p = 0.042$); however, this amount made up a similar percentage of the original DON present in the pristine and polluted wetlands ($p = 0.11$; Table 3).

The pristine and polluted wetlands had different seasonal DOM utilization patterns. In the pristine wetlands, DOC utilization (amount and percentage) was higher in the spring than the fall ($p \leq 0.031$) (Table 3, Fig. 3A), whereas the greatest consumption of DON was observed in the fall, followed by the spring and summer ($p \leq 0.003$) (Table 3, Fig. 3B). In the polluted wetlands, DOC utilization was similar in the fall and spring ($p \geq 0.26$) and was higher than in the summer ($p \leq 0.047$) (Table 3, Fig. 3A). The bioavailability of DON in the polluted wetlands was significantly different during each season ($p < 0.001$); DON utilization was highest in the fall, followed by the summer and spring ($p \leq 0.001$) (Table 3, Fig. 3B).

BGEs on DOM from the pristine and polluted wetlands varied across four orders of magnitude (Fig. 4). BGE was

Table 3. Seasonal utilization of DOC, DON, and DIN from pristine and polluted cedar bogs. DOM bioavailability is calculated as both the absolute amount (consumption rate) and the percentage of the initial DOC and DON concentration used after 4 d. DIN utilization is calculated as the combined net decrease in both NH_4^+ and $NO_3^- + NO_2^-$ concentrations during the experiments. Average ± SD for replicate flasks (site only for DIN) and wetland types are shown.

Season and wetland site	Initial DOC concentration (μmol C L^{-1})	Amount of DOC used (μmol C L^{-1})	% DOC bioavailable	Initial DON concentration (μmol N L^{-1})	Amount of DON used (μmol N L^{-1})	% DON bioavailable	Initial DIN concentration (μmol N L^{-1})	Amount of DIN used (μmol N L^{-1})
Spring								
Pristine-1	628±7	164±23	26±3	21±0	6±2	30±10	24.6±0.1	0.5±0.2
Pristine-2	458±5	143±16	31±4	11±0	4±0	32±2	10.3±0.1	0.4±0.02
Pristine average		153±20	29±4		5±2	31±6		
Polluted-1	1,149±9	114±14	10±1	19±0	0.4±0.5	2±3	24.1±0.5	1.1±0.1
Polluted-2	1,007±22	167±12	17±1	13±2	0±0	0±0	35.4±1.1	4.2±0.1
Polluted average		140±32	13±4		0.2±0.4	1±2		
Summer								
Pristine-1	547±16	63±18	11±3	30±0	3±1	11±5	40.0±1.1	0.6±0.6
Pristine-2	435±11	137±45	32±9	31±0	0±0	0±0	49.2±0.4	2.1±3.0
Pristine average		100±51	22±13		2±2	5±7		
Polluted-1	1,199±27	88±26	7±2	41±0	7±0	17±1	28.0±1.0	3.6±0.5
Polluted-2	396±22	35±3	9±0	24±0	8±1	34±5	45.3±0.2	5.2±1.8
Polluted average		61±34	8±2		8±1	26±10		
Fall								
Pristine-1	461±15	73±18	16±5	17±1	10±1	62±12	32.1±2.2	4.2±2.7
Pristine-2	429±9	75±2	17±1	22±2	14±3	64±7	37.3±1.3	0.6±0.9
Pristine average		74±11	17±3		12±3	63±8		
Polluted-1	1,871±28	297±6	16±0	30±0	14±0	47±2	31.2±0.6	13.7±0.5
Polluted-2	1,213±42	137±66	11±5	35±1	23±4	65±9	44.0±0.7	10.3±0.7
Polluted average		217±100	14±4		19±6	56±12		
Overall								
Pristine average		109±45	22±9		6±5	33±25		
Polluted average		139±88	12±4		9±8	28±25		
Wetland average		124±70	17±9		8±7	30±25		

significantly ($p < 0.001$) higher in the polluted than the pristine bogs. The pristine and polluted wetlands had different seasonal BGE patterns ($p < 0.001$; Fig. 4). In the pristine sites, each season had significantly different BGEs from one another ($p < 0.001$). BGE was greatest during the fall, followed by the summer and spring. In the polluted sites, BGE was highest in the summer, followed by the fall and spring.

