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Microbial Biomass and Utilization of Dissolved Organic Matter in the Okefenokee Swamp Ecosystem†

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The Okefenokee Swamp exhibited levels of microbial biomass and aerobic glucose uptake comparable to those of other organically rich, detritus-based aquatic ecosystems. In contrast to other peat-accumulating systems, this acidic (pH 3.7), low-nutrient environment does not show diminished water column or surface sediment microbial biomass or heterotrophic activity. The total particular ATP varied between 0.03 and 6.6 μg liter^{-1} (mean, 1.6 μg liter^{-1}) in water and between 1 and 28 μg g (dry weight)$^{-1}$ (mean, 10.0 μg g [dry weight]$^{-1}$ in sediments. The turnover times for dissolved D-glucose were 1.26 to 701.25 h (mean, 110.25 h) in aerobic waters and 2.4 to 72 min (mean, 10.2 min) in aerobic surface sediments. Water column bacterial secondary production, measured as the incorporation of [^{3}H]thymidine into cold-trichloroacetic acid-insoluble material, ranged from 0.06 to 1.67 nmol liter^{-1} day^{-1} (mean, 0.45 nmol liter^{-1} day^{-1}). The kinetics of D-glucose uptake by water column microflora are multiphasic and suggest the presence of a diverse microbial population capable of using labile substrates at nanomolar concentrations and at substantial rates. The presence of a large and active aerobic microbial community in the Okefenokee Swamp is indicative of an important role for microbes in swamp geochemistry and strongly suggests the existence of a detritus-based food web.

The Okefenokee Swamp, located in southeastern Georgia and northeastern Florida, is one of the largest freshwater wetlands in the United States. The Okefenokee Swamp is an acidic (pH 3.1 to 4.4), black-water, peat-accumulating environment consisting primarily of forested swamp and open marsh prairies (G. T. Auble, Ph.D. thesis, University of Georgia, Athens, 1982; E. R. Blood, Ph.D. thesis, University of Georgia, Athens, 1981). Concentrations of inorganic micronutrients (e.g., nitrate, phosphate, ammonia, etc.) in the water column are very low; most of the nutrients in the ecosystem are sequestered in organic form as plant biomass or peat (32; G. T. Auble, Ph.D. thesis, University of Georgia, Athens, 1982), which is as much as several meters thick in the sediments of the swamp interior (11). Apparently, very little of the plant biomass is grazed directly by animals, but rather most of it dies and becomes a component of the organic detritus. Presumably, microbial transformation of the two major reservoirs of organic matter (biomass and peat) is an important mechanism controlling both nutrient availability and the conversion of relatively refractory organic matter to microbial biomass that is highly assimilable by animals. However, to date essentially no information is available regarding either the rates of microbially mediated processes or the sizes of microbial populations in the swamp.

The results of some previous studies of the microbiology of acidic peat bogs suggest that these systems exhibit reduced microbial biomass compared with more neutral grassland soils (24). The combination of low pH, low redox potential, and the refractory nature of the available organic substrates appears to limit both microbial numbers and activity in these environments (12, 25). On the basis of previous studies then, one might expect that microbial biomass and the rates of microbially mediated processes, such as production of particulate organic material (POM) and turnover of dissolved organic compounds in sediment and water, would be lower in the Okefenokee Swamp than in more-neutral-pH detritus-based ecosystems.

Although little is known about detritus processing in the Okefenokee Swamp, information is available from other acknowledged detritus-based ecosystems. The salt marsh, for example, is a more neutral detritus-based ecosystem in which less than 10% of the plant biomass is consumed as living material (29). Most of the plant material first becomes a component of the organic detritus or dissolved organic matter and is subsequently degraded by the luxuriant natural microbial assemblages of the marsh water and sediment. In the process, some of the low-protein, high-fiber plant material is converted to high-protein microbial biomass, which is readily ingested and assimilated by marsh animals (19, 27). For detritus-based food webs to be major contributors to the overall trophodynamics of the Okefenokee Swamp, there would have to be a microbial community supporting rates of organic matter turnover and secondary production that is comparable to those in other wetland ecosystems, not the diminished populations often thought to exist in peat-forming ecosystems.

The above-ground input of plant-derived organic material, which is then potentially available to aquatic microheterotrophs, that is due to the fall of leaf litter can range between 233 g (dry weight) m^{-2} year^{-1} in shrub swamp to 468 g (dry weight) m^{-2} year^{-1} in cypress stands and is comparable to that of other wetlands in the Eastern United States (G. T. Auble, Ph.D. thesis, University of Georgia, Athens, 1982). Cohen (10) suggested that the below-ground production of plant biomass is a major additional input of organic matter; 35 to 75% of the organic matter in Okefenokee peat is distinguishable as root derived. A significantly higher percentage of newly produced organic detritus is leached (solubilized) in the initial weeks of exposure to the water in the Okefenokee Swamp than in non-peat-forming wetlands (G. T. Auble, Ph.D. thesis, University of Georgia, Athens, 1982). Although its composition has not been determined, the leachate is a potential source of labile organic com-

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pounds for the microbial assemblages in the water column. Some preliminary data indicate that the residual insoluble fraction of the organic matter in the Okefenokee Swamp may be more resistant to microbial degradation than comparable detrital material in other wetlands (G. T. Auble, Ph.D. thesis, University of Georgia, Athens, 1982).

