ORIGINS, FATES, AND RAMIFICATIONS OF NATURAL ORGANIC COMPOUNDS OF WETLANDS

Robert G. Wetzel[1]

Abstract—Much of the organic carbon for heterotrophic metabolism in aquatic ecosystems is soluble and derived from structural compounds of higher plants of terrestrial and wetland-littoral sources of both lake and river ecosystems. The chemical recalcitrance of this organic matter and its oxidative utilization are fundamentally different from many sources within the aquatic ecosystems. Within the lake or river, complex physical interactions occur that can greatly modify rates of utilization and biochemical reactions. Natural photolysis by photosynthetically active radiation, UV-A and UV-B, can result in the partial degradation of these macromolecules, reactivate complexed enzymes, and generate simple organic compounds and nutrients. A portion of the dissolved organic matter is photolytically degraded completely to CO_2. The chemical recalcitrance of these organic compounds (a) represents a fundamental, often dominant, subsidy of organic matter that drives metabolism in fresh waters and (b) is an essential aspect of metabolic stability in aquatic ecosystems. Climatic changes affect this metabolic stability in both positive and negative ways but, generally, will increase instabilities.

INTRODUCTION

The land-water interface region of aquatic ecosystems is always the most productive per-unit area along the gradient from land to open water. Because most aquatic ecosystems occur in terrain of gentle slopes and are small (mean area < 100 ha) and shallow (mean < 5 m), the wetland-littoral components usually dominate in productivity and the synthesis of organic matter (Wetzel 1990, 1992). Because of the predominance of small, shallow freshwater bodies, most dissolved organic carbon (DOC) of lacustrine and riverine ecosystems is derived from photosynthesis of higher plants and microflora associated with detritus, including sediments, and is only augmented by releases of organic matter produced by phytoplankton.

The dissolved organic compounds generated by the production and partial degradation of organic matter produced in terrestrial soils, wetland, and littoral interface regions move downgradient toward the open-water regions of the lake or river. En route, partial utilization by largely attached microorganisms effects a selective decomposition of more labile organic compounds and an increase in organic substrate recalcitrance.

Dissolved and colloidal organic matter of inland waters can be separated on the basis of many criteria. Although separated from particulate organic matter by filtration at a size of 0.5 mm, for an array of practical reasons (Wetzel and Likens 2000), dissolved organic matter is often further separated by molecular-sized categories (Cabaniss and others 2000, Gustafsson and Gschwend 1997). The molecular weight of humic substances influences their proton and metal-binding characteristics, partitioning behavior, and adsorption properties. Average molecular weight distributions of aquatic fulvic acids vary from 2.7 to 3.0 kD and include low-MW fractions of 1,110 D and a high-MW fraction of ca. 4,890 D. Less that 10 percent of dissolved humic substances were found to be greater than 5 kD. The low-MW fractions are more hydrophilic, exhibit faster diffusion and increased mobility, and are commonly more bioavailable. In contrast, the high-MW components have increased uptake of hydrophobic organic compounds, greater aromatic components, decreased mobility and diffusion, and are usually appreciably less susceptible to biological utilization. Molecular-sized fractionation helps little, however, in relation to bioavailability of different compounds, particularly above the size of effective cellular membrane permeability of ca. 500 D. Bonding structure, particularly of aromatic components, and availability of sites for enzyme hydrolysis are of greater importance. The present study addresses aspects that alter bioavailability of these humic compounds as they are imported to lakes and rivers.

Because of the relatively high utilization and turnover rates of simple, low molecular weight compounds by microbes, as much as 80 percent of dissolved organic matter in inland waters is composed of organic acids. Many of these compounds originate from higher aquatic and terrestrial plants. Of these organic acids, some 30 to 40 percent are composed of aromatic carbon compounds originating from structural plant tissues (Malcolm 1990).

Because of their large size and limited accessibility of large portions of these molecules to enzymatic hydrolysis, degradation rates of many of these commonly acidic macromolecules is slow. As a result, these heterogeneous compounds exhibit long turnover times with relatively long environmental residence times. Dissolved macromolecules are often of considerable age (months) but are mixed with variable and rapidly changing inputs of younger humic and nonhumic substances. Recent studies indicated that humic substances, particularly relatively recalcitrant fulvic acids, are also generated by algae and decomposing

[1] Bishop Professor of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487-0206, Present address: Professor, Environmental Sciences and Engineering, The University of North Carolina, Chapel Hill, NC 27599-7431.

