Extracellular Organic Carbon from *Eichhornia crassipes* (Mart.) Solms: Does Water Hyacinth Leak Organic Carbon?

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ABSTRACT

Aquatic macrophytes leak photosynthetically-fixed carbon as dissolved organic molecules. This leakage of organic carbon appears to be an energetically inefficient process. However macrophyte extracellular organic carbon (EOC) may be evidence of a symbiotic association between macrophytes and epiphytic bacteria. Bacteria colonize macrophytes, and EOC is a potential energy source for heterotrophic bacteria.

I investigated extracellular release of organic carbon from Eichhornia crassipes (Mart.) Solms (water hyacinth), and utilization of that EOC by epiphytic bacteria. I reduced the number of bacteria on water hyacinth roots using a combination of sodium hypochlorite (bleach) and chloramphenicol (antibiotic). Using ^{14}C-labelled CO_2, I then compared the percentage of photosynthetically-fixed carbon released from plants having intact epiphytic communities with the percentage released from plants having artificially-reduced epiphytic communities.

Water hyacinth roots supported a large and active microbial community. The mean density of epiphytic bacteria was 1.4×10^7 cm^{-2} in the spring and 1.8×10^7 cm^{-2}

in late summer. Approximately 24% of the bacteria on water hyacinth roots were metabolically active.

Water hyacinth released 0.02-0.15% of photoassimilated carbon during a 12-hour light period. Bacterial uptake of EOC did not appear to mask true EOC release, because more EOC was not recovered from plant-epiphyte complexes having reduced epiphytic communities.

Leakage of organic carbon from water hyacinth was an insignificant portion of the plant's carbon budget. Thus leakage from water hyacinth is unlikely to represent a significant portion of an aquatic system's total carbon budget. However water hyacinth EOC may have enhanced the development of the plant's epiphytic community. The bacterial population on treated roots grew quickly, almost doubling in 12 hours. In turn, bacteria on water hyacinth roots appeared to stimulate EOC production. When more bacteria were present on water hyacinth roots, more EOC was released from the plant-epiphyte complex.

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INTRODUCTION

Freshwater ecosystems are among the most productive ecosystems on earth. These bodies of water are fueled by particulate and dissolved organic carbon from numerous sources. Allochthonous, or external, sources include terrestrial plants, animal excretions, and soil leachates. Autochthonous, or internal, sources primarily are autotrophs such as algae and aquatic vascular plants (macrophytes). By the time allochthonous material enters a body of water, most of the material's labile organic compounds have been removed via microbial degradation (Wetzel 1983). Thus allochthonous carbon compounds typically are resistant to further breakdown and are not an immediate source of energy for aquatic heterotrophic bacteria (Wetzel 1983). In contrast, autochthonous matter from primary producers includes labile dissolved organic compounds that are potential carbon sources for bacteria.

This study focused on organic carbon from Eichhornia crassipes (Mart.) Solms (water hyacinth). Water hyacinth is a floating, perennial weed that thrives in tropical and subtropical aquatic environments. The plant grows quickly and often clogs waterways and crowds out other vegetation (Parija 1934; Penfound and Earle 1948; Holm et al. 1969).

Due to the productivity and abundance of water hyacinth, the plant may be an important source of autochthonous carbon in some freshwater ecosystems. Although dead or senescent macrophytes are obvious organic matter sources, extracellular release, defined as the leakage of dissolved organic compounds from healthy cells (Nalewajko 1977), also is a source of organic carbon. The objective of this investigation was to determine whether water hyacinth leaked organic carbon, and if so, did epiphytic bacteria utilize the organic leakage.

Extracellular Organic Carbon

Extracellular release of organic compounds first was discovered in phytoplankton (see reviews by Fogg 1966, 1971; Nalewajko 1977; Sharp 1977). Extensive studies have shown that phytoplankton commonly release 0.3-35% and sometimes more than 50% of the total carbon fixed during photosynthesis (see reviews by Fogg 1971; Nalewajko 1977). Estimates of extracellular organic carbon (EOC) from macroalgae range from undetectable levels to 40% of photosynthetically-fixed carbon (Khailov and Burlakova 1969; Moebus and Johnson 1974; Harlin and Craigie 1975).

Algal EOC can be an important carbon source for bacteria (Blaauboer et al. 1982; Cole et al. 1982; Nalewajko et al. 1980). For example, Coveney (1982) found that bacterial uptake of phytoplankton EOC supported 32-95%

of bacterial production in two eutrophic lakes in Sweden. Similarly, algal EOC accounted for 24-45% of bacterial production in an oligotrophic lake in Sweden (Bell and Kuparinen 1984) and 14% of bacterial production in an eutrophic Wisconsin lake (Brock and Clyne 1984).

