Biogeochemistry **31:** 99–120, 1995.

Dissolved organic carbon and its utilization in a riverine wetland ecosystem

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Received 3 July 1995; accepted 26 July 1995

Abstract. Variations in dissolved organic carbon (DOC) concentrations of surface waters and subsurface interstitial groundwater of riparian and wetland soils to 1.2 m depth were evaluated in a riverine wetland ecosystem over one year. DOC was monitored at seven sites within the wetland pond, two sites on the inflow stream, and one site on the outflow stream. Surface concentrations in the inflow stream ranged from 0.74 to 11.6 mg C L^{-1} and those of the outflow from 2.1 to 8.0 mg C L^{-1}. Average DOC from stream floodplain hydrosoils (3.1 to 32.1 mg C L^{-1}) was greater than DOC from the sediments below the stream channel (1.6 to 6.8 mg C L^{-1}). Surface DOC within the wetland varied seasonally, with greatest fluctuations in concentrations through the summer and autumn (range 4.8 to 32.6 mg C L^{-1}) during intensive macrophyte growth and bacterial production. DOC was less variable during the winter months (1.7 to 3.3 mg C L^{-1}). Within the wetland pond, average DOC concentrations (7.1 to 48.2 mg C L^{-1}) in the subsurface waters were significantly greater ($p < 0.05$) than average surface concentrations. The microbial availability of surface and subsurface DOC to bacteria was evaluated from losses of DOC by wetland bacteria grown on the DOC. Bacterial growth efficiencies ranged from 5 to 20% and were negatively correlated to the percentage of DOC removed by bacteria ($r^2 = 0.93$). Throughout the ecosystem, DOC concentrations were greatest in the subsurface waters, but at most depths this DOC was a less suitable substrate than surface DOC for utilization by bacteria.

Key words: organic carbon, riverine wetland ecosystem

Introduction

Dissolved organic carbon (DOC) is the dominant form of organic carbon in most aquatic ecosystems (Wetzel 1983; Thurman 1985). Seasonal variations of DOC concentrations in streams (Kaplan et al. 1980; Ford & Naiman 1989; Ivarsson & Jansson 1994), wetlands (Dalva & Moore 1991; Briggs et al. 1993), and subsurface waters (Ford & Naiman 1989; Dalva & Moore 1991; Easthouse et al. 1992; Vervier & Naiman 1992) have been studied extensively. Some of the studies cited also quantified changes in DOC concentration as water moved through the ecosystems. DOC in surface waters may increase significantly when enriched by DOC from wetlands (Dalva & Moore 1991), whereas DOC transported through a forested stream ecosystem was found to be relatively stable (Fisher & Likens 1973; McDowell & Likens 1988).

In addition to seasonal and spatial variations in DOC concentration, variations in the chemical composition of DOC from surface (Thurman 1985; David et al. 1992) and subsurface (Thurman 1985; Easthouse et al. 1992) waters have been evaluated. The chemical composition of DOC is important because it affects substrate availability for bacterial growth. Bacterial metabolism and the role of bacteria in the microbial loop can be regulated by the structural complexity and the nutrient content of substrates (Linley & Newell 1984). As DOC compounds pass through aquatic ecosystems, the quality and quantity of DOC can be altered by physical (adsorption and chelation), chemical (UV radiation), and biological (decomposition) mechanisms. These alterations of DOC typically act to remove the more labile components of DOC and result in higher concentrations of more recalcitrant DOC in natural waters.

Variations in the chemical composition (Thurman 1985; David et al. 1992) and bacterial utilization (Tranvik & Höfle 1987; Tranvik 1988; Markosova 1991; Middelboe et al. 1992) of DOC from surface waters have been studied. Variations in the chemical composition of DOC from subsurface waters have been studied, but there are few data on the availability of this DOC to natural bacterial assemblages (cf. review of Kaplan & Newbold 1993). Release of DOC from terrestrial and floodplain sources into streams can represent a major source of organic carbon to the stream ecosystem (Fisher & Likens 1973; Wallis et al. 1981; Kuserk et al. 1984; Meyer 1994). In wetlands, DOC can leach from subsurface waters into wetland surface waters or stream surface waters (Mulholland & Kuenzler 1979; Dosskey & Bertsch 1994). The overall ecological impact of subsurface DOC flux into the surface water is poorly understood, because bacterial metabolism of subsurface DOC has not been evaluated appreciably.

This study examined variations in DOC concentration in the surface and subsurface waters of a small forested stream that passes through a wetland ecosystem. The microbial availability of DOC from the surface and subsurface water from the wetland pond was evaluated by measuring the changes in DOC by an assemblage of wetland bacteria metabolizing the DOC. Bacterial growth efficiencies were determined as the ratio of carbon incorporated into bacterial biomass to that carbon removed from the DOC.

Materials and methods

Study sites

Surface and hydrosoil subsurface water was collected from a riverine wetland ecosystem located in the Talladega Wetland Ecosystem (TWE) in the