The little available information on the organic geochemistry of the Okefenokee Swamp does not support the assumption of reduced activity but rather suggests active microbial decomposition. For example, the microscopic structure of peat cores reveals that surface peat is highly decomposed (11). The existence of a significant microbial population in sediment is suggested by the presence of microbial cellular components such as muramic acid and certain amino sugars (8; D. Casagrande, A. Ferguson, J. Boudreau, R. Predy, and C. Foldin, Presentation to Proceedings-Ninth International Congress of Carboniferous Stratigraphy and Geology, 1979).

This report presents the initial results of an intensive investigation of the microbial biogeochemistry of the Okefenokee Swamp ecosystem. We have used several widely applied microbial biomass and activity measurements chosen because of the availability of comparative data from a variety of other aquatic environments. Some of the methods are currently the subjects of discussion in the literature regarding what, exactly, they measure and how results should be interpreted. However, the results of the several techniques used to date are in general agreement; they indicate that, far from having depressed microbial populations and activity, the waters and surface sediment of the Okefenokee Swamp support microbial biomass and turnover rates of simple dissolved organic compounds equal to or greater than those of other wetland ecosystems.

MATERIALS AND METHODS

Sample sites. The Okefenokee watershed encompasses an area of 3,781 km^2 of which 46% is swamp and 54% is upland pine plantation (1). The major input of water to the swamp is precipitation, and drainage is via two rivers, the Suwanee, which flows to the Gulf of Mexico, and the St. Marys, which flows to the Atlantic. Flow through the watershed is slow; evapotranspiration is the major mechanism of water loss from the system, as described by Patten and Mates (*in* A. D. Cohen, D. J. Casagrande, M. J. Andrejko and G. R. Best, ed., *Okefenokee Swamp: its natural history, geology, geochemistry*, in press). Swamp vegetation is a mosaic of several distinctive habitats (15; D. B. Hamilton, Ph.D. thesis, University of Georgia, Athens, 1981), including freshwater marshes, shrub swamps, and forested areas dominated by cypress, black gum, and a number of species of bay trees. Representative areas of four major habitats, which together represent 78% of the total vegetation cover of the swamp, were sampled as part of this study.

(i) Chesser Prairie. The Chesser Prairie is an aquatic macrophyte prairie dominated by white water lily (*Nuphar advena*), golden club (*Orontium aquaticum*), floating heart (*Nymphoides* spp.), and a number of bladderwort species (*Ultricularia* spp.). Water was absent from this site during the last 3 months of sampling, and the usual macrophyte vegetation began to be replaced by growth of the sedge *Rhynchospora* sp. Aquatic macrophyte prairies, together with grass and sedge prairies, cover 21% of the swamp area.

(ii) Chesser Shrub. Chesser Shrub is a shrub swamp island in Chesser Prairie. Dominant shrubs are titi (*Cyrella racemiflora*), federbush (*Leucothoe racemosa*), and sweet spire (*Itea virginiana*). The shrub swamp habitat constitutes 34% of the Okefenokee Swamp.

(iii) Grand Cypress. Grand Cypress is a forested swamp dominated by pond cypress (*Taxodium ascendens*) with an understory of white bay (*Persea borbonia*), magnolia (*Magnolia virginiana*), loblolly bay (*Gordonia lasianthus*), and poor man's soap (*Clethra alnifolia*). *Sphagnum* moss is present in scattered mats. The cypress bay habitat covers 23% of the swamp.

(iv) Mizell Prairie. The Mizell Prairie is a grass and sedge prairie dominated by maidencane (*Panicum hemitomon*), sedge (*Carex* spp.), and *Sphagnum* spp.

Sample collection. Water and sediment samples were collected in autoclaved polyethylene bottles. Okefenokee surface sediments are highly organic (over 90%), extremely flocculent, and easily resuspended by any movement of the overlying water. When standing water was present, surface sediment was collected as a slurry by gently passing the mouth of the sample bottle over the sediment surface. When standing water was absent, sediment was obtained by scraping the upper centimeter of the aerobic zone with a sterile spatula, and slurries were prepared by vigorously mixing 10 ml of sediment with 90 ml of swamp water (filtered through a 0.2-μm pore-size filter) collected from as close to the sampling site as possible. All samples were processed within 4 to 12 h of collection.