Fewer studies have focused on EOC from aquatic macrophytes or utilization of that EOC by bacteria. Compared with phytoplankton, aquatic macrophytes release a smaller proportion of their photosynthetically-fixed carbon as EOC -- usually less than 10% (Wetzel and Manny 1972; Wetzel 1983). For example, Lemna minor (duckweed) in the River Frome leaked 1-3% of photoassimilated carbon (Baker and Farr 1987). Axenic Myriophyllum spicatum released 0.6-1.3% of photoassimilated carbon (Godmaire and Nalewajko 1986). Najas flexilis and Scirpus subterminalis from a hard-water lake in Michigan released 0.2-3% of photosynthetically-fixed carbon (Hough and Wetzel 1975). Similarly, Sondergaard (1981a) found that release rates for six macrophyte species were less than 0.9%. Wetzel and Manny (1972) suggested that 4% was a conservative approximation for EOC from aquatic macrophytes.

Low EOC estimates from macrophytes may be misleading, however. Macrophytes are more productive than phytoplankton in some freshwater ecosystems, such as shallow lentic systems having extensive littoral zones

(Likens 1973; Rich et al. 1971; Westlake 1974; Westlake 1975; Wetzel 1964). Thus macrophyte EOC may be a significant component of autochthonous carbon supporting bacterial populations.

Macrophyte EOC is utilized readily by heterotrophic bacteria. Sondergaard (1983) found that epiphytic bacteria utilized 12-30% of macrophyte EOC, and bacterioplankton consumed approximately 20%. In another study, Sondergaard (1981b) reported that bacteria utilized a maximum of 10% of macrophyte EOC. Baker and Farr (1987) compared organic compounds released from axenic duckweed with organic compounds occurring in the plant's natural habitat. They found more low molecular weight (labile) material in the macrophyte EOC, and concluded that labile EOC from macrophytes was metabolized quickly by bacteria.

Macrophytes and Epiphytic Bacteria

Macrophyte EOC may fuel epiphytic communities. Leaked organic carbon is an energy source for bacteria, and bacteria colonize the submerged portions of macrophytes heavily (Allen 1971, Baker and Orr 1986, Zuberer 1984). For example duckweed growing in the River Frome harbored 10^6-10^7 bacteria cm^{-2} (Hossell and Baker 1979a). Kudryavtsev (1984) reported bacterial densities of 10^7-10^8 bacteria cm^{-2} on emergent macrophytes growing in the

Rybinsk reservoir. In some environments epiphytic bacteria outnumber bacteria in the water (Fry and Humphrey 1978).

The association between macrophytes and epiphytes is complex. Although some researchers believe macrophytes merely provide a large surface area for attachment of epiphytic bacteria (Carignan and Kalff 1982; Cattaneo 1983; Fontaine and Nigh 1983), the plants may function as more than neutral substrates (Burkholder and Wetzel 1989; Coler and Gunner 1969). For example, epiphytic bacteria have been shown to be more active metabolically than planktonic or benthic bacteria (Fry and Humphrey 1978; Strzelczyk and Mielczarek 1971). Some epiphytic bacteria produce growth-promoting substances that may stimulate EOC release (Patil and Iswaran 1980). Other epiphytes are nitrogen-fixers (Finke and Seely 1978). Nitrogen-fixing epiphytes could supply 15-20% of the nitrogen required by duckweed (Zuberer 1982), 10% of the nitrogen required by Typha sp. and 50% of that needed by Glyceria borealis (Bristow 1975). Nitrogen fixation by epiphytes associated with Hydrilla verticillata, Hydrocotyle umbellata, Cyperus tetragonus and Eichhornia crassipes also has been reported (Silver and Jump 1975). Thus some researchers suggest that macrophytes and their epiphytes are symbiotic: plants provide the bacteria with substrates and organic nutrients, and bacteria supply plants with inorganic nutrients and

micronutrients (Allen 1971; Wetzel and Hough 1973; Wetzel 1983).

The macrophyte-epiphyte complex is associated closely with invertebrate grazers. Because bacteria are more palatable and easier to consume than macrophytes, epiphytic bacteria shield plants from invertebrate grazers such as snails (Bronmark 1985; Carpenter and Lodge 1986; Hutchinson 1975). However macrophyte EOC attracts snails (Bronmark 1985; Pip and Stewart 1976). In fact, grazers benefit macrophytes by harvesting epiphytes; if epiphytes become too dense, they interfere with gas exchange and nutrient uptake and may reduce a plant's photosynthetic efficiency (Carpenter and Lodge 1986). Rogers and Breen (1983) suggest that grazers may benefit macrophytes by eating potentially harmful bacteria. Furthermore, if bacteria invade a macrophyte, grazers may extend the life of the plant by eating dead material and thus reducing the risk of pathogens invading live tissue (Sterry et al. 1983). In turn, macrophytes offer grazers a constant food source of epiphyton, refuge from prey and an abundance of oviposition sites (Carpenter and Lodge 1986). Thus, macrophyte EOC appears to play an important and complex role in structuring aquatic communities.