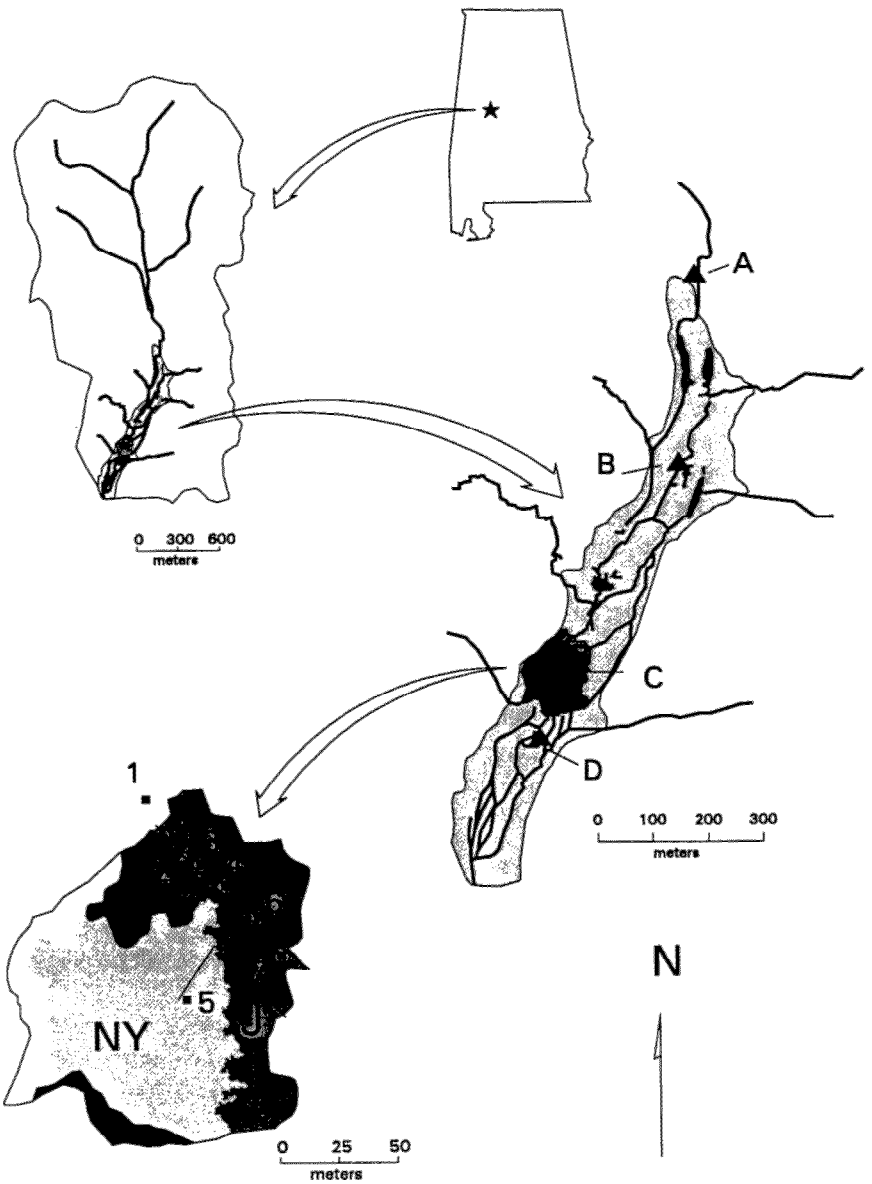

Fig. 1. Talladega Wetland Ecosystem (TWE) study area: lysimeters 725 m upstream from the wetland pond (A), lysimeters 400 m upstream from the wetland pond (B), lysimeters within the wetland pond (C) and lysimeters 50 m downstream from the wetland pond (D); west hill slope (1), open water channel (2), west *Juncus* (3), central *Juncus* (4), *Nymphaea* pond (5), east *Juncus* (6), and far east *Juncus* (7). Within the wetland pond, solid lines note boardwalk constructed to minimize disturbance from repeated samplings; dominant aquatic macrophytes in the wetland pond: *Juncus effusus* = J; *Nymphaea odorata* = NY; *Proserpinaca palustris* = P.

Talladega National Forest (Hale County, Alabama) (Fig. 1). Elevational gradients and fluctuations in hydrological stage within the wetland create a longitudinal vegetative zonation from an up-gradient pine-deciduous riparian mixture, to pure stands of alder, to zones of emergent *Juncus effusus* L., floating-leaved *Nymphaea odorata* Aiton, and down-gradient to deeper open water areas. Subsurface water was sampled with vacuum lysimeters positioned along transects within the wetland pond and in the primary inflow and outflow stream to the wetland pond (Fig. 1). Within the wetland pond, lysimeters were placed along a transect that transversed a population of the emergent rush *Juncus effusus*. Additionally, series of lysimeters were placed in the sediments of two open water areas; one area dominated by the white water lily *Nymphaea odorata* and the second area dominated by *Proserpinaca palustris* L. (mermaid weed). Another series of lysimeters was placed on the steep western hill slope (within 0.5 m of the wetland pond) to monitor potential terrestrial inputs of DOC to the wetland pond.

To evaluate DOC gradients within the entire wetland ecosystem, lysimeters were placed in the sediments of the primary inflow and outflow stream and within the sediments of the stream floodplains (Fig. 1). Lysimeters 725 m upstream from the wetland pond were placed 3 and 6 m from the center of the stream channel on the west floodplain and 6 and 10 m from the center on the east floodplain. Lysimeters on the east and west floodplain 400 m upstream from the wetland pond were ca. 6 m from the center of the stream. The floodplain watershed 725 m upstream from the wetland pond contained a mixed deciduous-pine forest and then shifted to a reach that was dominated by *Alnus serrulata* (Aiton) Willd. and other wetland plant species some 400 m upstream from the wetland pond. Outlet water 50 m downstream from the wetland pond flowed through a forest of mixed wetland species (*Nyssa sylvatica* var. *biflora* (Walt.) Sarg., *A. serrulata*). Surface water samples were collected if standing water occurred within a 0.5 m radius of any series of lysimeters.

Vacuum lysimeters were constructed of porous ceramic cups (Soil Moisture Corporation, nominal pore size of 0.1 μm) epoxied onto polyvinyl chloride (PVC) pipes. Lysimeters were nested in series at sediment depths of 20, 30, 60, 90 and 120 cm within the wetland pond and sediment depths of 20, 50 and 100 cm in the inflow and outflow stream channel and the stream floodplains. To reduce DOC contamination of the first samples, the lysimeters were thoroughly cleaned and rinsed with organic-free water (Milli-Q Water System; Millipore Corporation) prior to placement in the field.

Surface and subsurface water samples were collected every two weeks for 1.5 years. On the day prior to sampling, the water in the lysimeters was evacuated and discarded. A vacuum of 30 cm of Hg was left on the

lysimeters and water was allowed to infiltrate for 24 h. After 24 h, samples were collected and stored in acid-washed, organic carbon-free containers and placed on ice until filtering could be completed (<4 h). To prepare samples for DOC analysis, samples were filtered through pre-combusted (520 °C for 2 h) Whatman GF/F filters (pore size 0.6 μm), and then sample pH was lowered to 2.0 with the addition of ultra-pure 2N HCl. DOC analyses were performed on a Shimadzu T5000 Total Organic Carbon Analyzer calibrated against organic standards.

For the first 20 weeks of sampling, water that accumulated in the lysimeters between sample dates was collected and analyzed for DOC concentration. DOC concentrations from these samples were then compared to the DOC samples from water that collected with the standard method (after 24-h of vacuum). Similar comparisons were conducted on samples collected from the inflow and outflow stream for the first 10 weeks of the study period. There were no significant differences in DOC between the two data sets from the wetland pond (Wilcoxon signed-rank test; $p < 0.001$; $n = 193$) and between the two DOC data sets from lysimeters on the inflow and outflow streams ($p < 0.001$; $n = 92$). Consequently, only DOC values that were collected after a 24-h infiltration period are presented in this study.

Bacterial productivity and DOC consumption measurements

Surface and subsurface DOC samples were collected in November 1993 and July 1994 to evaluate the fraction of total DOC that was available to wetland bacteria during short-term incubations (96 h). Bacterial productivity was evaluated by the uptake and incorporation rates of [^{3}H]leucine (range of specific activity: 5661–6327 kBq mL^{-1}; Amersham Corporation) into bacterial protein (Wetzel & Likens 1991; Kirchman 1993). To determine rates of [^{3}H]leucine incorporation, subsamples of 10 mL were removed from each inoculated water sample after incubation periods of 0.5, 24, 48, 72, and 96 h. At each time interval four 10-mL subsamples were removed from each treatment, three replicates for active [^{3}H]leucine incorporation and one killed control (5% formalin) for abiotic uptake (modified from Wetzel & Likens 1991; Kirchman 1993). [^{3}H]leucine was added to all four replicates from each treatment to achieve a final concentration of 10 nM. Live samples and controls were incubated at 25 °C for 0.5 h, with incubations of live samples terminated with the addition of 5% formalin. All samples were then treated with 3.25 mL of 15% TCA (5% final concentration) and placed in a hot water bath (90 °C) for 30 min. Samples were filtered through Millipore GS filters (pore size 0.22 μm) followed by one rinse with organic-free water (10 mL), two rinses with 5% TCA (3 mL), and two rinses with 80% EtOH (2 mL). Filters were then placed into scintillation vials and dissolved with 1 mL

of ethyl acetate. Within 24 h, 10 mL of Aquasol-2 Universal LSC Cocktail (Dupont) was added and dpm determined on a calibrated Beckman LS 5801 liquid scintillation spectrometer.

Bacterial protein production (BPP) was calculated from the moles of exogenous leucine incorporated, the mole percentage of leucine in protein, the molecular weight of leucine, and the intracellular isotope dilution. Independent methods demonstrated isotopic dilution to be twofold (Simon & Azam 1989; Thomaz & Wetzel 1995). Ratios of protein:dry weight and of carbon:dry weight were found to be quite constant (Simon & Azam 1989; Kirchman 1993). BPP measurements for all cell sizes were converted to rates of dry mass production by multiplying by 1.6, and to bacterial carbon production by a factor of 0.86.