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microorganisms, and these contribute to the multitude of diverse compounds of the composite dissolved organic matter. This latter source is particularly important in wetland and littoral areas, where attached algal productivity is often many orders of magnitude greater than that of phytoplankton, e.g., review of Wetzel 1996.

Early detailed studies of fluxes of organic carbon in lake ecosystems indicated both that dissolved organic matter did not accumulate or precipitate, and that large quantities of CO_2 evaded to the atmosphere, e.g., Cole and others 1994, Kling and others 1992, Otsuki and Wetzel 1974, Wetzel 1995). The CO_2 evasion of respiratory origins within the lakes was always greatly in excess of autochthonous photosynthetic organic carbon production by phytoplankton. In spite of this evidence, for decades in aquatic ecology, the apparent chemical recalcitrance of fulvic and humic hydrophobic organic substances that dominated the instantaneous bulk DOC of standing and running waters was believed to result in poor utilization by microbiota. Loss rates are slow but consistently in the range of 0.5 to 2 percent per day under many different environmental conditions and, often, are faster.

A number of studies have demonstrated that ultraviolet irradiance (UV) can photolyse portions of proteinaceous and humic macromolecules, e.g., Moran 2000, reviews of Moran and Zepp 1997, Wetzel and others 1995. The resulting dissolved organic substances generated by photolysis resulted in immediate stimulation of and sustained bacterial growth, e.g., Lindall and others 1995; Moran and Zepp 1997; Stewart and Wetzel 1981, 1982; Wetzel and others 1995. Chemical analyses of these phytolytic transformations indicated that small organic fractions, particularly numerous small fatty acids that serve as excellent bacterial substrates, were generated by partial photolysis of the humic substances.

In the present study, dissolved organic matter leached from decomposing foliage of several wetland and floodplain plants was examined at intervals during their individual decomposition over an annual period. The dissolved organic matter was exposed to different durations of spectral fractions of photosynthetically active radiation, UV-A, and UV-B of natural sunlight. Photolytic products of organic matter and CO_2 were examined in relation to bacterial growth on these products under standardized growth conditions with natural wetland bacterial consortia.

MATERIALS AND METHODS

Dissolved organic compounds were isolated from whole leachates from decomposing foliage of emergent littoral aquatic plants and floodplain trees. These plants are dominant species in the Talladega Wetland Ecosystem (TWE) of the Talladega National Forest, Hale County, AL (Mann and Wetzel 1995, 2000). Emergent aquatic plants included: (a) the common cattail (*Typha latifolia* L.), which exhibits four or more cohorts per year (Dickerman and Wetzel 1985) and much standing dead tissue that undergoes considerable leaching of DOC; and, (b) the spike rush (*Juncus effusus* L.), a dominant within the TWE that exhibits at least seven cohorts per year at this latitude and contains much standing dead tissue that is constantly leaching DOC (Mann and Wetzel 1996, Wetzel and Howe 1999). Floating leaves of the common water lily (*Nymphaea odorata* Aiton) served as a representative of a rapidly decaying species that exhibits a leaf turnover rate of about every 30 days throughout the active growing season (March through November) at this latitude [Carter, S.; Ward, G.M.; Wetzel, R.G.; Benke, A.C. Growth, production, and senescence of *Nymphaea odorata* Aiton in a southeastern (U.S.A.) wetland. Manuscript in review. Aquatic Botany]. Leaves of the common semiaquatic alder [*Alnus serrulata* (Ait.) Willd.] were also collected during late summer and autumnal abscission. Alder is well known for rapid leaching of DOC and fast rates of decomposition in streams. Leaves of the riverine red oak (*Quercus rubra* L.) are retained for long periods after senescence, and usually abscise in spring (February at this latitude) after lengthy periods of slow leaching of DOC during the autumnal and winter rainy period.

Combined fresh and partly senesced culms and leaves of the two emergent and the floating-leaved aquatic angiosperms and mature leaves of the semiaquatic alder and riparian oak were separately decomposed anaerobically in closed chambers at 20 °C in the laboratory. At different intervals over a period of decomposition of over 2 years, prefiltered (precombusted at 500 °C Whatman GF/F glass filters, 0.6-µm pore size) whole leachate was then sterile filtered (0.2-µm pore size) and held aseptically at 4 °C until use. Final concentrations were in the range of 10 to 20 mg DOC-C l^{-1} to simulate natural concentrations, as determined by a Shimadzu TOC-5000 analyzer.