Water Hyacinth

As mentioned earlier, the objective of this study was to determine whether water hyacinth leaked organic carbon, and whether epiphytic bacteria utilized that EOC. Water hyacinth is an aquatic weed that occurs in almost all tropical and subtropical aquatic environments. Although the plants usually form large floating mats, water hyacinth also lives rooted in muddy soils (Center and Spencer 1981; Forno and Wright 1981; Parija 1934; Penfound and Earle 1948).

Mature plants consist of roots, rhizomes, leaves, inflorescences and stolons (Penfound and Earle 1948). Roots are adventitious, unbranched and have large, conspicuous root caps. Each main root bears many lateral, or side roots. Root length ranges from several centimeters to more than a meter (Forno and Wright 1981) and may be related inversely to the availability of nutrients (Knipling et al. 1970; Doersam, personal communication).

The rhizome is a vegetative stem from which roots, leaves, inflorescences and stolons originate. The rhizome usually is submerged and functions as a starch storage organ (Penfound and Earle 1948).

A leaf consists of a bulbous petiole referred to as a float, a broad leaf lamina, and a narrow leaf isthmus

connecting the petiole and the blade. A membranous ligule occurs at the base of each float.

Inflorescences bear varying numbers of pale purple flowers. A conspicuous yellow spot occurs on the upper petal of each flower and may function as a nectar guide (Forno and Wright 1981). Although water hyacinth produces viable seeds, the plant typically reproduces asexually by vegetative stolons (Barrett 1980; Forno and Wright 1981; Parija 1934; Robertson and Thein 1932; Penfound and Earle 1948).

Experimental Overview

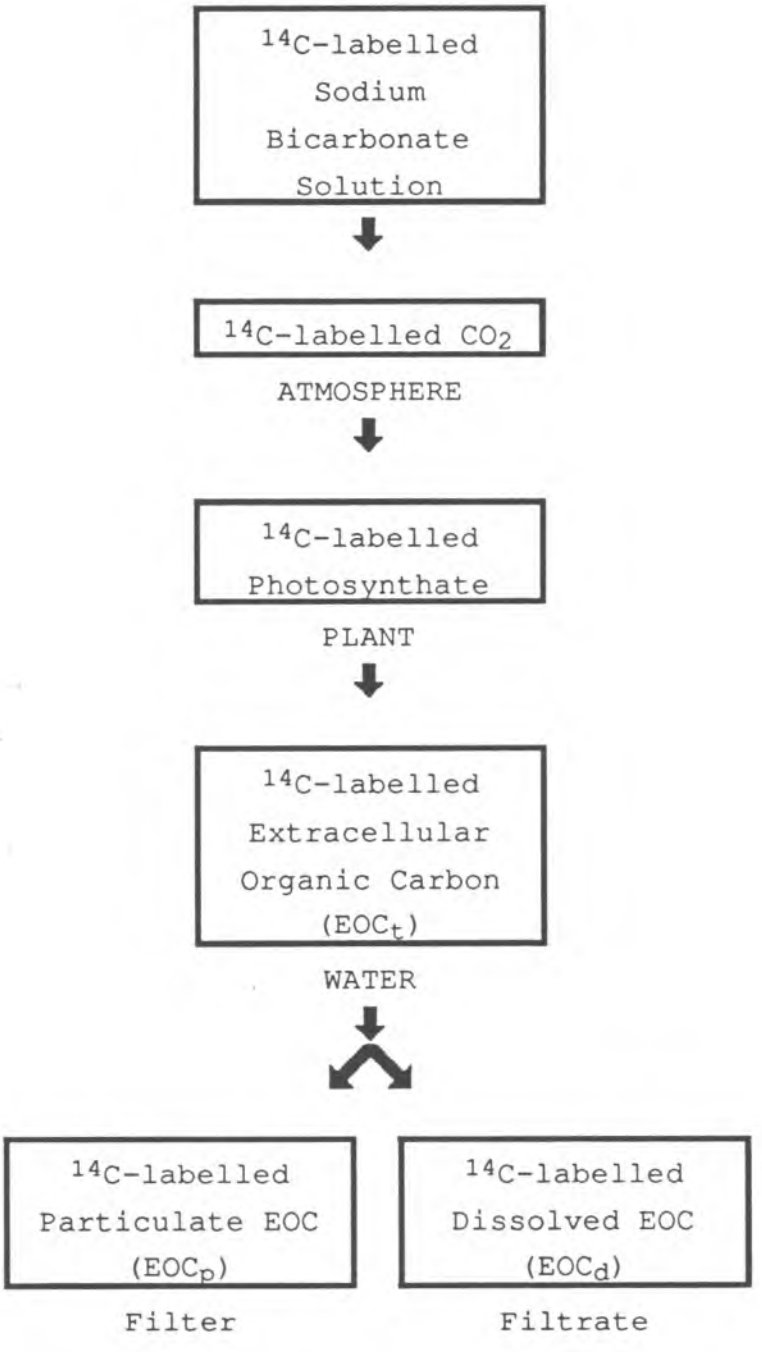
Because bacterial epiphytes are situated near the source of macrophyte EOC, EOC release and uptake may occur simultaneously. Therefore EOC studies may measure net rather than gross amounts of release. To uncouple EOC production and assimilation, researchers have removed epiphytes mechanically (Sondergaard 1981a; Sondergaard 1981b; Carignan and Kalff 1982; Sondergaard 1983), used antibiotics to inhibit bacterial metabolism (Jensen 1984; Jensen and Sondergaard 1985), worked with axenic plants (Wetzel and Manny 1972; Hough and Wetzel 1975; Godmaire and Nalewajko 1986) or used a combination of chemical and mechanical treatments (Baker and Farr 1987). Each method has disadvantages. For example, mechanical cleaning does not remove all epiphytes and may damage plants (Godmaire