Surface and subsurface water samples were inoculated with bacterial cultures isolated from the detrital layers found in the surface waters at TWE. Bacterial pellets were collected by centrifuging surface waters for 10 min at 10,000 rpm. The bacterial pellets were placed into a 20% glycerol solution and stored in liquid nitrogen until the experiments were conducted. Prior to inoculating treatments, frozen bacterial pellets were allowed to warm to room temperature, suspended in MOPS solution and centrifuged three times (10 min at 10,000 rpm) to remove DOC present in the culture. The cells were rinsed once with organic-free water and resuspended in organic-free water prior to addition to surface and subsurface water samples.

Surface and subsurface water samples collected in July 1994 were evaluated for changes in DOC in parallel with bacterial productivity over the 96-h incubation. DOC samples from treatments were collected prior to inoculation and after 24, 48, 72 and 96 h incubation periods. For DOC analysis, samples were filtered through pre-rinsed (200 mL organic-free water) Millipore GS and the pH was lowered to 2.0 (ultra-pure 2N HCl). DOC was determined on a Shimadzu T5000 Total Organic Carbon Analyzer calibrated against organic standards. The change in DOC of samples inoculated with bacteria (initial DOC minus plateau DOC) has been used as an indication of biodegradable DOC (BDOC) (Servais et al. 1987; Servais et al. 1989; Boissier & Fontvielle 1993).

Bacterial growth efficiency (BGE) of surface and subsurface DOC was evaluated on DOC samples collected in July 1994. BGE was defined as the ratio of bacterial carbon produced per mg DOC utilized. BGE was calculated by the following equation (Linley & Newell 1984):

$$\text{BGE} = \frac{\text{carbon incorporated into bacterial biomass}}{\text{carbon removed from substrate}} \times 100$$

Bacterial production and the change in DOC for the first 24 h of incubation were used in calculating BGE, because after this time increases in DOC

Table 1. Average DOC (mg C L^{-1} ± 1 SE; n = 33) from stream and floodplain lysimeters at four groundwater depths located on the inflow and outflow stream, collected June 1993 to August 1994).

	0 cm	20 cm	50 cm	100 cm
725 m Upstream				
West floodplain (6 m)	11.10 ± 0.07	5.41 ± 0.67	5.13 ± 0.93	4.32 ± 0.48
West floodplain (3 m)	8.39 ± 0.21	12.18 ± 3.84	6.37 ± 1.25	3.07 ± 0.43
Stream channel	1.95 ± 0.23	4.18 ± 0.86	1.56 ± 0.17	3.12 ± 0.44
East floodplain (6 m)	7.38 ± 2.18	15.56 ± 2.81	8.28 ± 1.20	5.96 ± 0.88
East floodplain (10 m)	3.65 ± 1.13	12.22 ± 2.85	6.61 ± 1.77	3.88 ± 0.69
400 m Upstream				
West floodplain	4.33 ± 0.98	6.27 ± 1.03	4.83 ± 0.64	3.52 ± 0.31
Stream channel	2.60 ± 0.35	6.43 ± 1.19	3.93 ± 0.44	5.69 ± 1.01
East floodplain	6.62 ± 1.25	7.38 ± 1.63	7.25 ± 0.84	7.36 ± 0.62
50 m Downstream				
West floodlpain	7.47 ± 0.13	3.76 ± 0.69	4.26 ± 0.79	32.05 ± 4.69
Stream channel	4.56 ± 0.30	5.35 ± 0.85	6.84 ± 1.00	4.37 ± 0.49
East floodplain	6.61 ± 0.05	5.12 ± 1.40	12.10 ± 0.67	19.16 ± 5.51

concentrations occurred in some inoculated treatments possibly from bacterial senescence.

Statistical analyses

Statistical analyses were conducted on SYSTAT for Windows, Version 5. Parametric statistics could not be used to analyze these data because critical assumptions of homogeneity of variance were violated. Therefore, variations in DOC concentrations and bacterial productivity measurements were analyzed with a nonparametric Wilcoxon signed-rank test.

Results

Surface DOC concentrations

DOC concentrations in the surface water of the stream at TWE increased as the water flowed through a less productive forested ecosystem to a more productive wetland ecosystem (cf. Wetzel 1983). Average DOC in stream water increased significantly ($p < 0.001$) from 725 m upstream (1.95 mg C L^{-1}) to 400 m upstream (2.60 mg C L^{-1}) from the wetland pond (Table 1).

Table 2. Average DOC (mg C L^{-1} ± 1 SE; n = 34) for surface and subsurface waters from the wetland pond at TWE, collected May 1993 to August 1994.

	0 cm	20 cm	30 cm	60 cm	90 cm	120 cm
West hill slope	NA	NA	6.32 ± 1.01	7.78 ± 1.40	19.55 ± 7.58	5.97 ± 0.49
Stream channel	4.68 ± 0.62	33.99 ± 1.68	20.51 ± 1.35	11.05 ± 1.12	16.03 ± 1.65	8.81 ± 0.61
West *Juncus*	7.09 ± 1.10	17.80 ± 1.00	7.88 ± 0.63	7.10 ± 0.54	7.49 ± 0.36	8.46 ± 0.44
Central *Juncus*	9.08 ± 1.54	NA	19.59 ± 1.28	48.17 ± 2.52	27.23 ± 1.33	24.28 ± 1.18
Nymphaea pond	4.76 ± 0.41	20.32 ± 1.56	21.46 ± 1.43	39.23 ± 6.17	30.90 ± 1.73	31.14 ± 5.42
East *Juncus*	9.70 ± 1.67	19.54 ± 1.98	13.83 ± 0.61	19.35 ± 0.71	23.46 ± 1.87	26.59 ± 1.54
Far east *Juncus*	4.43 ± 0.52	23.65 ± 2.34	12.31 ± 1.04	16.29 ± 1.13	18.62 ± 0.87	25.30 ± 1.45

Surface DOC increased significantly ($p < 0.001$) to an average of 4.68 mg C L^{-1} in the open water channel of the wetland pond (Table 2). Average DOC from the open water channel and the *Nymphaea* pond (4.43 mg C L^{-1}) were not significantly different ($p = 0.30$). Average DOC in the outflow stream (4.56 mg C L^{-1}) was not significantly different ($p = 0.48$) from the open water areas of the wetland pond, but was significantly higher ($p < 0.001$) than DOC of the inflow stream. A net input of DOC occurred to the surface waters from the wetland pond, and some of this DOC is exported from the wetland pond via the surface water in the outflow stream.

Surface DOC concentrations from the stream channel and open water areas of the wetland pond increased as flow deviated during flood periods of moderate to heavy precipitation events onto the floodplain and through regions of emergent macrophyte growth. At both inflow stations and the outflow station, average DOC in over bank flow across the floodplain was significantly greater than the average DOC from the stream channel (Table 1). Potential inputs of DOC from the floodplain to the stream were limited to periods of increased rainfall between November 1993 and April 1994. In the surface waters of the wetland pond, average DOC concentrations were greater in water associated with dense tufts of *J. effusus* than the open water areas. Average DOC was greater in isolated, frequently flooded pools of water associated with *J. effusus* (west, central, and east *Juncus*; means 7.09, 9.08, and 9.70 mg C L^{-1}, respectively) than in the open water areas or less frequently flooded areas (stream channel, *Nymphaea* pond, and far east *Juncus*; Table 2).