Discussion

Riverine DOM originates from numerous natural and anthropogenic watershed sources, atmospheric deposition, and autochthonous production. The amount and chemical composition of DOM from these sources to the river affects its bioavailability and metabolic role in the river. Currently, little is known about how the bioavailability of DOM in rivers varies with watershed land cover (Hopkinson et al. 1998; Findlay et al. 2001). This information is important for determining the contribution of DOM from different land covers to coastal eutrophication and hypoxia. The results of recent work have suggested that DON that is released from soils is less bioavailable than DON that is not subject to soil processing (Seitzinger et al. 2002). It is possible that season and anthropogenic activities modify soil processes and their effects on the bioavailability of DON. The lack of DOC bioavailability data across sources makes it difficult to evaluate whether this trend exists for DOC (Hopkinson et al. 1998; Findlay et al. 2001). Both season and anthropogenic activities affected the bioavailability of DOC and DON in the cedar bogs that we examined. We used DOC and DON bioavailability data from the present and previously published studies to evaluate the effects of soil processing, season, and anthropogenic activities on the bioavailability of DOM across sources and to examine the factors controlling its bioavailability in aquatic systems.

Effects of source on the bioavailability of DOM—The bioavailability of DOC in cedar bogs (17% ± 9; Table 3) is comparable to that measured in other freshwater bogs (average: 18%, range: 16–20%), forests (average: 21%, range: 4–44%), and agricultural areas (average: 18%, range: 9–27%; e.g., Satoh and Abe 1987; Qualls and Haines 1992; Wiegner and Seitzinger 2001). In contrast, freshwater marsh DOC is more bioavailable (average: 45%, range: 24–69%; Mann and Wetzel 1995) than DOC from the pristine and polluted cedar bogs. The freshwater marsh water was col-

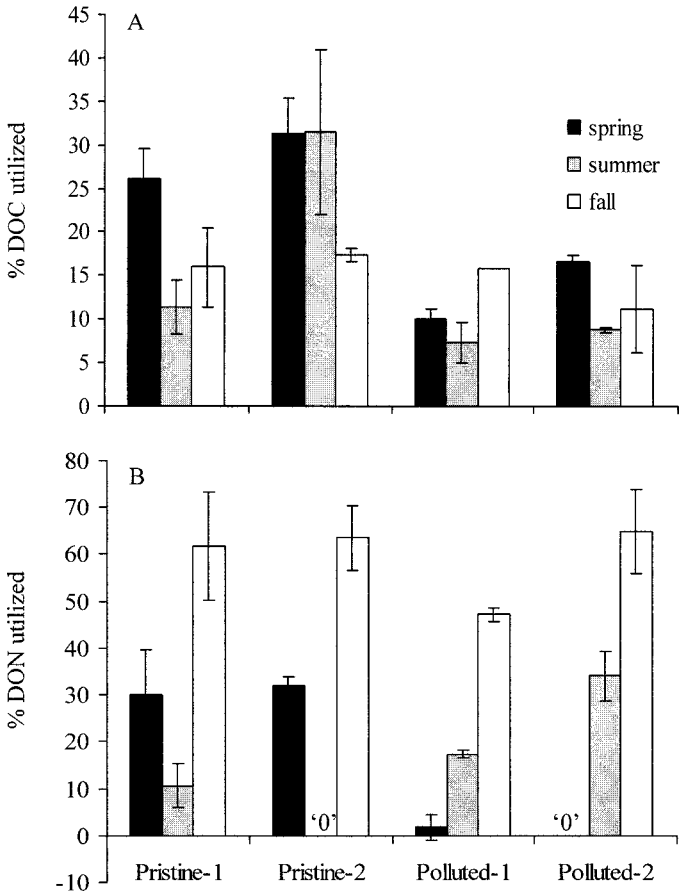

Fig. 3. Net (A) DOC and (B) DON utilization during wetland DOM bioavailability experiments in spring, summer, and fall. Bioavailability was calculated as the percentage of the initial DOC and DON concentrations utilized after 4 d. Averages (±SD) for replicate flasks are shown. Note the different *y*-axis scales.