Microbial biomass. The total microbial biomass was measured as the concentration of particulate ATP in the water column and the total ATP in surface sediments. The ATP technique was chosen because of the relative ease it affords in processing large numbers of samples and the availability of comparative data from a variety of aquatic ecosystems. The acidic conditions, high concentration of humic material, and flocculent surface sediments of the Okefenokee Swamp necessitated modification of existing methods. Water column samples (100 ml) were filtered on 0.2-μm Nuclepore filters and extracted by immersing the filters for 2 min in 5 ml of boiling 0.1 M sodium bicarbonate buffer, pH 8.5 (21). Sediment slurry samples (1 ml) were added to 16 ml of boiling 0.1 M sodium bicarbonate buffer at pH 8.5 and extracted for 2 min (6). The efficiency of ATP recovery from sediment extracts was determined by adding 500 or 1,000 ng of reagent-grade ATP Disodium Salt (Sigma Chemical Co.) to one of the three replicate sediment samples just before extraction. Once extracted, sediment samples were centrifuged at 20,000 × *g* for 15 min, and the supernatants were analyzed for ATP. Water column and sediment sample extracts were diluted with 0.1 M Tris buffer (pH 7.8) to a final bicarbonate/Tris ratio of 2:3, and 0.2 ml was assayed for ATP by the firefly luciferase method (22) with a Science Applications Inc. model 3000 ATP photometer. An additional internal standard consisting of 100 ng of ATP was added to a 1-ml portion of each sample after the initial assay, and the spiked portion was reassayed as a check on any inhibition of the luciferin-luciferase luminescence which might result from the presence of inhibitory compounds in the extracts. The high concentrations of humic substances in black-water systems necessitated rigorous monitoring of ATP recovery and of the efficiency of the luciferin-luciferase enzyme. The mean recovery of ATP from sediment was 44% (±22%). The mean inhibition of the luciferin-luciferase enzyme was 24% for sediment extracts and 9% for extracted water samples. After corrections for ATP recovery and luciferase inhibition, the mean coefficient of variation for replicates within a single slurry was 12%. This is a realistic estimate of the precision of the ATP technique for swamp sediments.

Microbial activity. The microbial turnover of dissolved D-glucose was used as an index of the relative rate at which

the microbial community utilized labile organic substrates in the water column and in aerobic surface sediments. The basic procedure followed the Azam and Holm-Hansen (5) modification of the initial Wright and Hobbie (33) technique. Incubations were carried out in the dark at in situ temperatures in either sterile Wirl-pak (Nasco) bags or sterile VACUTAINER (Becton Dickinson Vacutainer Systems) tubes. For each incubation procedure, preliminary time course experiments were run to determine appropriate incubation periods during which the uptake of turnover rates were constant. Glucose turnover times in the water column were determined by the incubation of triplicate 25-ml water samples for 30 min with 1.3 nM D-[6-^{3}H]glucose (New England Nuclear Corp.). For experiments aimed at the determination of glucose uptake kinetics, glucose concentrations were increased by the addition of unlabeled glucose to give final concentrations ranging from 1.3 nM to 0.8 nM. To determine glucose turnover time in sediments, slurries (3 ml) were incubated for 5 min with 11.1 nM D-[6-^{3}H]glucose. Preliminary experiments revealed that, with sediments, observed glucose turnover time was directly proportional to the dilution factor used in the preparation of sediment slurries (i.e., 10-fold-diluted sediment had a turnover time which was 10 times greater than that of undiluted sediment). Therefore, the total incorporation by the slurry was corrected for the degree of dilution of the surface sediment. All incubations were terminated by the addition of Formalin to a final concentration of 2%. Samples were then collected by filtration on Nuclepore filters (47 mm; 0.2 μm pore size) and rinsed twice with several milliliters of filtered swamp water. Filters were air dried and then combusted in an OX-300 biological oxidizer (R. J. Harvey Co.) to oxidize the incorporated radiolabel to ^3H_2O. Tritiated water was collected in 14 ml of Scintiverse counting medium (Fisher Scientific Co.), and the samples were radioassayed in a Beckman LS 9000 liquid scintillation spectrometer. Quench corrections were made by using either the external standard or sample channels ratio method, and counts per minute were converted to disintegrations per minute. Filter combustion proved to be a highly effective method for the recovery of radiolabeled material from the humic-rich waters and sediments of the Okefenokee Swamp. Filtered material which was not combusted could not be effectively counted, whereas combusted samples could be counted at high efficiency and without significant quench or self-absorption. Compared with combusted samples, filtered water samples which were not combusted had microbial turnover rates that were underestimates by a factor of 2.8 (n = 14). Likewise, sediment samples which were not combusted had turnover rates that were underestimates by a factor of 26.1 (n = 18).

Bacterial secondary production. The incorporation of [^{3}H]thymidine into cold-trichloroacetic acid (TCA)-insoluble material (primarily DNA) was used as an index of water column bacterial secondry production (18). Triplicate 10-ml water samples were incubated with 5 nM [*methyl*-^{3}H]thymidine (40 to 60 Ci/mmol; New England Nuclear Corp.) at in situ temperatures for 2 h. The samples and formalin-killed controls were processed by the technique developed by Fuhrman and Azam (18). The incubations were terminated by the addition of 10 ml of ice-cold 10% TCA. The samples were filtered on 0.2-μm pore-size Millipore filters, washed twice with 5% TCA, air dried, and then combusted and radioassayed as described above. Thymidine incorporation by bacteria in the water samples was linear over the 2-h incubation period. The additions of 5 nM thymidine were adequate to produce maximum labeling of cold-TCA-insoluble material. Riemann et al. (30) reported that thymidine concentrations up to 10 nM may be required in some freshwater systems. In Okefenokee waters, incubation with 20 nM thymidine increased incorporation by only 12%, suggesting that 5 nM was sufficient to saturate extracellular and intracellular thymidine pools.