Distinct seasonal fluctuations in DOC concentration occurred in the surface waters of the wetland pond (Fig. 2). From May through late September 1993, DOC concentrations were higher and more variable (range 7.02 to 13.93 mg C L^{-1}) than during the period of October 1993 to April 1994 (range 2.90 to 4.03 mg C L^{-1}). DOC concentrations increased from May 1994 through the end of the study, with average DOC ranging from 4.24 to 14.33 mg C L^{-1}. The fluctuations and average concentration during high DOC periods were more pronounced in the isolated pools than in the open water areas. Such seasonality was not observed in the surface waters at either site on the inflow stream (Figs. 3 and 4), but was present in the surface water of the outflow stream (Fig. 5). This seasonal increase in DOC in the outflow stream supports the suggestion that part of the net export of DOC from TWE occurred through the outflow stream.

When relationships among surface DOC concentrations of all wetland pond sample stations and daily average air, water, and sediment temperature, the daily average stage of the wetland pond, and average precipitation for each sample date were analyzed, significant positive correlations were found

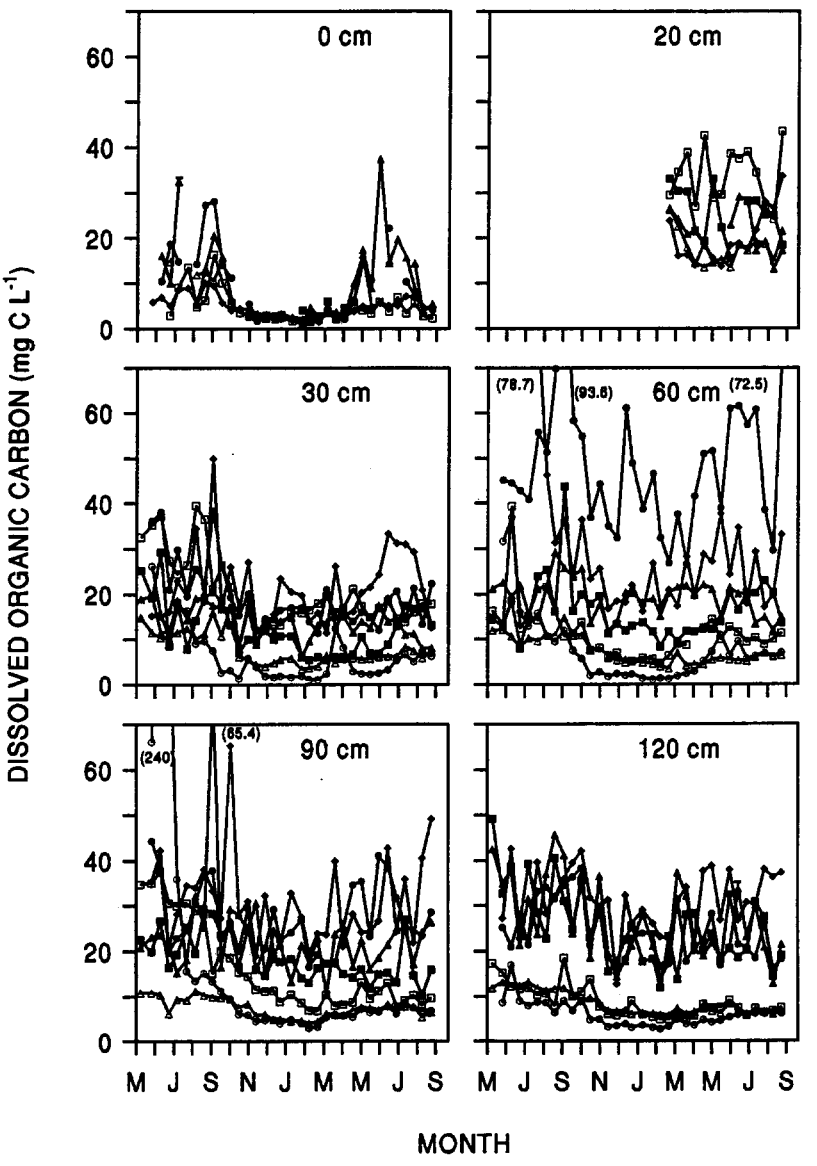

Fig. 2. DOC from surface and subsurface water collected from the wetland pond; May 1993 through August 1994: west hill slope (○), open water channel (□), west *Juncus* (△), central *Juncus* (●), *Nymphaea* pond (◆), east *Juncus* (▲), far east *Juncus* (■). Sampling was on a rigorous 14-d schedule; missing data points represent dates in which insufficient water volume was collected for DOC analyses.

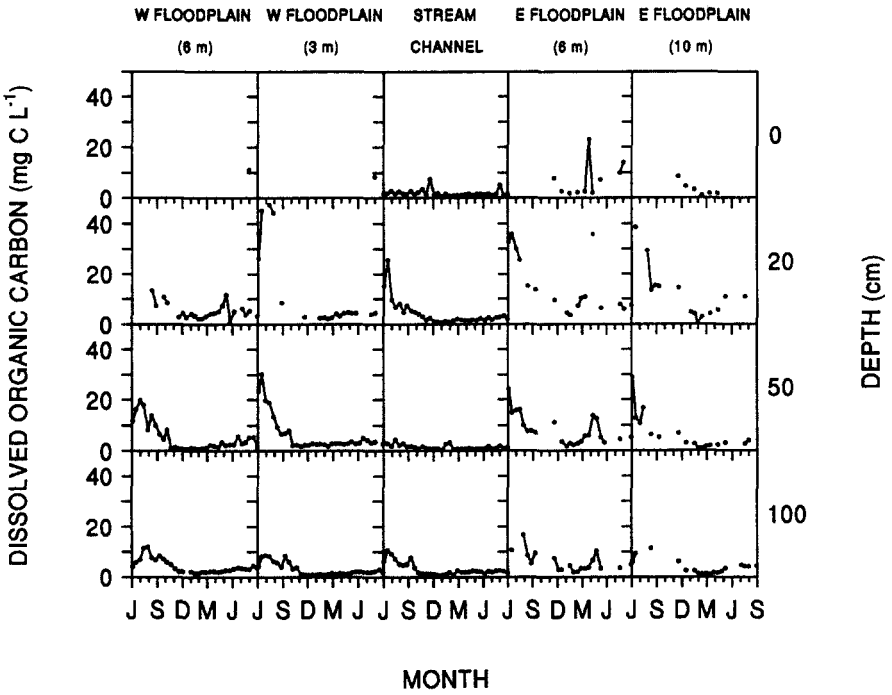

Fig. 3. DOC from surface and subsurface water collected 725 m upstream from the wetland pond; June 1993 through August 1994. Sampling was on a rigorous 14-d schedule; missing data points represent dates in which insufficient water volume was collected for DOC analyses.

between surface DOC concentrations in the *Nymphaea* pond and average air temperature (r^2 = 0.77; n = 26), average water temperature (r^2 = 0.81; n = 26) and average sediment temperature (r^2 = 0.76; n = 26). A negative correlation was found between DOC concentrations of the *Nymphaea* pond and wetland pond stage (r^2 = 0.57; n = 26). No other significant correlations were observed among the stage of the wetland pond, average air, water, and sediment temperature, and DOC concetrations from other surface sites within the wetland pond.

Subsurface waters of the inflow stream

DOC increased laterally from the stream channel into the floodplain at the lysimeters 725 m upstream from the wetland pond (Fig. 3). For example, DOC concentrations from 20 and 50 cm depths were significantly greater than DOC from the same depth in the stream sediments. DOC concentrations at 100-cm depth did not exhibit this pattern and were more constant within these samples from the stream sediments to those of the floodplain sediments.