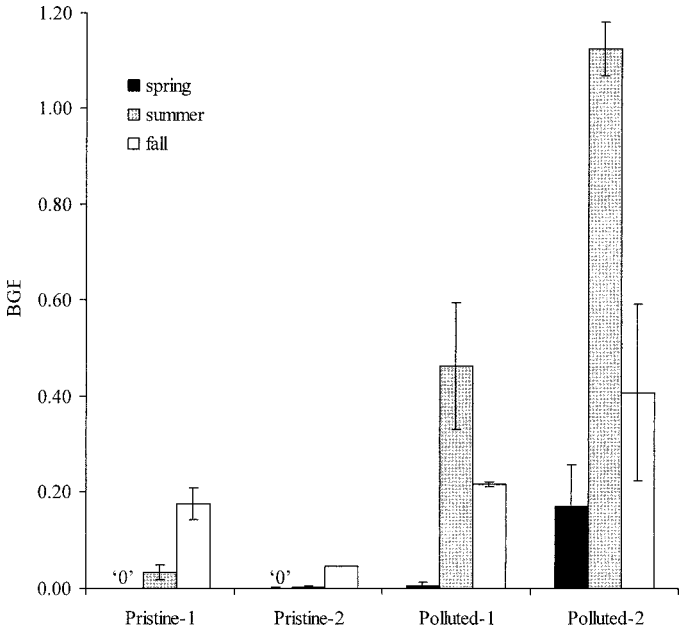

Fig. 4. BGE during wetland DOM bioavailability experiments in spring, summer, and fall. BGE was calculated as the integrated bacterial production divided by the net decrease in the DOC concentration during the experiments. Averages (±SD) for replicate flasks are shown.

lected from a water lily (*Nymphae odorata*) pond where a large fraction of the DOC was macrophyte-derived and very bioavailable (i.e., Findlay et al. 1986). DOC from cedar bogs is also less bioavailable than DOC that originates from sewage treatment plants and industrial effluent (average: 66%, range: 57–75%; Markosova 1991). This data synthesis suggests that DOC released from soils, like DON, is more refractory than DOC that is not subject to soil processes.

The bioavailability of cedar bog DON (30% ± 25) is comparable to that of DON in forest (24%) and agricultural (29%) runoff (Wiegner and Seitzinger 2001; Seitzinger et al. 2002). In contrast, cedar bog DON is ~30 times more bioavailable than that from Swedish freshwater wetlands (1%; Stepanauskas et al. 1999). Differences in the bioavailability of wetland DON may be due to differences in vegetation, soils, and quantification methods. The cedar bogs we studied were cedar forests with shallow muck soils, whereas the Swedish wetlands were spruce forests with sandy soils and flooded meadows with peat soils (Stepanauskas et al. 1999). Additionally, the bioavailability of DON was estimated from changes in bacterial-nitrogen biomass in the Swedish wetlands, whereas changes in the concentration of DON were measured in the cedar bogs (Stepanauskas et al. 1999). In comparison, the bioavailability of DON in the cedar bogs (30 ± 25%) is lower than that measured in urban/suburban runoff (59 ± 11%; Seitzinger et al. 2002). Our data further support the idea that soil processes alter the bioavailability of DON (Seitzinger et al. 2002); however, more data are needed to evaluate this pattern across different sources and seasons for both DON and DOC.

Effects of season on the bioavailability of DOM—The bioavailability of DOM varies seasonally within a number of watershed sources, and the most comprehensive analyses have been done for DON (Qualls and Haines 1992; Yano et al. 2000; Seitzinger et al. 2002). Unique seasonal DON bioavailability patterns have been observed for natural and anthropogenic watershed DOM sources (Seitzinger et al. 2002); seasonal DOC bioavailability measurements from these sources are generally lacking. Seasonal changes in the relative amount and quality of DOM from autochthonous and allochthonous sources can affect the overall bioavailability of riverine DOM and its metabolic role in downstream ecosystems. Hence, a working knowledge of how the bioavailability of DOM varies with source and season is needed to better understand and predict the role of riverine DOM in eutrophication and hypoxia.

Consistent seasonal DON bioavailability patterns were observed for the two wetland types; however, the patterns observed for the pristine and polluted sites differed (Fig. 3B). Unique seasonal DON bioavailability patterns have been observed in forest, agricultural, and urban storm water runoff (Seitzinger et al. 2002); the patterns observed for these sources are different from the ones measured in the pristine and polluted wetlands (Fig. 3B; Seitzinger et al. 2002). The

bioavailability of DON was highest in the fall for both wetland types and was generally lower in forest, agricultural, and urban storm water runoff (Seitzinger et al. 2002). Fall wetland waters were collected when soils were freezing and thawing; under these conditions, bioavailable DON may have been released into solution from particulate detritus and/or lysed microbial cells (e.g., Deluca et al. 1992). In contrast, seasonal land use practices probably contributed to different spring DON bioavailability patterns in the pristine and polluted wetlands. Nitrogen fertilization enhances the consumption of DOM by soil microbes and, thus, lowers the concentration of bioavailable DOM in soil water (Chantigny et al. 1999). During spring, the fertilization of nearby blueberry fields may have lowered the bioavailability of DON in the polluted wetlands relative to the pristine wetlands. Our results, in conjunction with those of Seitzinger et al. (2002), demonstrate that season and anthropogenic activities affect the bioavailability of DON in runoff from natural and anthropogenic watershed sources. These effects need to be incorporated into bioavailable riverine and estuarine nitrogen budgets.