DOC solutions were transferred aseptically to gas-tight sealed, sterilized UV-transparent quartz chambers (4 cm in diameter by 50 cm in length, curved upward at one end) that contained ports for the periodic sampling of water and of gases in the headspace above the water. The lower one-third of the tubes was incubated in a water bath to maintain temperatures constant at 25 °C but not to interfere with light penetration into the solutions. Four conditions were assayed: (a) full sunlight (photosynthetically-active radiation, 720 to 400 nm (PAR), + UV-A at 400 to 320 nm + UV-B at 320 to 285 nm; (b) PAR + UV-A only; (c) PAR only; and (d) darkness (Al foil double wrapped). Filters used for these separations were Pyrex glass, Mylar D, and Plexiglas (Acrylite OP-2) (fig. 1). Quartz glass absorbed essentially none of the natural UV and PAR light above 240 nm. Samples for organic compounds in the water and CO_2 in the overlying headspace were removed at 30-minute intervals throughout the incubation periods of 6 hours (usually 09.00 to 15.00 hours).

Organic acids were analyzed by high performance liquid chromatography (HPLC) collected through Teflon microtubing from the quartz incubation chambers at time zero and each subsequent interval. HPLC methodology follows that described in Wetzel and others (1995). Of the numerous fatty acids generated by photolysis of the humic macromolecules, calibration with known compounds of highest available quality in identical media allowed quantitative evaluations. All error estimates are standard deviations (± SD) based on separate analyses or incubations for each variable and time interval (minimally n = 3 to 5).

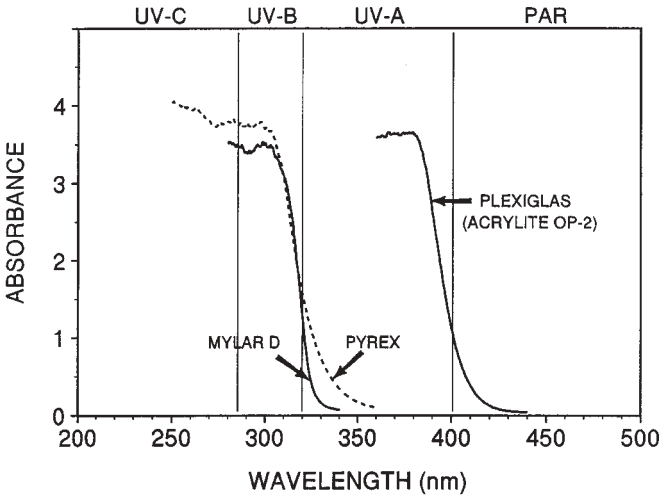

Figure 1—Selective UV absorption of Pyrex glass, Mylar D, and Plexiglas (Acrylite OP–2).

CO_2 gas samples (250 µl) were analyzed with a Varian gas chromatograph (series 3400) equipped with a flame ionization detector (Hayes Sep N column) and an auxiliary oven containing a small nickel catalyst column (column: 60 °C; injector: 150 °C; auxiliary oven: 390 °C; detector: 180 °C; duration: 3 minutes). Calibration was against a standard gas mixture (405 ppm CO_2).

Natural consortia of bacteria were obtained from the TWE, inoculated into modified lakewater medium (inorganic components only, pH 7.0, slightly modified from Veerkamp and others 1980), and grown for 48 hours at 25 °C. Eight hundred µl of the consortium was pipetted into sterile cryogenic (Nunc) vials with 200 µl of sterile glycerol. Tubes were mixed, frozen, and stored in liquid nitrogen (Sambrook and others 1989). These parent cultures were used in all experimentation. For experiments, one frozen tube of the consortium was thawed and added to 150 ml of modified lakewater medium, grown at 25 °C for 24 hours and pelleted (10,000 rpm, 10 minutes). The supernatant was decanted and the pellet washed with 20 mM MOPS (pH 7), a procedure that was repeated twice with MOPS and once with sterile ultrapure water (Millipore Q-pyrogen-free). The final pellet was suspended in 5 ml of sterile Q-water. Dissolved organic C (DOC) content of the Q-water was below detection by high temperature combustion with a Shimadzu TOC-5000 analyzer. Of the final culture suspension, 100 µl was inoculated aseptically into each experimental flask of UV-treated DOC water.