TABLE 1. Ranges of ATP concentrations in the water column and surface sediments of the Okefenokee Swamp and other wetlands and in terrestrial soils

Location	ATP concn[a]	Reference
Water column		
South Carolina salt marsh	0.3–3.5	16
Cheseapeake Bay salt marsh	0.02–2.7	2
Sapelo Island salt marsh, Ga.	1.0–4.0	20
Okefenokee Swamp, Ga.	0.3–6.6[b]	This study
Sediments		
Long Island Sound, N.Y.	1.1–7.6	34
Sapelo Island salt marsh, Ga.	1.2–9.8	9
13 Australian soil types (agricultural and forest)	0.6–9.0	23
9 New Zealand tussock grasslands	2.2–10.7	31
Freshwater marsh, Mich.	5.3–16.7	14
Okefenokee Swamp, Ga.	1.0–28.0[c]	This study

[a] The water column concentrations are given in micrograms liter^{-1}; sediment concentrations are given in micrograms gram (dry weight)$^{-1}$.

[b] The arithmetic mean ATP concentration in the Okefenokee Swamp water column was 1.6 μg liter^{-1}.

[c] The arithmetic mean ATP concentration in the Okefenokee Swamp sediments was 10.0 μg g (dry weight)$^{-1}$.

RESULTS AND DISCUSSION

The Okefenokee Swamp exhibited levels of microbial biomass, rates of dissolved glucose turnover, and bacterial secondary production comparable to those of other aquatic ecosystems which receive inputs of detrital material. The resident microbial community did not show reduced biomass or activity notwithstanding the prevailing acidic, low-nutrient conditions.

Microbial biomass. Microbial biomass in the water column and aerobic surface sediments was substantial in all habitats throughout the year. A summary of Okefenokee water column and sediment microbial biomass values compared with similar data from several other ecosystems is presented in Table 1. The means and ranges of biomass values from the Okefenokee Swamp were compiled from over 100 samples collected over a period of 1 year from the four major swamp habitats. Water column microbial biomass encompassed the ranges reported for the more-neutral-pH salt-marsh wetlands. Likewise, the range of values for Okefenokee sediment ATP spanned the ranges reported for a variety of freshwater and marine sediments and terrestrial soils. The Okefenokee mean ATP concentration of 10.0 μg g (dry weight)$^{-1}$ is at the upper end of the ranges for most systems. It should be noted that the highly organic (over 90% by weight) composition of swamp surface sediments makes direct comparison, on a dry weight basis, with other sediment systems difficult. Each gram (dry weight) of Okefenokee sediment represents a much larger initial volume of wet sediment than does a gram (dry weight) of sediment from the other environments. This difference is the result of the low-density, flocculent nature of swamp sediments. For exam-

TABLE 2. Concentrations of microbial ATP in the water column and surface sediment of four Okefenokee Swamp habitats, 1980 to 1981

Habitat	ATP concn[a]										
	August	September	December	February	March	April	May	June	July	August	Mean[b]
Water column											
Chesser Prairie	0.30 ± 0.01	0.03 ± 0.01	2.63 ± 0.35	0.40 ± 0.10	0.44 ± 0.10	0.36 ± 0.02	1.29 ± 0.12	—	—	3.77 ± 1.12	1.15 ± 1.35
Chesser Shrub	2.87 ± 0.10	0.21 ± 0.01	0.48 ± 0.06	1.75 ± 0.13	1.05 ± 0.22	6.63 ± 0.88	—	—	—	—	2.16 ± 2.39
Grand Cypress	*	*	2.63 ± 0.04	1.32 ± 0.15	2.12 ± 0.09	—	—	—	—	—	2.02 ± 0.66
Mizell Prairie	3.33 ± 0.04	0.420 ± 0.06	—	0.97 ± 0.07	1.39 ± 0.27	—	—	—	—	—	1.52 ± 1.27
Surface sediment											
Chesser Prairie	*	23.88 ± 2.30	24.29 ± 0.18	14.43 ± 1.14	9.35 ± 0.65	15.41 ± 0.01	19.13 ± 1.02	2.66 ± 0.06	5.46 ± 0.21	5.37 ± 0.84	13.33 ± 8.11
Chesser Shrub	*	13.74 ± 0.29	3.55 ± 0.37	2.84 ± 1.69	1.66 ± 0.11	2.90 ± 0.33	1.85 ± 0.11	2.01 ± 0.18	5.72 ± 0.31	17.56 ± 0.60	5.76 ± 5.82
Grand Cypress	*	12.89 ± 0.48	6.70 ± 0.07	*	7.33 ± 1.07	2.55 ± 0.01	15.58 ± 1.30	*	9.10 ± 0.80	12.85 ± 2.81	9.57 ± 4.49
Mizell Prairie	*	27.94 ± 3.87	*	26.27 ± 2.46	3.38 ± 0.08	<1.00	13.06 ± 0.56	4.42 ± 0.23	9.24 ± 0.72	5.60 ± 0.71	11.36 ± 10.40

[a] The water column concentrations are given in micrograms liter^{-1}; sediment concentrations are given in micrograms gram (dry weight)$^{-1}$. —, No standing water; *, not sampled.
[b] Mean values are arithmetic means.