Seasonal DOC bioavailability patterns are not as well established as those for DON. In our study, season significantly affected the bioavailability of wetland DOC ($p \leq 0.036$; Fig. 3A). In general, the bioavailability of wetland DOC, in both the pristine and polluted wetlands, was higher during spring and fall than summer (Fig. 3A; 3 of 4 sites), possibly because of soil freezing and thawing (e.g., Deluca et al. 1992). In contrast, no consistent seasonal DOC bioavailability patterns have been observed in forest runoff (Qualls and Haines 1992; Yano et al. 2000). The presence or absence of seasonal DOM bioavailability patterns is ultimately linked to the processes and factors that control the production and consumption of DOM; currently, these controls in soils are poorly understood (Kalbitz et al. 2000). In addition, the processes and factors that regulate the bioavailability of DOC may differ from those that regulate DON. Environmental and age gradients differentially affect concentrations of DOC and DON in soils (reviewed in McDowell 2003) and may also affect their bioavailability in water, as is suggested by our results (Fig. 3A,B).

Controls of DOM bioavailability—A number of factors can affect the percentage of DOM consumed by bacteria; these include temperature, light, nutrient and micronutrient availability, the composition of the bacteria community, the chemical composition of DOM, and exposure time to the material (del Giorgio and Davis 2003). The physical and biological factors, as well as the exposure time, were similar among our experiments. The initial composition of the bacterial community was the same for both the pristine and polluted wetlands each season; the inoculum consisted of an equal mixture of river water from the four wetland sites. The composition of the bacterial community may have changed seasonally; this may explain, in part, the differences in DOM utilization among seasons, but it does not explain differences in DOM bioavailability patterns between the pristine and polluted wetlands. Therefore, differences in the composition of DOM (quality) and/or the absence of some nutrients and micronutrients are responsible for differences in the DOM bioavailability patterns between pristine and polluted cedar bogs.

The results of several studies have suggested that aliphatic carbon is more bioavailable than aromatic carbon (e.g., Sun et al. 1997; Hopkinson et al. 1998). Bacterial production has been positively correlated with the aliphatic content and negatively correlated with the aromatic content of the DOC pool (Sun et al. 1997; Hopkinson et al. 1998). To examine this relationship with our data, we used molar absorptivity at 280 nm (ε_{280nm}) as a proxy for the aromaticity of DOM (Chin et al. 1994); the aromaticity of fulvic acids from a variety of American rivers and an Antarctic lake has been found to be strongly correlated with ε_{280nm} (% DOM aromaticity = $0.05 \times \varepsilon_{280nm} + 6.74$, $r^2 = 0.90$; Chin et al. 1994). DOM from the pristine wetlands (54 ± 19%) was more aromatic percentwise than DOM from the polluted wetlands (27 ± 4%; Fig. 2) and other freshwater ecosystems (26 ± 6%, calculated from Chin et al. 1994; Sun et al. 1997; Hopkinson et al. 1998). In our study, ε_{280nm} was not correlated with bacterial production ($r^2 = 0.10$), BGE ($r^2 = 0.10$), the percentage of bioavailable DOC ($r^2 = 0.08$), or the percentage of bioavailable DON ($r^2 = 0.03$), which suggests that the aromaticity of DOM was not a major factor affecting its bioavailability and that some other factor was more important.