Bacterial production was determined on nonirradiated (time 0) and sunlight-irradiated DOM of leachates of known DOC concentrations from the known time intervals of progressive decomposition over a period of a year. Bacterial protein production (biomass production) was determined with the [^{3}H]leucine uptake and conversion to protein technique with 0.5-hour incubations (dark, 20 °C) on subsamples taken from cultures immediately (day 0) and at 24-hour intervals thereafter for several days. Methods follow Wetzel and Likens (2000) with very high specific activity [^{3}H]leucine (range of many batches 5 to 7 TBq mmole^{-1} or 35 to 47 GBq mg^{-1}) at a final concentration of 10 nM. Calculations of protein production follow Wetzel and Likens (2000).

RESULTS AND DISCUSSION

Photolytic alterations of the recalcitrant dissolved organic compounds were manifested in two important ways, both of which have major significance to the metabolism of organic matter within the ecosystems.

Partial Photolysis of Humic Substances to Simple Organic Compounds

Photolytic changes to DOM of whole leachates, as well as humic and fulvic acid fractions separated by ion chromatography, released from decomposing plant materials, were examined by solid-state ^{13}C nuclear magnetic resonance and low-temperature pyrolytic gas chromatography-mass spectrometry before and after photolysis. These analyses revealed subtle but important qualitative changes to the bulk DOM and small but progressive declines in DOM quantity during photolysis (Wetzel and others 1995). Small organic fractions generated by natural photolysis of DOM leachates showed marked, progressively increasing release of numerous small fatty acids, particularly acetic, levulinic, propionic, pyruvic, formic, and citric tartaric, among others. This common pattern resulted from photolysis by natural sunlight or if controlled UV-A only (Pyrex filtered) light source of leachates from different plant species. An example of the phytolytic generation of fatty acid substrates from humic substance leachates of the aboveground foliage of the rush *Juncus* decomposing over an annual period (fig. 2). Levulinic and formic acids were generated consistently from humic substances over the annual period, whereas acetic, propionic, and pyruvic/malic acids were generated more

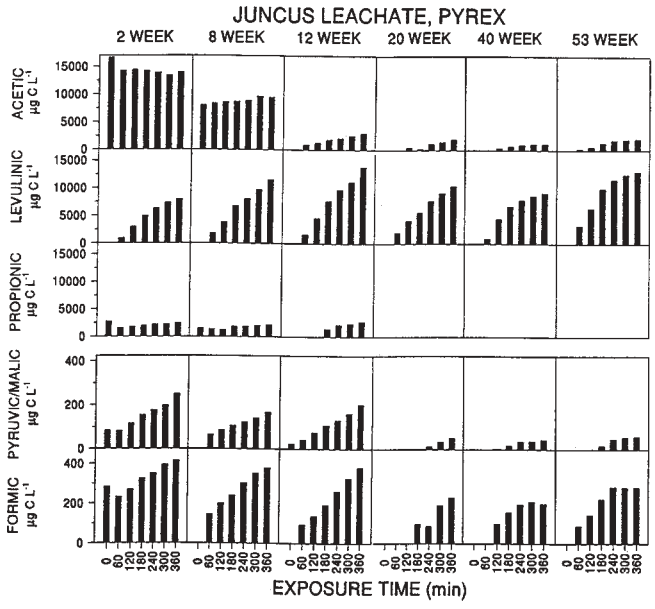

Figure 2—Fatty acid substrates occurring in the solutions of DOM (20 mg C l^{-1}) leachate (0.6-µm pore-size filtrate) from decomposing *Juncus effusus* at intervals over an annual period during exposure (minutes) to UV irradiance of increasing durations (minutes). Pyrex glass shielding simulating UV-A and PAR portions of the spectra.

strongly in earlier stages of decomposition and were less effectively cleaved from the macromolecules after several months of decomposition.

Changes in the photolytic generation of fatty acids from the humic substances leached from other plant species were significantly different from those patterns seen in *Juncus* (Wetzel 2000). Fatty acids generated from photolysis of cattail (*Typha*) leachate exhibited persistent generation of acetic and formic acids over the annual period of decomposition, but other substrates, particularly levulinic, propionic, and pyruvic/malic were of lesser quantitative importance (fig. 3). Levulinic and tartaric acids were generated photolytically early in the decomposition chronology but were not found among the photolytic products of the more recalcitrant DOM.