TABLE 3. Spatial distribution of ATP in surface sediment[a]

Slurry	No. of replicates	ATP (μg g [dry wt]$^{-1}$)[b]
1	3	9.25 ± 1.42
2	2	12.38 ± 0.84
3	3	5.12 ± 0.54
4	3	13.89 ± 1.78
5	3	5.42 ± 0.50

[a] All samples were collected on the same day from randomly selected sites over a 10,000-m^2 area in Chesser Prairie.
[b] Mean ± 1 standard deviation. Arithmetic mean of all samples is 8.99 ± 3.79 (n = 14).

ple, a gram (dry weight) of Sapelo Island, Ga., salt-marsh sediment is equivalent to 2.5 ml of original wet sediment, although a gram (dry weight) of Okefenokee sediment is equivalent to 15.0 ml. Thus, an organism foraging for microbes in the swamp sediments would have to ingest a much larger volume of sediment to obtain an equal amount of biomass. It is clear from Table 1, however, that a substantial microbial population is associated with the surface sediment.

Water column and sediment ATP concentrations obtained during routine sampling of one site from each habitat over a 1-year period are presented in Table 2. Microbial biomass exhibited considerable temporal variability in all habitats and did not show any obvious correlation with season or water temperature. The Chesser Prairie water column exhibited its highest biomass in December, May, and August. The highest water column ATP value recorded during the study was obtained at the shrub site in April, when much of the site was dry and most of the remaining water was concentrated in small pools. Thus, the high biomass may result from the concentration of microbial populations as a result of the decreasing water level. The Grand Cypress and Mizell Prairie sites were the driest of the four habitats; standing water could be sampled only intermittently. Neither site exhibited any clearly definable trends with respect to biomass, on the basis of the small number of samples obtained. Sediment microbial biomass (Table 2) ranged from less than 1.0 to 27.9 μg g (dry weight)$^{-1}$. Biomass decreased markedly in June, July, and August at Chesser Prairie, corresponding to a period when virtually no standing water was present on the site although the suface sediments remained moist. Sediment biomass varied less from month to month at the shrub site except for large peaks in September and August. High sediment microbial biomass values were observed from May to September at the cypress site and during September and February at the Mizell Prairie site. Again, these habitats were the driest of the four habitats sampled. The annual means of ATP (micobial biomass) concentrations for both water and sediment samples did not differ significantly among the four sites as indicated by one-way analysis of variance. Although substantial differences between habitats were observed on some sampling occasions, general trends were not apparent. A likely cause of the absence of identifiable differences between habitats is the spatial and temporal patchiness of the microbial populations within each habitat. Preliminary results indicated that surface sediment microbial biomass can vary by a factor of two at any given time at various sites within a habitat. Table 3, which shows the ATP biomass of surface sediments collected on the same day from randomly selected sites over a 10,000-m^2 plot in the Chesser Prairie habitat, illustrates the degree of spatial patchiness within a habitat. Considering the patchy distribution of

microbial biomass, it is apparent that intensive sampling within each habitat will be required to more critically assess the question of habitat differences in water column and sediment biomass.

Microbial activity. Turnover times for dissolved D-glucose in the water column of the Okefenokee Swamp (Table 4) varied between 1.26 and 701.25 h (mean, 110.25 h). No significant turnover was detected in Formalin-killed samples, indicating that turnover in unamended samples was microbially mediated. The longest turnover time (701.25 h) was recorded in December at the shrub site, and the shortest turnover time (1.26 h) was recorded in August at the Chesser Prairie site. Activity was highest during the spring and summer months. The mean glucose turnover time in the water column for all habitats between September and February was 185.07 h, and between March and August it was 18.81 h. Sediment glucose turnover times were considerably shorter than those of the water column and varied between 2.46 and 69.96 min (mean, 9.24 min). Glucose turnover in surface sediments was slowest in December at the shrub and cypress sites and in April at the two prairie sites. All sites exhibited short (less than 9.24-min) turnover times during the summer months. D-Glucose turnover was fast at Chesser Prairie between June and August despite the lack of standing water on the site and the observed decrease in ATP biomass. Site differences in water column and surface sediment glucose turnover times were not observed. Annual means for glucose turnover time at the four sites were not statistically different (one-way analysis of variance).

Overall, the Okefenokee water column community exhibited glucose turnover times comparable to those reported for eutrophic estuarine waters (13, 20, 28). Glucose turnover was generally faster in the warmer months, although this trend could not be followed during much of the summer owing to the drying of the swamp. The turnover of glucose in the surface sediment was much faster than in the water column, and the rates varied less over the year. The consistency of the sediment turnover rates suggests that surface sediment microbial processes are not tightly coupled to annual cycles of litter fall or water column primary production.