and Nalewajko 1986). Because bacteria differ in their susceptibility to antibiotics, antibiotics usually inhibit bacterial uptake of EOC by less than 50% (Jensen 1984; Jensen and Sondergaard 1985). Studies of axenic plants may not approximate natural conditions because bacteria may influence EOC production in macrophytes (Patil and Iswaran 1980) as they do in algae (Nalewajko 1977) and terrestrial plants (Curl and Truelove 1986).

I investigated extracellular release of organic carbon from nonaxenic water hyacinth. To estimate EOC release and heterotrophic uptake, I first reduced the number of bacteria on water hyacinth roots using a combination of chemical and mechanical treatments. I then compared the percentage of photosynthetically-fixed carbon released from plants having intact epiphytic communities with the percentage released from plants having artificially-reduced epiphytic communities.

I used a radioisotope tracer to follow the movement of carbon into and out of the plant-epiphyte complex (Fig. 1). Twelve hours after labelling the atmosphere surrounding a plant with $^{14}CO_2$, I measured the amount of ^{14}C-labelled photosynthate in the plant and the amount leaked into the water. I measured net carbon fixation as the amount of labelled photosynthate in the plant plus the amount of ^{14}C-labelled extracellular organic carbon in the water. I

Fig. 1. Procedure for the investigation of EOC production by water hyacinth and bacterial uptake of that EOC. Carbon-14 was introduced into the atmosphere surrounding water hyacinth. Carbon fixation, leakage of organic carbon and bacterial uptake of EOC were measured after a 12-hour light period.


^{14}C-labelled Sodium Bicarbonate Solution
^{14}C-labelled CO_2
ATMOSPHERE
^{14}C-labelled Photosynthate
PLANT
^{14}C-labelled Extracellular Organic Carbon (EOC_t)
WATER
^{14}C-labelled Particulate EOC (EOC_p)
Filter
^{14}C-labelled Dissolved EOC (EOC_d)
Filtrate

measured total leakage (EOC_t) and then separated EOC_t into particulate EOC (EOC_p) and dissolved EOC (EOC_d). Particulate EOC is a measure of EOC incorporated into bacterial particles and therefore is an estimate of bacterial uptake (Sondergaard and Jensen 1986). Dissolved EOC is dissolved organic carbon not utilized by bacteria.

MATERIALS AND METHODS

Young, healthy specimens of E. crassipes were collected from the San Marcos River near Aquarena Springs in San Marcos, Hays Co, Texas. Plants were maintained under natural lighting in the Baylor University greenhouse in plastic tubs containing water from the collection site. During experiments, plants were incubated at constant conditions: 15h/9h light/dark cycle; 29°C/21°C light/dark temperatures; 9688 lux light intensity. All experiments were begun within one month of plant collection.

Experimental containers were 3-L plastic soda bottles (top 10 cm cut off) that had been washed, rinsed with 5% HCl and rinsed with deionized water. These containers were covered with either plastic wrap secured with a rubber band or an inverted half of a plastic petri dish lined with petroleum jelly. The plastic bottles, plastic wrap and petri dish halves were sterilized with UV light prior to use.