Dissolved organic matter from leachates of decomposing oak (*Quercus*) leaves was considerably more resistant to photolytic generation of fatty acids than were those of other species evaluated. Acetic and propionic acids were found early in the sequence (fig. 3), but levulinic acid, a dominant product from partial photolysis of other plant DOM sources, was not detected. Citric and succinic acids were generated weakly only from the most recalcitrant DOM after long periods of microbial degradation.

It is important to note that although the UV portion of the spectrum is of major significance in the partial photolysis of DOM with the release of simple substrates from the macromolecules, PAR is also contributing to the photolysis and generation of simple organic compounds. For example, one of the dominant fatty acids, levulinic acid, released from the photolysis of leachates from *Juncus* is quite effectively generated by PAR only (fig. 4). These results indicated that over half of this substrate was generated by PAR irradiance.

Many studies have shown that bacterial production is markedly enhanced when exposed to organic substrates generated by solar insolation and partial photolysis of humic substances from decomposing plant materials (Moran and Zepp 1998, Wetzel and others 1995). Despite the heterogeneous assemblage of simple organic substrates generated photolytically among the many diverse plant sources under many different stages of organic recalcitrance, the common pattern of enhanced bacterial productivity occurred. The photolytic effect of producing substrates that enhanced bacterial productivity increased with increasing age of the DOM. In the example given in figure 5, only the bacterial production is presented in response to photolytically-decomposed DOM of *Juncus* leachates at the beginning and after 6 hours of exposure to sunlight or controlled UV simulations. This same pattern was found with humic macromolecules released in leachates of the other species as well, although the rates at which they occurred differed markedly. For example, this pattern was found among leachate sources from the water lily *Nymphaea* already at week 5, whereas among *Juncus*, *Quercus,* and *Typha* the effects were most pronounced from the more recalcitrant DOM (Wetzel 2000).

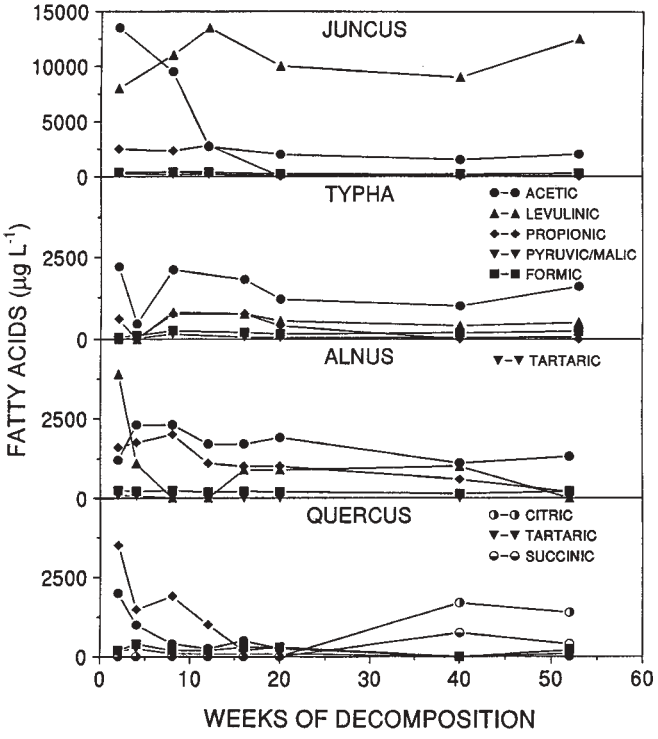

Figure 3—Fatty acid substrates occurring in the solutions of DOM (20 mg C l^{-1}) leachate (0.6-µm pore-size filtrate) from decomposing *Juncus effusus, Typha latifolia, Alnus serrulata,* and *Quercus rubra* at intervals over a 1-year period during exposure to UV-A and PAR portions of the spectra at the maximum exposure duration (360 minutes) (from Wetzel 2000 by permission).