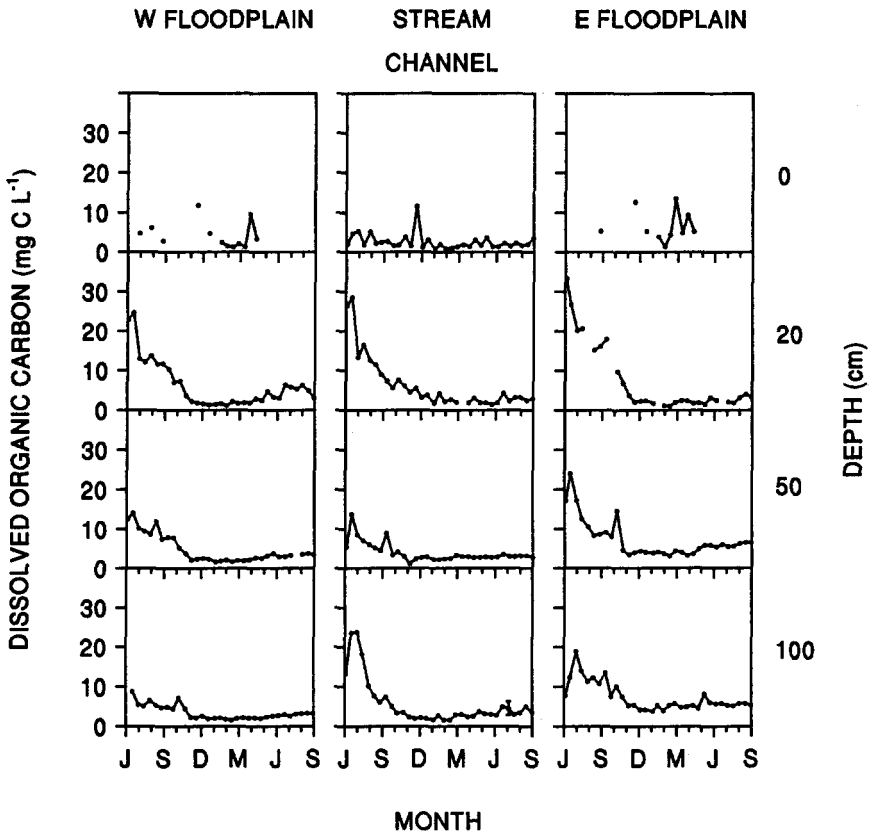

Fig. 4. DOC from surface and subsurface water collected 400 m upstream from the wetland pond; June 1993 through August 1994. Sampling was on a rigorous 14-d schedule; missing data points represent dates in which insufficient water volume was collected for DOC analyses.

No consistent increase in DOC was found in a lateral progression into the floodplain at the lysimeters 400 m upstream from the wetland pond (Fig. 4). At 20-cm depths there was no significant difference in DOC from the sediments of the stream channel and the floodplain hydrosoils. At 50 cm, there was no significant difference in average DOC between the west floodplain and the stream channel, but both were significantly less than DOC from the east floodplain hydrosoils. At 100-cm depths, DOC from the west floodplain groundwater was significantly less than from the stream channel, and both contained significantly less DOC than from the east floodplain.

Average DOC concentrations decreased from 20 to 100-cm depths at all floodplain sites 725 m upstream from the wetland pond and on the west floodplain 400 m upstream from the wetland pond. DOC concentrations on the east floodplain (400 m upstream) did not change significantly with depth, which

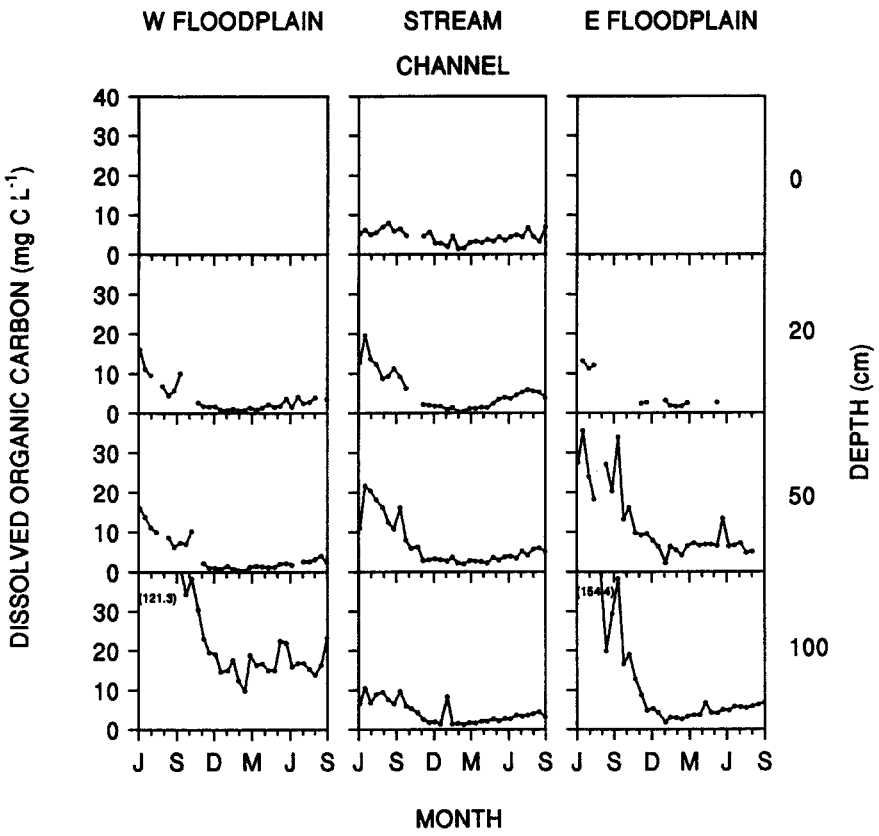

Fig. 5. DOC from surface and subsurface water collected 50 m downstream from the wetland pond; June 1993 through August 1994. Sampling was on a rigorous 14-d schedule; missing data points represent dates in which insufficient water volume was collected for DOC analyses.

suggested different imports or hydrologic regimes on the east floodplain. In the hyporheic sediments of the stream channel, DOC concentration declined among samples from 20 to 50 cm but increased in DOC among samples from 50 to 100 cm.

DOC concentrations in the subsurface waters of the inflow stream were higher from May 1993 through October 1993 (Figs. 3 and 4), a period of lower precipitation, high evapotranspiration, and lower outflow. Subsurface DOC concentrations remained lower and more stable until late April 1994, when they began to increase again. These increases in floodplain hydrosoil DOC concentrations continued through the end of the study period.

Subsurface waters of the wetland pond

Within the wetland pond, average annual DOC concentrations of subsurface waters were 1 to 8-fold higher than in the surface waters (Table 2). A diversity of patterns of DOC concentration with depth were found within the hydrosoils of the wetland pond. DOC in subsurface waters from the two eastern series of lysimeters (located in areas of dense *J. effusus*) increased significantly ($p < 0.01$) with depth (Table 2; Fig. 2). In contrast, in the central area of the wetland pond (central *J. effusus* and *Nymphaea* pond lysimeters), subsurface DOC was greatest at 60 cm and declined markedly at 90 and 120 cm depths. The two series of lysimeters associated with the open water channel (west side of the wetland pond) had maximum subsurface DOC concentrations at the 20-cm depth. On the western hill slope, highest subsurface DOC is present at 90 cm.

Subsurface DOC concentrations from 60, 90 and 120 cm were greater on the eastern side of the wetland pond than the western side (Fig. 2). This trend was most pronounced at 120 cm. DOC concentrations in hydrosoils at the hill slope, instream channel, and west *Juncus* sites were markedly lower than among the other sites.

Subsurface waters of the outflow stream

DOC concentrations from the sediments of the east floodplain were significantly greater than DOC from the same depth in the stream channel sediments (Table 1; Fig. 5). This pattern was not observed in sediments of the west floodplain, where DOC concentrations did not increase laterally from the stream channel to the floodplain at 20 and 50-cm depths. Average DOC was significantly greater from the west floodplain at the 100-cm depth than from the east floodplain.