The incubation medium in all experiments was water from the collection site filtered through a Gelman capsule filter (0.2 μm pore) within 24 hours after collection. This filter-sterilized water was refrigerated in 3-L autoclaved, plastic bottles.

Statistical Procedures

Prior to performing parametric statistical tests, the assumption of normally distributed data was tested with Kolmogorov-Smirnov goodness-of-fit tests for normality. Variance ratio tests also were performed to test the assumption that the samples came from populations having equal variances. When one of the assumptions was not met, transformations or nonparametric tests were performed.

Procedures to Reduce Numbers of Epiphytic Bacteria

I reduced the number of epiphytic bacteria on water hyacinth roots using a combination of sodium hypochlorite (bleach) and chloramphenicol (antibiotic). Plants were agitated in a sodium hypochlorite solution (1% v/v) for 8 minutes (Baker and Farr 1987), rinsed in autoclaved deionized water and placed in 600 ml chloramphenicol solution (2.5 mg/L) in experimental containers. Containers were covered with plastic wrap and incubated for 24 hours. Alternative sterilization procedures involving exposure to various combinations of tetracycline (5 mg/L), iodine tablets (1.3 tablets/L), colloidal silver (5 drops/L) and copper sulphate (0.2 mg/L) were not used because they did not reduce bacterial densities effectively and frequently damaged the plants.

I verified the efficacy of the bacterial reduction treatment by comparing the density of epiphytic bacteria on root caps of 32 plants before and after treatment. Before treatment, epiphytic bacteria were aggregated too densely to count in a reasonable period of time. Therefore bacterial densities before treatment were estimated from micrographs taken using bright field microscopy. For each plant, side roots were excised from the distal end of a main root approximately 3 cm long. Side roots were stained with filter-sterilized phenolic aniline blue (PAB) for 1-2 minutes (Hossell and Baker 1979b; Baker 1981; Baker 1988) and mounted in a drop of the stain. Ten randomly selected root caps were photographed for each plant. These photographic slides later were projected randomly onto a screen divided into 12 quadrats. Bacteria in three randomly selected quadrats (100 μm^2 each) were counted for each photograph until a total of 15 quadrats had been counted for each plant. Slides were discarded if four or more quadrats were not in focus or were obstructed (eg. by air bubbles, algal cells or darkly-stained plant nuclei). Counts for the 15 quadrats then were averaged to determine bacterial densities before treatment.

Bacterial densities after treatment were determined using bright field microscopy of wet mounts. Side roots were stained with and mounted in PAB as described above.

Epiphytic bacteria on root caps were counted at a magnification of 1500X. Fifteen randomly-selected, eyepiece graticule quadrats were counted for each of the 32 plants and averaged to determine bacterial densities after treatment. Bacterial densities before and after the bacterial reduction treatment were compared using a paired-sample t test.

Effects of Bacterial Reduction Treatment on Plant Growth

I compared growth characteristics of treated and untreated water hyacinth to determine whether the bacterial reduction treatment affected the plants. Nine randomly selected plants were exposed to sodium hypochlorite solution and rinsed as described above. They then were placed in experimental containers with 600 ml chloramphenicol solution (5 mg/L), covered with plastic wrap and incubated for 24 hours. Nine randomly selected control plants were handled similarly but were not exposed to sodium hypochlorite or chloramphenicol. After incubation, the treated and untreated plants were removed from the containers and placed randomly in two aquaria in the greenhouse. Growth characteristics including number of new leaves produced, increase in total leaf length (the sum of the length of each leaf measured from its insertion point on the rhizome to the distal apex of the lamina) and

occurrence of flowering were recorded periodically for treated and untreated plants (method modified from Center and Spencer 1981).

I compared growth characteristics of treated and untreated plants after three weeks. The number of leaves produced by treated and untreated plants was compared using the Mann-Whitney test. The increase in total leaf length was compared using the two-sample t test. The occurrence of flowering in treated and untreated plants was not compared statistically.

Extracellular Organic Carbon from Water Hyacinth

I used ^{14}C-labelled CO_2 to investigate EOC release in E. crassipes having reduced epiphytic communities (treated plants) and E. crassipes having intact epiphytic communities (untreated plants). All ^{14}C-labelled samples were counted three times in a Beckman LS-1800 liquid scintillation counter. Samples were counted to a preset error of 2% whenever possible. Sample errors never were greater than 5%. Quench correction was by automatic external standard, and efficiencies were determined from quench curves using carbon tetrachloride as a quenching agent. Radioactivity detected in control samples was subtracted from treated and untreated sample activities in all calculations.