D-Glucose uptake kinetics. The relationship between substrate concentration and the rate of uptake of various dissolved organic compounds has been used to assess the degree of kinetic diversity with which a natural aquatic bacterial population interacts with its potential carbon and energy sources. This approach (4, 33) involves deriving kinetic parameters graphically from uptake data via a modified Lineweaver-Burk plot wherein t/f (incubation time [t] divided by the fraction [f] of added substrate used during t) is plotted on the y axis against [A], the concentration of added substrate, on the x axis. Over a relatively small range of [A] (e.g., 10- to 20-fold), data from freshwater and marine environments often fall on a straight line (4, 33) with a single, finite V_{max} and $K_t + S_n$ (sum of K_t, the concentration of substrate resulting in half maximal uptake, and S_n, the in situ substrate concentrations). Linearity is taken as indicating that uptake is predominantly via a single transport system or several kinetically indistinguishable uptake mechanisms. However, for some aquatic environments, when the relationship between t/f and added substrate concentration is examined over a much wider range of [A] (e.g., nanomolar to millimolar), the resulting data plot is curvilinear (4, 33). Azam and Hodson (4) have interpreted such multiphasic kinetic patterns as indicating the presence and activity of multiple uptake systems with different kinetic parameters. In

TABLE 4. Turnover times for dissolved D-glucose in the water column and surface sediments of four Okefenokee Swamp habitats, 1980 to 1981

Habitat	Turnover time[a]								Mean[b]
	September	December	February	March	April	May	June	July	August	
Water column										
Chesser Prairie	243.85 ± 37.64	66.47 ± 6.48	4.86 ± 0.33	1.57 ± 0.24	7.97 ± 3.57	16.37 ± 1.75	—	—	1.26 ± 0.04	48.91 ± 88.97
Chesser Shrub	39.77 ± 1.15	701.25 ± 251.73	37.97 ± 4.02	15.42 ± 0.93	8.95 ± 0.73	—	—	—	—	160.67 ± 302.50
Grand Cypress	—	509.85 ± 31.23	105.87 ± 6.92	8.87 ± 0.58	—	—	—	—	—	41.53 ± 55.72
Mizell Prairie	259.64 ± 13.29	53.75 ± 42.92	12.48 ± 4.90	3.40 ± 0.47	105.51 ± 13.77	—	—	—	—	86.96 ± 104.64
Surface sediment										
Chesser Prairie	2.46 ± 0.36	4.62 ± 0.78	5.46 ± 0.72	12.54 ± 6.00	26.04 ± 7.32	8.76 ± 2.94	2.04 ± 0.48	3.36 ± 0.42	5.76 ± 0.84	7.89 ± 7.56
Chesser Shrub	2.52 ± 0.54	24.12 ± 3.00	11.64 ± 2.10	7.80 ± 2.16	7.80 ± 3.18	8.22 ± 3.78	7.92 ± 2.16	6.12 ± 0.84	2.22 ± 0.06	8.71 ± 6.48
Grand Cypress	7.86 ± 1.26	69.96 ± 27.36	6.66 ± 0.42	12.66 ± 5.40	10.20 ± 1.98	9.24 ± 2.40	*	5.46 ± 2.40	2.46 ± 0.36	15.56 ± 22.20
Mizell Prairie	2.88 ± 0.24	4.68 ± 1.20	5.58 ± 0.60	22.74 ± 2.34	24.30 ± 2.34	8.10 ± 2.52	4.80 ± 0.66	5.10 ± 0.12	2.34 ± 0.42	8.95 ± 8.43

[a] The water column turnover times are given in hours; surface sediment turnover times are given in minutes. —, No standing water; *, not sampled.
[b] Mean values are arithmetic means.

such cases no single K_t or V_{max} value describes the kinetics of uptake of the particular substrate by the entire microbial population. Rather, the dominant K_t and V_{max} values shift in response to changes in in situ substrate concentrations. High-affinity (low K_t), low-capacity (low V_{max}) uptake systems may predominate at low (nanomolar) substrate concentrations. Lower-affinity, higher-capacity systems may predominate during uptake at higher (micromolar) substrate concentrations. Thus, the microbial assemblage can rapidly respond to fluctuations in substrate concentration that may occur spatially and temporally.

The kinetics of microbial D-glucose uptake in water from one important habitat in the Okefenokee Swamp, the Chesser aquatic macrophyte prairie, were examined in the manner of Azam and Hodson (4). Water column kinetics were clearly multiphasic when examined over a wide range of substrate concentrations (Fig. 1). We graphically determined the lowest and highest observable values for $K_t + S_n$ and V_{max} by linear extrapolation through the data points corresponding to the three lowest and three highest glucose concentrations. The values for $K_t + S_n$ varied between 5.1 nM and 38.5 μM with V_{max} values ranging from 4.96 to 700 nmol liter^{-1} h^{-1}. Even assuming that the 5 nM value is due totally to K_t, it is clear that very high affinity uptake systems are present in some of the bacteria inhabiting this environment.