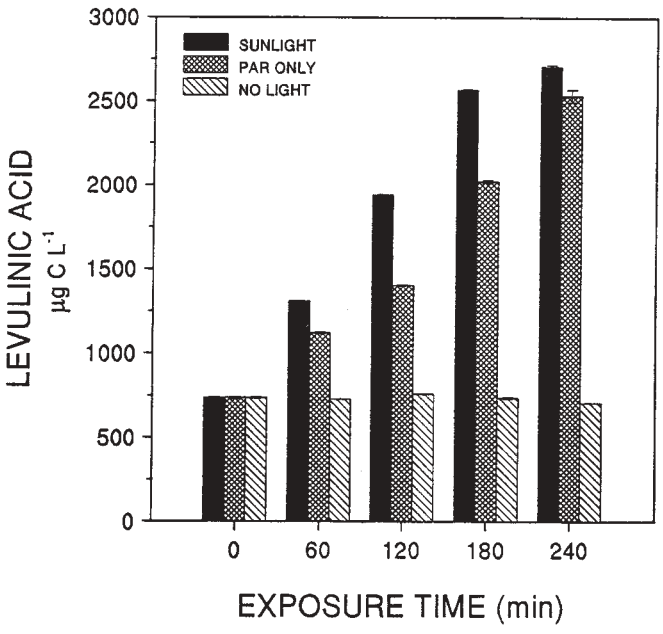

Figure 4—Net generation of levulinic acid from the partial photolysis of sterile whole leachate (0.2-µm pore-size filtrate) of *Juncus effusus* (10 mg C l^{-1}) after 4 weeks of microbial decomposition at 20 °C in the dark. Exposed to full natural sunlight (total insolation over 4-hour period = 13.05 mol m^{-2}), PAR only, or incubated simultaneously in the dark.

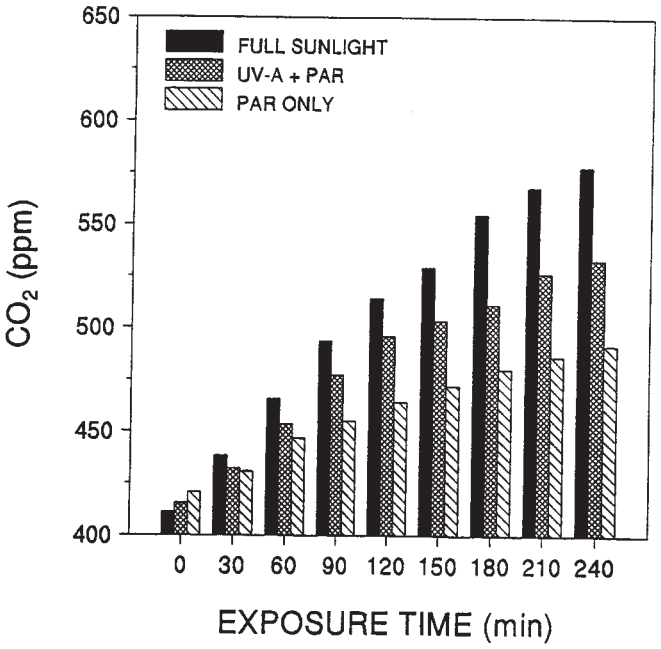

Figure 5—Photolytic degradation of sterile *Juncus* whole leachate (0.2-µm pore-size filtrate) after 4 weeks of microbial decomposition to CO_2 under replicated, aseptic conditions exposed to full sunlight (15.53 mol m^{-2} over the 4-hour period), UV-A + PAR, and PAR only.