The quality of DOM can influence BGE and the subsequent transfer of carbon and nitrogen to higher trophic levels (del Giorgio and Cole 1998). The C : N ratio of the bulk DOM is often used as a measure of DOM quality (e.g., Sun et al. 1997; del Giorgio and Cole 1998; Hopkinson et al. 1998). A DOM molecule with a C : N ratio lower than that of a bacteria cell (~5 : 1; e.g., Fagerbakke et al. 1996) is generally considered to be of higher quality than one with a higher C : N ratio. BGE and bacterial production have been negatively correlated with the C : N ratio of the bulk DOM (Sun et al. 1997; del Giorgio and Cole 1998; Hopkinson et al. 1998), which suggests that the consumption of molecules with low C : N ratios (e.g., proteins) results in more efficient growth; however, few studies have actually measured the C : N ratio of the DOM consumed by bacteria (Wiegner and Seitzinger 2001). In our study, we measured both the C : N ratio of the bulk and bioavailable DOM (the amount of DOC : DON consumed) and found that, although the C : N ratio of the bulk DOM was not significantly related to either BGE ($r^2 = 0.04$) or bacterial production ($r^2 = 0.03$), the C : N ratio of the DOM consumed was related to these parameters ($P \leq 0.05$; Fig. 5A,B). Our results support the idea that bacteria grow more efficiently on organic substrates with low C : N ratios and demonstrate this capability with naturally occurring complex molecules.

Like DOM quality, the availability of nutrients and micronutrients (i.e., N, P, and Fe) can also affect BGE (del Giorgio and Cole 1998). In our experiments, BGE (Fig. 5C; $r^2 = 0.59$, $P < 0.01$) and bacterial production ($r^2 = 0.30$, $P = 0.075$) were inversely correlated with the percentage of DOC consumed. A similar relationship has been reported for bacteria that grow on DOM in surface and subsurface waters from a freshwater marsh (Mann and Wetzel 1995). This pattern occurs when bacterial production is limited by some substrate other than carbon, such as a nutrient (del Giorgio and Cole 1998). The highest percentage of DOC utilization