Seasonal trends in subsurface DOC concentrations were most apparent in the subsurface water of the stream channel hydrosoils. Higher DOC concentrations occurred at the beginning of the study followed by progressive declines until November 1993. Lower concentrations remained until March 1994, when steady increases occurred. These patterns were also observed in the subsurface waters of the floodplains, but more variation was noted during the period between October 1993 and March 1994. DOC in excess of 40 mg C L^{-1} was found at the 100-cm depths on both the east and west floodplain.

Bacterial utilization of DOC of surface and subsurface water

Different patterns of bacterial DOC utilization were observed with samples collected from the *Nymphaea* pond in November 1993 and July 1994. In

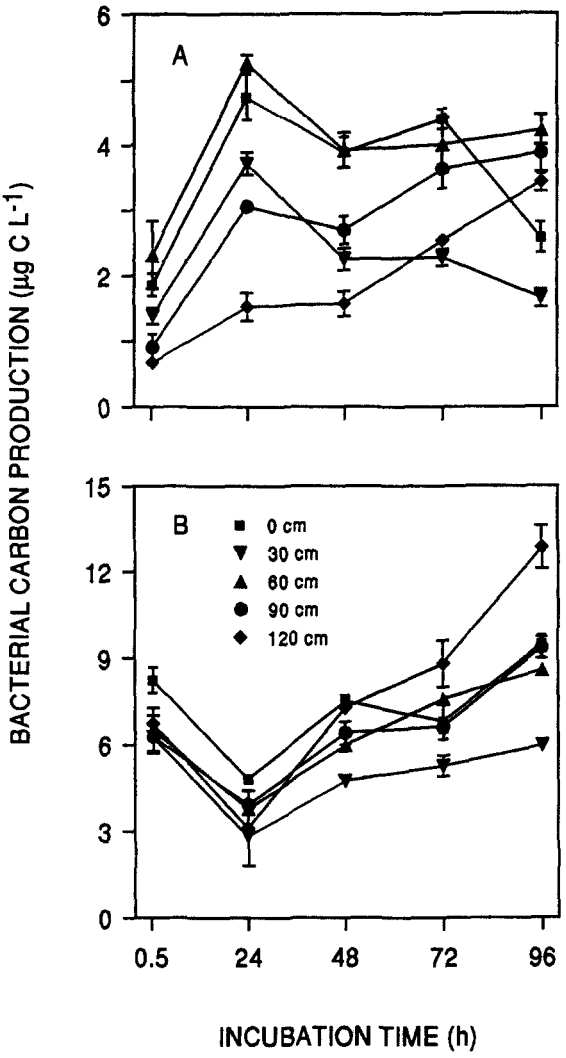

Fig. 6. Bacterial productivity on DOC from surface and subsurface water samples from the wetland pond collected in November 1993 (A) and July 1994 (B).

November 1993, bacterial productivity increased significantly from 0.5 to 24 h on DOC from all depths ($p < 0.05$) (Fig. 6). Additionally, there were significant increases in productivity from 24 to 96-h of incubation on DOC collected from 90 and 120 cm depths. In contrast, there were significant decreases in productivity from 24 to 96 h on DOC in samples collected from the upper strata (0, 30 and 60 cm).

In July 1994, rates of bacterial productivity significantly decreased from 0.5 to 24-h of incubation (Fig. 6). This decrease was followed by significant

Table 3. Comparison of the percent of total productivity on DOC from the *Nymphaea* pond. Values are the percent of total productivity for each specific depth based on total bacterial production estimated for the entire 96-h incubation.

	November 1993	July 1994
0 cm	25.0	22.3
30 cm	16.0	15.1
60 cm	27.1	19.8
90 cm	19.3	19.7
120 cm	12.6	23.1

Table 4. Utilization of DOC of surface and subsurface waters collected from the *Nymphaea* pond in July 1994. DOC removed, biodegradable DOC (BDOC), bacterial production (BP) and bacterial growth efficiency (BGE) was calculated for the first 24 h of incubation.

	Initial DOC (mg C L^{-1})	DOC removed (mg C L^{-1})	BDOC (%)	BP (mg C L^{-1})	BGE (%)
0 cm	3.20	0.86	27	0.15	17
30 cm	3.00	2.07	69	0.11	5
60 cm	2.51	0.60	24	0.12	20
90 cm	2.71	1.66	61	0.12	7
120 cm	3.00	1.31	44	0.12	9

increases in productivity after 48 h at all depths. In samples from 0 and 30 cm, bacterial productivity at 96 h was not significantly different from initial values (0.5 h). At the lower depths (60, 90 and 120 cm) there was significantly higher productivity after 96 h than after 0.5 h incubation.

Bacterial production over the entire 96-h incubation period was integrated to compare the availability of DOC in surface and subsurface water to bacteria. The productivity for all depths (from the same sample period) was summed to give total productivity on DOC from all depths. Productivity for a specific depth was then analyzed as a percentage of the total depth-integrated productivity. The percentage of total productivity at a specific depth could then be examined for seasonal variations. Bacterial production on DOC from 0, 30 and 90-cm depths was similar on both sample dates (Table 3). However, the percentage of overall productivity differed seasonally on DOC collected from 60 and 120-cm depths. At 60 and 120-cm depths, annual variability in DOC concentrations was large, which may have contributed to variability in the quality of DOC at these two depths.

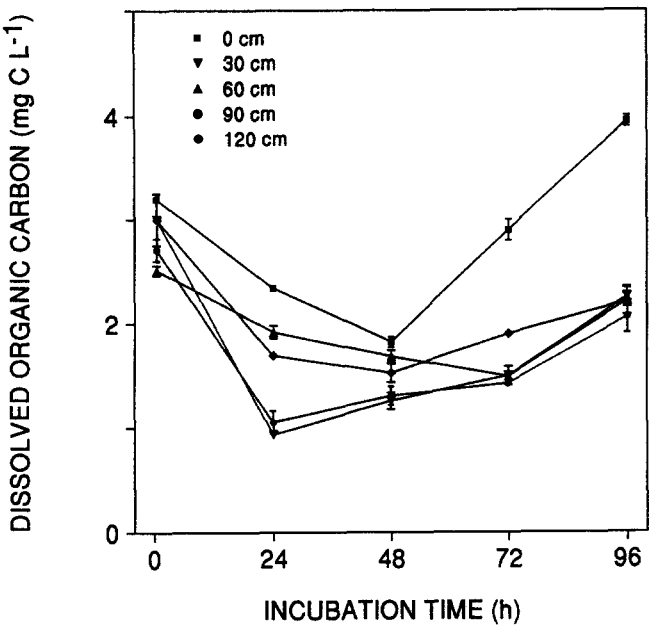

Fig. 7. Change in DOC concentration in the inoculated surface and subsurface water samples from the wetland pond collected July 1994.

DOC analyses during bacterial utilization

Consumption of DOC in bacterially-inoculated surface and subsurface water was monitored in samples collected in July 1994. In samples from all depths, there was a significant decrease ($p < 0.05$) in DOC concentration within the first 72-h of incubation (Fig. 7). The decrease was followed by increases in DOC concentrations. Because DOC decreases were variable over a 72-h period, DOC for BDOC and BGE determinations were evaluated only for the first 24-h of incubation. BDOC of samples collected in July 1994 ranged from 27 to 69% of total DOC (Table 4). Lower percentages of DOC were removed from 0 and 60 cm depths with higher removal rates of DOC from 30, 90 and 120-cm depths.