Exposure of Plants to $^{14}CO_2$

A plant having a reduced epiphytic community (treated plant) and a plant having an intact epiphytic community (untreated plant) each was placed in 600 ml water in an experimental container. A third container with 600 ml water but no plant was a control. To create an atmosphere labelled with $^{14}CO_2$, a small plastic cap (1.5 cm diameter) was glued to the inner surface of each container, and 186 µl $Na^{14}CO_3$ solution (specific activity 0.066 µCi μl^{-1}) was pipetted into each cap. Containers were covered with an inverted half of a plastic petri dish. Five microliters of 1N H_2SO_4 then were pipetted into each cap through a small hole in the side of each container (method modified from Allen 1971). The acidification of the sodium carbonate solution liberated 12.3 µCi ^{14}C as $^{14}CO_2$ in each container. Immediately after acidification, the holes were sealed with cloth tape coated with petroleum jelly, and the containers were incubated for a 12-hour light period.

After incubation, the amount of inorganic ^{14}C remaining in each cap was measured by adding its contents to a liquid scintillation vial containing 10 ml Ready Safe® liquid scintillation cocktail and counting the activity of each sample. The amount of labelled organic carbon in the plants and in the water then was determined. The entire procedure for treated, untreated and control

containers was replicated 10 times. The total amount of ^{14}C accounted for in treated vs untreated experimental containers at the end of the 12-hour light period was compared using a Mann Whitney test.

Amount and Location of ^{14}C-Labelled Photosynthate in Plants

After incubation, plants were rinsed in deionized water adjusted to pH 3.0 with 2M HCl to remove adsorbed inorganic ^{14}C. Plants then were dried for 48 hours at 60°C. After drying, leaf, float, ligule, rhizome and root tissue from each plant was weighed to the nearest 0.01 mg. Each tissue then was homogenized using a mortar and pestle, and 5-10 tissue samples (range 1-16 mg dry wt) were taken. These samples were put in scintillation vials, wetted with 100 µl deionized water and dissolved in 1 ml quaternary ammonium compound (BTS-450®) for 60 hours at 60°C (Beer et al. 1982). Ready Organic® liquid scintillation cocktail (15 ml) was added to each vial, and sample activities were counted.

Activities of replicate samples for each tissue type for each plant were averaged to determine the amount of labelled photosynthate per milligram of tissue. Mean activities for leaf, float, ligule, rhizome and root tissues were multiplied by the total weight of each tissue to determine the total amount of labelled photosynthate in

the different parts of each plant. Activities in each tissue then were summed to determine total amount of labelled photosynthate in each plant. Percent of labelled photosynthate in each tissue also was calculated.

To compare percent allocation of labelled photosynthate in treated versus untreated plants, a mixed-model ANOVA having two fixed factors (treatment and tissue type) and one random factor (individual plants nested within tissue type) was performed using the maximum likelihood approach.

Measurement of Total Extracellular Organic Carbon

Total EOC (EOC_t) includes particulate EOC (EOC_p) and dissolved EOC (EOC_d). To determine the amount of ^{14}C-labelled EOC_t released from each plant, three 5-ml water samples were taken from each experimental container. Each water sample was pipetted into a 15-ml Pyrex® graduated centrifuge tube, acidified to pH 3.0 by the addition of 2M HCl (0.15 $\mu l\ ml^{-1}$) and bubbled with N_2 for 30 min to remove inorganic ^{14}C (Gachter and Mares 1979; Mague et al 1980; McKinley et al. 1977; Schindler et al. 1972; Sondergaard 1985; Theodorsson and Bjarnason 1975). Inorganic carbon occurs as CO_2 at pH 3.0, and bubbling with N_2 drives $^{14}CO_2$ out of the sample. After checking the final volume of each sample to correct for evaporation, two 1-ml samples from

each centrifuge tube were pipetted into 20-ml liquid scintillation vials containing 10 ml Ready Safe® liquid scintillation cocktail. Samples then were counted in a liquid scintillation counter.

Total leakage values were averaged to determine mean EOC_t from each treated and untreated plant. For each plant, mean EOC_t was added to the total amount of ^{14}C-labelled photosynthate to calculate total μCi ^{14}C fixed during the 12-hour light period. The percentage of photosynthetically-fixed ^{14}C recovered as EOC_t ($PEOC_t$) then was calculated.

To compare $PEOC_t$ in treated versus untreated plants, values of $PEOC_t$ were arcsine transformed, and an hierarchical ANOVA (replicate plants nested within treatment) was performed on the transformed estimates.