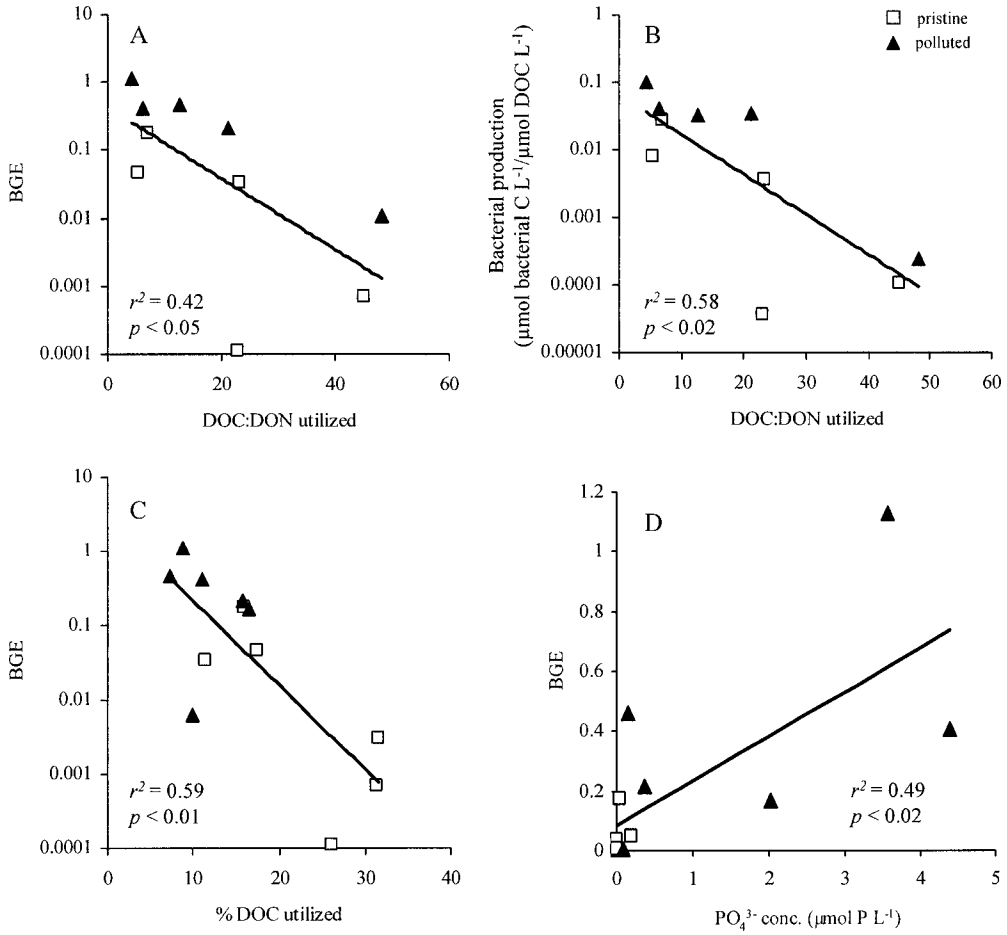

Fig. 5. Factors affecting BGE and bacterial production in wetland DOM bioavailability experiments in spring, summer, and fall. Relationships between (A) BGE and the DOC : DON ratio utilized by the bacteria, (B) integrated bacterial production (normalized to initial DOC concentrations) and the DOC : DON ratio utilized by the bacteria, (C) BGE and %DOC utilized, and (D) BGE and initial PO_4^{3-} concentrations are shown. Correlation analysis for BGE and bacterial production with the DOC : DON ratio utilized by the bacteria only includes data where there was both DOC and DON consumption. Averages for replicate flasks are shown.

and the lowest BGE were primarily observed in the pristine wetlands (Fig. 5C). Nitrogen availability can sometimes limit BGE (e.g., Kroer 1993); however, the amount of NH_4^+, the inorganic nitrogen form that is energetically preferred by most bacteria, was high in the pristine wetlands (Table 2), and its concentration was not correlated with BGE ($r^2 = 0.16$). Phosphorus may have limited bacterial growth; BGE was significantly ($P < 0.02$) correlated with the PO_4^{3-} concentrations at the beginning of the bioavailability experiments (Fig. 5D). This relationship was driven by data from all four sites and not just by data from Polluted-2, as demonstrated by the continued linearity and significance of the regression when data from Polluted-2 were removed. These results suggest that, as PO_4^{3-} concentrations increased in the wetland waters, bacteria utilized wetland DOC more efficiently. Similar results have been observed for bacteria growing on freshwater marsh DOM (Mann and Wetzel 2000), and this pattern is consistent with phosphorus limitation of plants in many bogs and freshwater marshes. Phosphorus limitation in cedar bogs may result from PO_4^{3-} sorption onto iron bog deposits (limonite (hydrous iron oxide)).

The bioavailability of DON, in contrast to DOC, was not significantly correlated with BGE ($r^2 = 0.04$). This relationship may be weak because bacteria use both DIN and DON for nitrogen (e.g., Wheeler and Kirchman 1986). In our experiments, ~60% of the nitrogen consumed by the bacteria in the pristine or polluted wetlands was DON (calculated from Table 3). BGE was also not correlated with DIN utilization ($r^2 = 0.15$) or, as mentioned above, initial NH_4^+ concentrations ($r^2 = 0.16$). These results suggest that nitrogen did not limit BGE on wetland DOM.

The nitrogen and carbon components of the DOM pool appear to cycle differently through the bacterial community. Other researchers have observed higher bacterial utilization of DON relative to DOC (Stepanauskas et al. 2000; Wiegner and Seitzinger 2001); our results provide further details on the disconnection between these two DOM components. Much of the DOC consumed by the bacteria in our experi-

ments was respired and was not made into biomass, as indicated by the negative relationship between BGE and the percentage of DOC utilization (Fig. 5C). The availability of phosphate seemed to affect how much of the consumed DOC was made into biomass (Fig. 5D). In contrast, DON was made into biomass and was not mineralized, as indicated by net decreases in DIN concentrations (Table 3). Furthermore, bacteria grew more efficiently on nitrogen-rich molecules (low C : N ratios), as demonstrated by the inverse relationship between BGE and the ratio of DOC : DON consumed (Fig. 5A). These results suggest that the fate of the nitrogen and carbon components of DOM differ within the bacterial community, which directly affects the amount of bacterial nitrogen and carbon derived from DOM available for transfer to higher trophic levels.

Implications—Anthropogenic activities and season both affect DOM in cedar bog wetlands. Specifically, anthropogenic activities alter wetland DOC and DON concentrations, chemical characteristics, and bioavailability. These results suggest that anthropogenic activities modify DOM cycling within natural wetlands and potentially affect their streamside pollutant removal abilities like denitrification, a process that is limited by the availability of bioavailable DOM. The chemical characteristics of DOM and its bioavailability in wetlands also change seasonally and alter the amount and composition of wetland DOM exported to rivers. Given the importance of wetlands as a DOM source to rivers in the Pine Barrens and worldwide (e.g., Mulholland 2003), anthropogenic-induced and seasonal changes in wetland DOM inputs to rivers should directly affect riverine DOM quality, metabolism, and food web dynamics.

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Received: 12 December 2003
Accepted: 4 March 2004
Amended: 19 April 2004