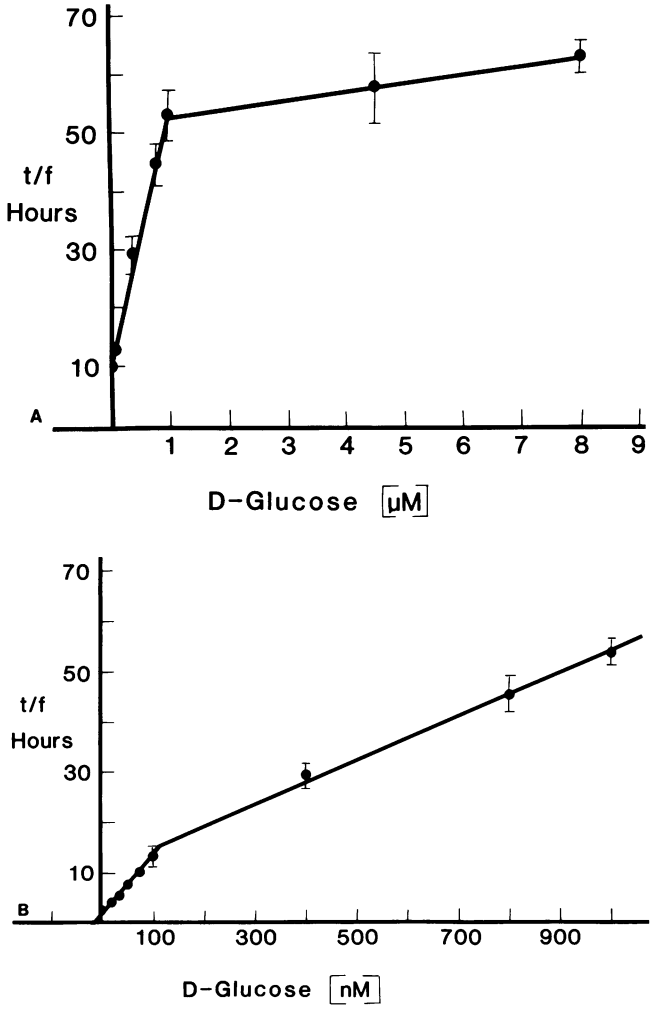

FIG. 1. (A) Modified Lineweaver-Burk plot of microbial D-glucose assimilation kinetics in Chesser Prairie water column. Each point represents mean of triplicates. Bars depict ±1 standard deviation. Points without visible bars indicate that ±1 standard deviation was less than the size of the point. (B) Modified Lineweaver-Burk plot of same data as in (A) except that the scale of the x-axis has been expanded to better show kinetics at nanomolar concentrations of glucose.

Not all of the systems examined to date exhibit kinetic diversity even when examined over a very wide range of substrate concentrations. Azam and Hodson (4) observed that glucose uptake in two extremely eutrophic desert pools obeyed simple Michaelis-Menten kinetics. The low kinetic diversity in the desert pools was interpreted as indicative of the predominance of a single bacterial species or multiple species with kinetically indistinguishable uptake systems for glucose. The microbial populations of Okefenokee waters and sediment exhibit high kinetic diversity similar to that of marine populations (4). Our data clearly show that the acidic, low-nutrient conditions in the swamp have not resulted in kinetic uniformity of the bacterial population. Rather, Okefenokee populations possess a diversity of kinetic types which allows them to respond efficiently to changes in the concentration of dissolved glucose and probably other substrates as well.

In the Okefenokee Swamp, the concentrations of labile dissolved substrates are likely to vary spatially between bulk water and organically rich microzones such as the immediate vicinity of living plants, the surfaces of suspended detrital particles, and the guts of aquatic animals. These regions might produce sustained high concentrations of dissolved organic substrates. In addition, transiently high concentrations might result from the fragmentation of plant material and organisms when they are fed upon and from the excretion of feces by aquatic animals. In the organically rich, vegetation-choked waters of the Okefenokee Swamp, local concentrations of dissolved organic compounds may thus, at times, greatly exceed the overall bulk concentration that would be measured after filtration (homogenization) of a water sample for organic analysis. Instead of monotonously low nanomolar levels, the microheterotrophs would be expected to encounter, at various times, a wide range of substrate concentrations from nanomolar to perhaps micromolar or even millimolar concentrations in enriched microzones. Thus, the observed kinetic diversity may be a means by which the bacterial assemblage, as a whole, can rapidly exploit high concentrations of dissolved organic compounds in rich microzones while efficiently scavenging the very dilute dissolved organic compounds of bulk water.