Bacterial growth efficiency

Bacterial growth efficiency was greatest on DOC from 0 and 60-cm depths (17 and 20%, respectively), with lower BGE on DOC from 30, 90 and 120-cm depths (5, 7 and 9%, respectively) (Table 4). A significant inverse correlation ($r^2 = 0.93$; $p < 0.01$) was found between percent DOC removed and BGE.

Discussion

The surface waters of small forested streams can become enriched with DOC as a result of the movement of water through a wetland ecosystem. Average surface DOC concentrations in the stream at TWE increased as water moved from the less productive mixed deciduous-pine forest through a more productive wetland ecosystem. Similar results were found in a forested swamp catchment in Quebec (Dalva & Moore 1991). Average DOC concentrations (mean annual concentration, not flow-weighted values) in the inflow stream at TWE were 1.95 and 2.60 mg C L^{-1} 725 and 400 m upstream from the wetland pond, respectively. These average surface DOC concentrations from TWE are similar to concentrations reported by Ford and Naiman (1989) for a forested stream in Quebec, Canada and for streams in the Marmot Basin (Alberta, Canada; Wallis et al. 1981), and greater than East and West Bear Brooks (David et al. 1992). DOC concentrations for both streams at TWE were less than those of a stream in northern Michigan (Hendricks & White 1991) and considerably lower than concentrations reported for blackwater rivers in the southeastern United States (Alberts et al. 1990).

Significant seasonal variations in surface water DOC concentrations were observed. DOC concentrations were lower from the winter through the spring with increased concentrations through the summer and fall. Seasonal DOC variations similar to these were found in the surface waters of two Australian wetlands (Briggs et al. 1993). Increased DOC during the summer period can be attributed to increased primary productivity of macrophytes (Wetzel & Howe 1995; Carter et al. 1995) and periphyton (Johnson & Ward 1995) and microbial conversion of POC to DOC.

Average DOC concentrations (mean annual concentration, not flow-weighted values) in the outflow stream at TWE was 4.56 mg C L^{-1}. Unlike the few previous studies that examined DOC exports from wetland ecosystems, DOC concentrations in the outflow of the wetland pond at TWE were comparably lower. DOC in the surface waters of streams draining swamps in North Carolina ranged from 10.0 to 19.7 mg C L^{-1} (Mulholland & Kuenzler 1979) while Day et al. (1977) measured DOC in outflowing water from swamps in Louisiana that averaged 12 mg C L^{-1}. The review of Thurman (1985) reported DOC concentrations in outflow streams of wetlands that ranged from 5 to 60 mg C L^{-1}. The outflow stream at TWE may have lower DOC concentrations because much of the high DOC of the surface waters of the wetland pond was decomposed or retained such that release downstream did not represent a major export of DOC from the wetland pond. Complementary studies of planktonic and attached bacterial productivity (Johnson & Ward 1995) and $CO_2$and CH_4 release from the sediments (Roden & Wetzel 1995) indicate the predominance of DOC degradation.

Concentrations of subsurface DOC were substantially greater than surface DOC concentrations, which indicated that appreciable amounts of DOC produced within the wetland pond were being metabolized or stored within the wetland pond instead of being exported in the surface waters of the outflow stream. Another possible fate of DOC produced in the wetland pond is via export through the subsurface waters. Subsurface DOC concentrations at the lysimeters 50 m downstream from the wetland pond ranged from a low of 1.89 to concentrations >100 mg C L^{-1}. These data suggest that a more significant export of DOC occurred from the subsurface waters compared to the surface waters of the outflow stream. However, the magnitude of this potential export cannot be determined without further knowledge of the hydrology at TWE.

Lateral movements of subsurface water through stream floodplain and wetland hydrosoils at TWE enriched DOC concentrations. Increases in DOC concentrations in the floodplains of streams similar to TWE were reported by Ford and Naiman (1989) and Hendricks and White (1991). The importance of this DOC to the stream and wetland ecosystems cannot be determined fully without increased knowledge of the hydrologic patterns associated with TWE.

Biodegradable DOC for the surface and subsurface waters (after 24-h incubation with wetland bacteria) at TWE (24 to 69% of the total DOC) were similar to those reported for surface waters of European rivers (17 to 54%; Servais et al. 1987; Servais et al. 1989) and surface waters of Lake Ontario and Hamilton Harbor (57–75%; Markosova 1991), but were greater than those found for a clear and humic water lake in Germany (Tranvik & Höfle 1987) and oligotrophic lakes in Sweden (Tranvik 1988).

The use of BDOC as an indicator of the relative lability of DOC must be interpreted cautiously but is congruous with recent analyses. A significant negative correlation was observed between biodegradable DOC, i.e., the bacterial degradation of DOC in 24 h, and bacterial growth efficiency as estimated by the ratio of carbon incorporated into bacterial biomass to carbon removed from the DOC. In experimental studies on utilization rates of DOC from decomposing macrophytes by periphytic bacteria from the same wetland ecosystem (TWE), a majority of DOC-derived C was assimilated and released from the periphyton communities in the form of $DO^{14}C$ (mean 69.6%) rather than as inorganic $^{14}CO_2$ (6.7%) (Bicudo et al. 1995). Retention of assimilated carbon from a mixture of amino acid substrates was more efficient (mean 43.4%) than with more recalcitrant substrates (mean 5.4% of whole leachate DOC of *Juncus effusus*, 3.7% of *Juncus* humic acids, and 2.7% of *Juncus* fulvic acids). These results are in accord with the observations that dissolved organic compounds are selectively removed by metabolic

and adsorptive processes as they pass through the periphyton complex down-gradient to the recipient lake or stream with an increase in the recalcitrance of the DOC en route (Wetzel 1992). Although growth efficiencies on the more recalcitrant DOC sources were smaller than on labile components, the concentrations of the recalcitrant DOC pools are very much larger by one to several orders of magnitude (e.g., Wetzel 1984). As a result, the collective metabolism of the recalcitrant DOC in the ecosystem dominates in all aquatic ecosystems (Wetzel 1995) and is particularly true in the land-water interface zones of high rates of photosynthetic production and decomposition.

BGE for the surface waters of the *Nymphaea* pond at TWE was 17% during the first 24 h. These results were less than growth efficiencies determined for surface waters from the Ogeechee River (Meyer et al. 1987), clear and humic lakes in Sweden (Tranvik 1988), and coastal waters (Middelboe et al. 1992). BGE measured by Tranvik and Höfle (1987) ranged from 17 to 22% on DOC from the surface waters of clear and humic lakes in Germany, similar to those from the surface water of the *Nymphaea* pond at TWE.

Few studies have evaluated bacterial growth efficiencies on DOC from subsurface waters. BGE on DOC collected from the soil waters of a beech-fir forest was 5.7% after 24 h of incubation with bacteria (Boissier & Fontvieille 1993). Bacterial growth efficiencies calculated for 30, 90 and 120 cm in this study (5 to 9%) were also similar to growth efficiencies on DOC from terrestrial leaf leachate (Linley & Newell 1984; Meyer et al. 1987). These data suggest that DOC from subsurface depths may be less available for bacterial growth relative to surface water samples examined.

A large fraction of the total DOC pool at TWE is apparently stored in the subsurface waters of streams, floodplains and wetland pond. This DOC pool likely represents the predominantly recalcitrant substrates for bacterial growth relative to growth efficiencies on DOC from surface waters. A more thorough understanding of bacterial utilization and growth efficiencies on DOC from subsurface waters is needed in order to obtain a more complete view of DOC availability to bacterial communities.