Measurement of Particulate Extracellular Organic Carbon

Particulate (>0.2 μm) ^{14}C-labelled EOC (EOC_p) was measured by filtering each of three 7-ml water samples from each experimental container through a Nuclepore® membrane filter (0.2 μm pore). After filtration, filters were rinsed with 1 ml 1% HCl to remove adsorbed inorganic ^{14}C and 2 ml deionized water to remove acid traces. All filtrations were performed at a low vacuum (<10 mm Hg) to prevent leakage of organic carbon from damaged bacterial

cells. Filters were placed in 20-ml liquid scintillation vials and dissolved with 250 µl chloroform. Ready Safe® liquid scintillation cocktail (10 ml) was added to each vial, and sample activities were counted.

Estimates of labelled EOC_p for the three subsamples were averaged for each treated and untreated plant. The percentage of photosynthetically-fixed ^{14}C recovered as EOC_p ($PEOC_p$) then was calculated. Particulate EOC also was expressed as a percentage of total leakage.

To compare $PEOC_p$ in treated versus untreated plants, the data were arcsine transformed, and an hierarchical ANOVA (replicate plants nested within treatments) was performed. An hierarchical ANOVA (replicate plants nested within treatments) was performed on the untransformed estimates of EOC_p as a percentage of EOC_t for treated versus untreated plants.

Measurement of Dissolved Extracellular Organic Carbon

Dissolved EOC (EOC_d) was measured as the activity in the filtrate passing through a 0.2-µm filter. Each of three 7-ml water samples from each experimental container was filtered through a Nuclepore® membrane filter (0.2 µm pore). A 5-ml sample of each filtrate was pipetted into a 15-ml Pyrex® graduated centrifuge tube. The filtrate was acidified and bubbled to remove inorganic ^{14}C, and the

final volume of each sample was corrected for evaporation. Each of two 1-ml samples from each centrifuge tube then was pipetted into a liquid scintillation vial containing 10 ml Ready Safe® liquid scintillation cocktail. Samples were counted in a liquid scintillation counter.

Estimates of ^{14}C-labelled EOC_d for the six subsamples were averaged for each treated and untreated plant. The percentage of photosynthetically-fixed ^{14}C recovered as EOC_d ($PEOC_d$) then was calculated. Dissolved EOC also was expressed as a percentage of total leakage.

To compare $PEOC_d$ in treated versus untreated plants, an hierarchical ANOVA (replicate plants nested within treatments) was performed on the untransformed values. An hierarchical ANOVA (replicate plants nested within treatments) also was performed on the untransformed estimates of EOC_d as a percentage of EOC_t for treated versus untreated plants.

Measurement of Metabolically Active Bacteria

Not all bacteria within a population are metabolically active at any given time. Respiring epiphytic bacteria were detected using the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT). An active bacterial electron transport system reduces water soluble, colorless INT to insoluble, red formazon crystals

that are deposited intracellularly (Baker 1988; Oren 1987; Zimmermann et al. 1978).

Ten plants were treated with sodium hypochlorite and chloramphenicol as described previously. Immediately after treatment they were rinsed in autoclaved, deionized water to remove traces of chloramphenicol. Ten additional plants were handled similarly but were not treated with sodium hypochlorite or chloramphenicol. All plants then were incubated in 600 ml water.

I measured the occurrence of active bacterial cells on treated and untreated roots at the beginning (0 h) and end (12 h) of a 12-hour light period. At 0 h and at 12 h one 3-cm root was removed from each treated and untreated plant using flame-sterilized forceps. Each root was placed in an autoclaved vial containing 8 ml of 0.04% (w/v) filter-sterilized INT solution. Vials were wrapped in foil and incubated in the dark for 40 minutes at room temperature (method modified from Swannell and Williamson 1988). After incubation, side roots were excised from the distal end of main roots, and their root caps were examined at a magnification of 1500X using bright field microscopy. Bacteria stained with INT in fifteen randomly-selected eyepiece graticule quadrats were counted for each of ten treated and ten untreated plants and averaged to determine the density of active bacteria.

Total Number and Percent Active Bacteria

To calculate percent active cells, the total number of epiphytic bacteria was estimated. After counting active cells, the coverslip was removed from each slide, and the side roots were stained with PAB. The root caps then were reexamined at a magnification of 1500X. Epiphytic bacteria in fifteen randomly-selected eyepiece graticule quadrats were counted and averaged for each of ten treated and untreated plants. The percentage of total bacteria that were metabolically active then was calculated.

Statistical Analyses of Active, Total and Percent Active Bacteria

Numbers of active and total bacteria on treated versus untreated plant roots at 0 h were compared using Mann-Whitney tests. Numbers of active and total bacteria on treated versus untreated plants at 12 h were compared using two-sample t tests. Paired-sample t tests were used to compare the number of active and total bacteria on treated plants at 0 h versus 12 h and the number of active and total bacteria on untreated plants at 0 h versus 12 h.