Bacterial secondary production. Bacterial secondary production of POM by the water column microflora was estimated as the rate of incorporation of [^{3}H]thymidine into cold-TCA-insoluble material (presumably DNA). These incorporation rates ranged from 0.5×10^{-10} to 16.7×10^{-10} mol liter $^{-1}$ day^{-1} (Table 5). The highest rate was recorded in Chesser Prairie in August, when standing water was lowest. Water levels throughout the swamp were decreasing during the sampling period; standing water was absent from all sampling sites during June and July. It is difficult to identify differences between sites owing to the small number of samples taken, but rates at the two prairie sites tended to be higher than those at the other two sites. In general, the water column bacterial thymidine incorporation rate was substantial and comparable to values reported for moderately eutrophic waters including Scripps Pier, the Southern

TABLE 5. Incorporation of [^{3}H]thymidine into cold-TCA-precipitable cellular material by microorganisms in the Okefenokee Swamp water column

Habitat	Incorporation[a] of [^{3}H]thymidine				
	March	April	May	August	Mean[b]
Chesser Prairie	4.84 ± 0.01	8.22 ± 0.06	1.73 ± 0.25	16.67 ± 1.43	7.87 ± 6.44
Chesser Shrub	1.69 ± 0.01	0.61 ± 0.18	—	—	1.15 ± 0.76
Grand Cypress	1.01 ± 0.01	—	—	—	1.01 ± 0.01
Mizell Prairie	6.69 ± 1.23	0.56 ± 0.02	—	—	3.63 ± 4.34

[a] Moles liter^{-1} day^{-1} ($\times 10^{-10}$). Each value represents the mean of three replicates. —, No standing water; water was absent from all sites during June and July.

[b] Mean values are arithmetic means.

California Bight, coastal Georgia (17, 18, 26), and a number of freshwater lakes (7, 30). Moreover, thymidine incorporation rates in the Okefenokee Swamp during the spring and summer were two orders of magnitude greater than those in oligotrophic systems such as the Antarctic Sea (18) and the Sargasso Sea (unpublished data).

In this report we have expressed the rates of thymidine incorporation by the Okefenokee microflora directly as moles liter^{-1} day^{-1}. Although we cannot yet convert these rates to rates of bacterial secondary production grams of C liter^{-1} day^{-1} or cells liter^{-1} day^{-1}) for comparison with the rates of the other ecosystems, our data do suggest that secondary production rates in the Okefenokee Swamp may be moderate to high relative to the rates of other aquatic ecosystems examined to date. These rates are consistent with our finding that microbial biomass and rates of microbial activity are high in the Okefenokee Swamp.

For animals, bacterial biomass represents one of the most readily utilizable and highest-quality (protein-rich) forms of POM in the aquatic habitats of the swamp ecosystem. Peat and detrital matter constitute the bulk of the POM, but most of this material is utilized by animals only after modification. Thus, the relatively small percentage of POM that consists of bacterial and other microbial biomass (autotrophic as well as heterotrophic) exerts a disproportionately large influence on production by swamp animals. The thymidine incorporation rates observed in Okefenokee Swamp water indicate that the bacterial biomass turns over, probably due to grazing by protozoa and larger zooplankton, and is replenished by new bacterial growth. The rates reported here are only for the predominantly free-living bacterioplankton, which grows at the expense of dissolved organic matter, and do not include production by bacteria attached to larger detrital particles or peat. Rates of secondary production by attached bacteria are unknown but may be high.

Free-living bacteria in phytoplankton-based marine systems, which exhibit levels of bacterial thymidine incorporation comparable to those in the Okefenokee, have been estimated to process from 10 to 50% of primary production before it enters the animal food web (3). Although we cannot yet quantify the percent contribution of bacterial secondary production to the total production of utilizable POM in the swamp, the observed substantial rates are suggestive of an important role for the water column bacterial community in the transfer and repackaging of readily utilizable organic matter.

Conclusions. The Okefenokee Swamp supports levels of microbial biomass in water column and surface sediments similar to those in other organically rich aquatic ecosystems. Bacterioplankton populations in the swamp exhibit significant rates of secondary production and of turnover of labile organic substrates at low concentrations. The kinetic diversity exhibited by the microbial assemblages would facilitate rapid, efficient uptake of transiently high concentrations of dissolved substrates when and if they occur. Thus, our results show the microbial biomass, activity, and secondary production can be high even in an acidic, low-nutrient aquatic environment.

It is difficult to directly compare our results with those from other acidic wetlands such as high-latitude peat bogs, which differ significantly from the swamp with regard to climate, pH regime, and vegetation type. Moreover, most of the studies of these systems used plate and dilution counting techniques to enumerate the total viable heterotrophic bacteria and specific groups of bacteria. These data were derived by methods no longer considered reliable when used in highly heterogenous aquatic environments. Thus, the results of earlier studies are difficult to relate to our measurements of total microbial biomass, turnover of dissolved organic compounds, and secondary production. However, results from our investigations in the Okefenokee Swamp are comparable to those from studies of salt marshes and other aquatic systems where similar measurements have been made.

Since the Okefenokee Swamp is a peat-forming ecosystem, our results clearly show that the formation of peat and the high rates of microbial activity in the water and aerobic surface sediments are not mutually exclusive. Peat deposition occurs when the rate of accumulation of organic matter exceeds, by however small an amount, the rate of removal or degradation or both. Several factors, which are unrelated to the activity of the microbial community, may influence peat formation. These include geomorphology, residence time of water in the system, and the mode of input of POM.

The substantial microbial biomass and the high rates of activity in the swamp water column and surface sediment are highly suggestive of the existence of a detritus-based food web. Our results demonstrate the existence of a large and active microbial population capable of using labile substrates and suggest that this is an important process in the flow of energy from primary production to the higher trophic levels of the food web.

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