Acknowledgements

The authors express their sincere appreciation to Mark Dedmon, Anne Bell, and Colleen McVicar for technical assistance with the many DOC analyses. Appreciable counsel was received from A.K. Ward, G.M. Ward, and E.E. Roden. The GIS Laboratory of the Department of Biological Sciences produced the maps of the Talladega Wetland Ecosystem of Fig. 1. G.M. Ward kindly provided hydrological and meteorological data from the research site used in certain analyses. The support of the Department of Energy (NIGEC-

91UOA55) and the National Science Foundation (OSR-91-08761 and DEB-92-20822) is gratefully acknowledged.

References

Alberts JJ, Ertel JR & Case L (1990) Characterization of organic matter in rivers of the Southeastern United States. Verh. Internat. Verein. Limnol. 24: 260–262

Bicudo DC, Ward AK & Wetzel RG (1995) Fluxes of dissolved organic carbon within attached aquatic microbiota. Verh. Internat. Verein. Limnol. (In press)

Boissier JM & Fontvielle D (1993) Biodegradable dissolved organic carbon in seepage waters from two forest soils. Soil Biol. Biochem. 25: 1257–1261

Briggs SV, Maher MT & Tongway DJ (1993) Dissolved and particulate organic carbon in two wetlands in southwestern New South Wales, Australia. Hydrobiologia 264: 13–19

Carter S, Ward GM, Wetzel RG & Benke AC (1995) Population dynamics and productivity of the water lily *Nymphaea odorata* in a wetland ecosystem. Ecology (In review)

Dalva M & Moore TR (1991) Sources and sinks of dissolved organic carbon in a forested swamp catchment. Biogeochemistry 15: 1–19

David MB, Vance GF & Kahl JS (1992) Chemistry of dissolved organic carbon and organic acids in two streams draining forested watersheds. Water Resour. Res. 28: 389–396

Day JW Jr., Butler TJ & Conner WH (1977) Production and nutrient export studies in a cypress swamp and lake system in Louisiana. In: Wiley M (Ed) Estuarine Processes 2 (pp 255–269). Academic Press, New York

Dosskey MG & Bertsch PM (1994) Forest sources and pathways of organic matter transport to a blackwater stream: a hydrologic approach. Biogeochemistry 24: 1–19

Easthouse KB, Mulder J & Christophersen N (1992) Dissolved organic carbon fractions in soil and stream water during variable hydrologic conditions at Birkenes, Southern Norway. Water Resour. Res. 28: 1585–1596

Fisher SG & Likens GE (1973) Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecol. Monogr. 43: 421–439

Ford TE & Naiman RJ (1989) Groundwater surface water relationships in boreal forest watershed: Dissolved organic carbon and inorganic nutrient dynamics. Can. J. Fish. Aquat. Sci. 46: 41–49

Hendricks SP & White DS (1991) Physiochemical patterns within a hyporheic zone of a northern Michigan river, with comments on surface water patterns. Can. J. Fish. Aquat. Sci. 48: 1645–1654

Ivarsson H & Jansson M (1994) Regional variation of dissolved organic matter in running waters in central northern Sweden. Hydrobiologia 286: 37–51

Johnson MD & Ward AK (1995) Seasonal trophic dynamics of microbial communities in a lotic wetland ecosystem. Ecology (In review)

Kaplan LA & Newbold JD (1993) Biogeochemistry of dissolved organic carbon entering streams. In: Ford TE (Ed) Aquatic Microbiology. An Ecological Approach (pp 139–165). Blackwell Scientific Publications, Oxford.

Kaplan LA, Larson RA & Bott TL (1980) Patterns of dissolved organic carbon in transport. Limnol. Oceanogr. 25: 1034–1043

Kirchman DL (1993) Leucine incorporation as a measure of biomass production by heterotrophic bacteria. In: Kemp PF, Sherr BF, Sherr EB & Cole JJ (Eds) Handbook of Methods in Aquatic Microbiology (pp 509–512). Lewis Publishers, Boca Raton, FL

Kuserk FT, Kaplan LA & Bott TL (1984) In situ measures of dissolved organic carbon flux in a rural stream. Can. J. Fish. Aquat. Sci. 41: 964–973

Linley EAS & Newell RC (1984) Estimates of bacterial growth yields based on plant detritus. Bull. of Mar. Sci. 35: 409–425

Markosova R (1991) Growth of bacterioplankton on dissolved organic carbon in Hamilton Harbour and western Lake Ontario. Water Pollut. Res. J. Can. 26: 173–185
McDowell WH & Likens GE (1988) Origin, composition, and flux of dissolved organic carbon in the Hubbard Brook Valley. Ecol. Monogr. 58: 177–195
Meyer JL (1994) The microbial loop in flowing waters. Microb. Ecol. 28: 195–199
Meyer JL, Edwards RT & Risley R (1987) Bacterial growth on dissolved organic carbon from a blackwater river. Microb. Ecol. 13: 13–29
Middelboe M, Nielsen B & Søndergaard M (1992) Bacterial utilization of dissolved organic carbon (DOC) in coastal waters – determination of growth yield. Arch. Hydrobiol. Beih. Ergebn. Limnol. 37: 51–61
Mulholland PJ & Kuenzler EJ (1979) Organic carbon export from upland and forested wetland watersheds. Limnol. Oceanogr. 24: 960–966
Roden, EE & Wetzel RG (1995) Regulation of methane flux from freshwater wetland sediments by competing anaerobic microbial processes. Limnol. Oceanogr. (In review)
Servais P, Billen G & Hascoët M (1987) Determination of the biodegradable fraction of dissolved organic matter in waters. Water Res. 21: 445–450
Servais P, Anzil A & Ventresque C (1989) Simple method for determination of biodegradable dissolved organic carbon in water. Appl. Environ. Microbiol. 55: 2732–2734
Simon M & Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51: 201–213.
SYSTAT for Windows: Statistics, Version 5 Edition. Evanston, Il: SYSTAT, Inc. (1992)
Thomaz SM & Wetzel RG (1995) ^{3}H-Leucine incorporation methodology to estimate epiphytic bacterial biomass production. Microbial Ecol. 29: 63–70.
Thurman EA (1985) Organic Geochemistry of Natural Waters. Nijhoff/Junk, Dordrecht 497 pp.
Tranvik LJ (1988) Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb. Ecol. 16: 311–322
Tranvik LJ & Höfle MG (1987) Bacterial growth in mixed cultures on dissolved organic carbon from humic and clear waters. Appl. Environ. Microbiol. 53: 482–488
Vervier P & Naiman RJ (1992) Spatial and temporal fluctuations of dissolved organic carbon in subsurface flow of the Stillaguamish River (Washington, USA). Arch. Hydrobiol. 123: 401–412
Wallis PM, Hynes HBN & Telang SA (1981) The importance of groundwater in the transport of allochthonous dissolved organic matter to the streams draining a small mountain basin. Hydrobiologia 79: 77–90
Wetzel RG (1983) Limnology. 2nd ed. Saunders College Publ., Philadelphia 860 pp.
Wetzel RG (1984) Detrital dissolved and particulate organic carbon functions in aquatic ecosystems. Bull. Mar. Sci. 35: 503–509.
Wetzel RG & Likens GE (1991) Limnological Analyses. 2nd ed. Springer-Verlag, New York 391 pp.
Wetzel RG (1992) Gradient-dominated ecosystems: Sources and regulatory functions of dissolved organic matter in freshwater ecosystems. Hydrobiologia 229: 181–198
Wetzel RG (1995) Death, detritus, and energy flow in aquatic ecosystems. Freshwat. Biol. 33: 83–89
Wetzel RG & Howe MJ (1995) Population dynamics and productivity of the emergent rush *Juncus effusus* of a lotic wetland ecosystem. Ecology (In review)