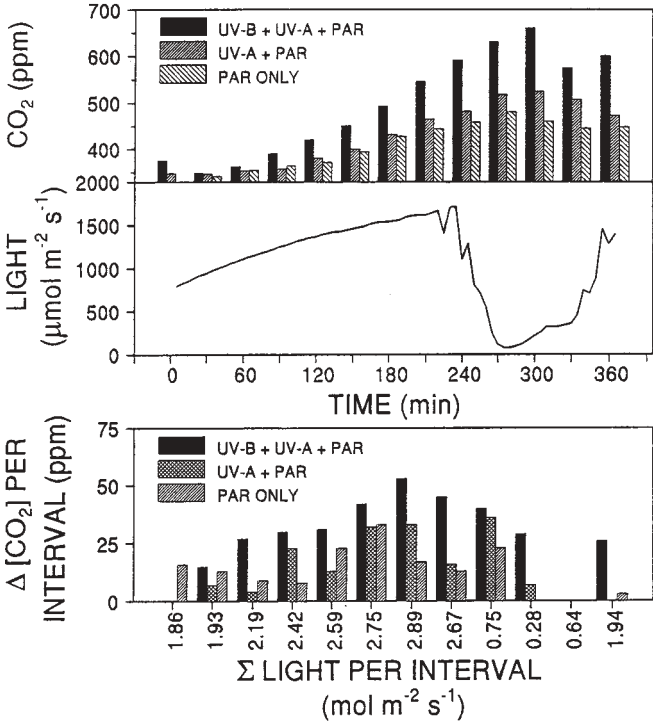

Figure 6—Photolytic degradation of sterile *Juncus* whole leachate (0.2-µm pore-size filtrate) after 20 weeks of microbial decomposition to CO_2 under replicated, aseptic conditions exposed to full sunlight, UV-A + PAR, and PAR only (upper). A severe rainstorm occurred during the incubations, which reduced light severely for an hour (middle). The net change in CO_2 production per amount of light received per interval under these conditions (lower).

Complete Photolysis of DOM to CO_2

Previous studies of the photolytic degradation of dissolved organic matter suggested that the dominant component of solar irradiance was UV-B and UV-A, and that photosynthetically active radiation (PAR) above 400 nm was of little consequence. Many of these studies, however, were not performed under sterile conditions, and, as a result, findings were confounded by microbial contributions to degradation and generation of CO_2. Furthermore, many of the DOM sources of these studies had been exposed to natural sunlight for long and noncomparable periods.

The present studies have employed recalcitrant dissolved organic matter from decaying plant sources over long periods (at intervals to a year or more) of many different types. Natural sunlight was used under many different conditions of light energy seasonally. Using the five terrestrial and aquatic plant species as sources in nearly 200 separate experiments, the results were consistently similar. The UV-B portion of the spectrum was always most effective in complete photodegradation to CO_2, but UV-A was also highly effective with small differences from the photolytic capacities of UV-B, e.g., fig. 5. Contrary to the promulgations of others, PAR is highly effective in photolytic degradation to CO_2.

The relative effectiveness of UV-B, UV-A, and PAR in this photodegradation to CO_2 varies with species and was more effective among leachates from relatively rapidly decomposing plant material such as *Alnus* and *Nymphaea*. Leachates from the cattail (*Typha*), rush (*Juncus*), and oak (*Quercus*) were consistently but less effectively photodegraded by PAR alone, e.g., fig. 6, upper. The effectiveness of relative photolysis by UV-B, UV-A, and PAR was more strongly correlated with species of plant DOM origins and the relative length of time to which the DOM had been degraded microbially before exposed to light rather than to total light intensity received upon exposure. For example, when natural sunlight was attenuated very rapidly, as by a severe thunderstorm, the rate of photolytic degradation of DOC to CO_2 declined precipitously (fig. 6, middle), but the photolytic capacities of PAR declined more rapidly than did the effects of UV-B (fig. 6, lower).

The high concentrations of DOM of inland waters, predominantly humic substances of plant structural tissue origin, serve as massive stores of relatively recalcitrant organic carbon and energy (Wetzel 1990, 1995). Photo-oxidation of DOM by natural sunlight, although recognized long ago, only recently has demonstrated the magnitude of this process of direct utilization and alteration chemically for enhanced microbial utilization. Findings that less energetic spectral components, particularly those of the PAR range, can also alter chemical availability, increase the dimensions of these photochemical processes. PAR penetrates water to a much greater extent than UV-A and especially UV-B and, as a consequence, can influence a large volume of aquatic ecosystems.

Reduction of ozone in the stratosphere and the associated increase in UV irradiance could lead to accelerated photolytic degradation of macromolecules of DOM by both abiotic and biotic pathways to CO_2. In addition, the photolytic

enhancement of substrates for bacterial metabolism can result in accelerated rates of biogeochemical cycling of nutrients and stimulated productivity of the ecosystems. Even if UV photoinhibition of microbes occurs in relatively clear surface waters (cf. review of Karentz and others 1994), the photolytic organic substrate products are readily transported by water circulation to photoprotected heterotrophic regions of greater depth. The resulting enhanced microbial respiration will certainly lead to enhanced generation of CO_2 and evasion to the atmosphere.

In addition, as the concentrations of CO_2 in the atmosphere increase, largely from anthropogenic combustion of fossil fuels, plant growth commonly accelerates. Concentrations of CO_2 will double to ca. 720 ppm yet in the 21st century. Experimental enhancement of CO_2 leads to increased rates of photosynthesis and growth by about 135 percent and commonly leads to nitrogen limitations. As a result, C:N ratios increase and commonly structural tissues, particularly lignin content, increase. As these plants grown in enriched CO_2 decay, DOM released from leachates into the surface waters contains greater amounts of dissolved humic substances. Photolysis of this DOM shows a conspicuous increase in CO_2 release from those plants grown under atmospherically CO_2 enriched conditions among both terrestrial plants (fig. 7) and emergent aquatic plants (fig. 8). Clearly, photodegradation of dissolved organic substrates is a major process altering rates of biogeochemical cycling in aquatic ecosystems.

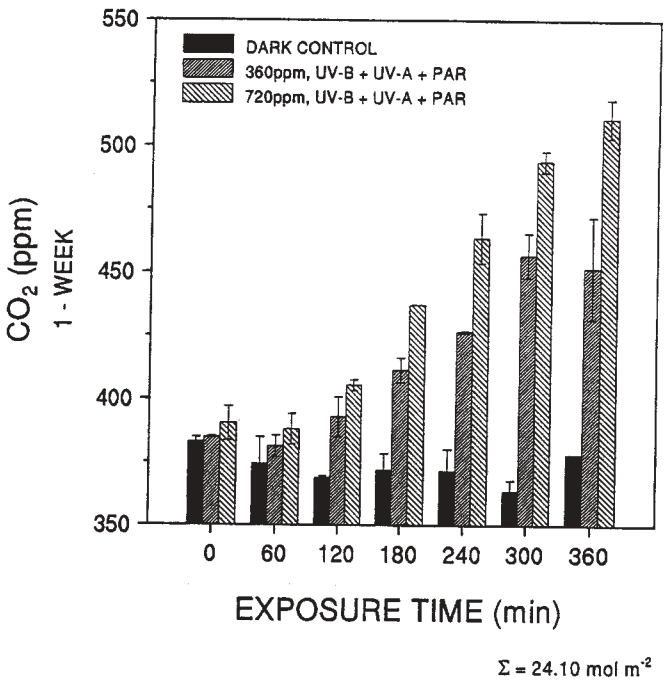

Figure 7—Release of CO_2 by complete photolytic degradation of sterile leachate from leaves of the poplar tree (*Populus tremuloides*), grown under ambient atmospheric CO_2 and doubled CO_2 concentrations, after 1 week of microbial decomposition.

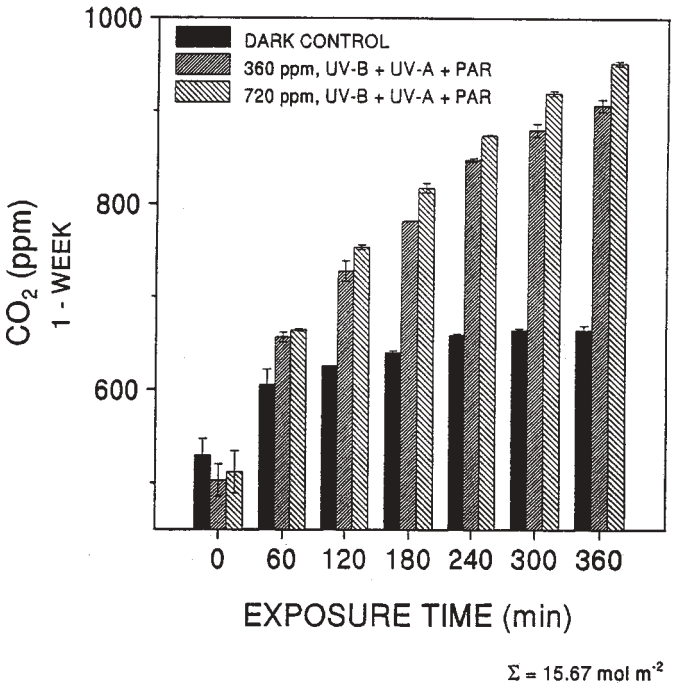

Figure 8—Release of CO_2 by complete photolytic degradation of sterile leachate from leaves of the cattail (*Typha latifolia*), grown under ambient atmospheric CO_2 and doubled CO_2 concentrations, after 1 week of microbial decomposition.

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