The percent active bacteria on treated versus untreated plant roots at 0 h and at 12 h was compared using two-sample t tests. Paired-sample t tests were used to compare the percent active bacteria on treated plants at 0

h versus 12 h and the percent active bacteria on untreated plants at 0 h versus 12 h.

Statistics used for comparisons of active, total and percent active bacteria are listed in Table 1.

Table 1. Statistics used for comparisons of active, total and percent active bacteria on roots of treated and untreated E. crassipes at the beginning (0 h) and end (12 h) of a 12-hour light period. Treated plants were exposed to sodium hypochlorite and chloramphenicol to reduce the number of epiphytic bacteria.

Parameter Estimated	Comparison	Statistical Test
Active Number at 0 h	Treated vs Untreated	Mann-Whitney
Active Number at 12 h	Treated vs Untreated	Two-sample t
Active Number on Treated Roots	0 h vs 12 h	Paired-sample t
Active Number on Untreated Roots	0 h vs 12 h	Paired-sample t
Total Number at 0 h	Treated vs Untreated	Mann-Whitney
Total Number at 12 h	Treated vs Untreated	Two-sample t
Total Number on Treated Roots	0 h vs 12 h	Paired-sample t
Total Number on Untreated Roots	0 h vs 12 h	Paired-sample t
Percent Active at 0 h	Treated vs Untreated	Two-sample t
Percent Active at 12 h	Treated vs Untreated	Two-sample t
Percent Active on Treated Roots	0 h vs 12 h	Paired-sample t
Percent Active on Untreated Roots	0 h vs 12 h	Paired-sample t

RESULTS

Reduction of Epiphytic Bacteria

Densities of epiphytic bacteria on root caps of E. crassipes before and after the bacterial reduction treatment differed significantly ($p<0.0001$) (Table 2). Treatment with sodium hypochlorite and chloramphenicol reduced bacterial densities approximately 65%.

Effects of Bacterial Reduction Treatment on Plant Growth

Growth characteristics of treated and untreated E. crassipes were not significantly different (Table 3). During the three weeks of observation, 33% of the treated plants and 44% of the untreated plants flowered, and all plants appeared healthy.

Extracellular Organic Carbon from Water Hyacinth

At the end of the EOC experiment, portions of the initial 12.3 µCi ^{14}C were recovered from plants, water and caps (Table 4). Free $^{14}CO_2$ was not recovered from the atmosphere. In experimental containers with treated plants, 93.05% of the initial 12.3 µCi ^{14}C was recovered. For untreated plants, 86.10% of the initial 12.3 µCi ^{14}C

Table 2. Bacterial densities ($x10^7$ cm^{-2}) on roots of E. crassipes before and after bacterial reduction treatment consisting of exposure to sodium hypochlorite and chloramphenicol.

	Mean	SE	N	t-value	p-value
Before Treatment	1.85	0.08			
			32	11.373	.0001
After Treament	0.64	0.06			

N is sample size, t-value is the result of a one-tailed paired-sample t test, and p-value is the significance level of t.

Table 3. Effects of bacterial reduction treatment on increase in leaf number and total leaf length in E. crassipes. Treatment consisted of exposure to sodium hypochlorite and chloramphenicol.

Leaf Number						Total Leaf Length					
Treated		Untreated				Treated		Untreated			
Mean	SE	Mean	SE	z	p	Mean	SE	Mean	SE	t	p
2.11	0.20	2.33	0.24	-0.793	>0.2	27.77	4.53	37.26	3.80	-1.60	0.128

Z is the normal approximation to the Mann Whitney test, t is the result of a two-sample t test, and p is the significance level of each statistic. N=9.

Table 4. Location of initial 12.3 µCi ^{14}C in treated and untreated experimental containers at the end of EOC experiments. Treated plants were exposed to sodium hypochlorite and chloramphenicol to reduce the density of epiphytic bacteria.

	Treated		Untreated	
	Mean	SE	Mean	SE
Plant	11.44	0.63	10.58	0.75
Water	0.0057	0.0008	0.0062	0.0014
Cap	0.00032	0.00005	0.00037	0.00007
Total	11.44	0.63	10.59	0.75

Data are µCi ^{14}C. N=10.

was recovered. The total amount of ^{14}C accounted for in treated versus untreated experimental containers did not differ significantly ($z=-0.907$; $p=0.3643$). Also, only 0.003% of the initial 12.3 µCi ^{14}C remained in the caps.

Amount and Location of Labelled Photosynthate in Plants

Labelled photosynthate was recovered from all tissues of treated and untreated plants (Table 5). The greatest amount of activity occurred in leaves and the least in ligules. Percent allocation of labelled photosynthate among tissues did not differ significantly in treated and untreated plants (chi-square=0.435; $p=0.510$) (Fig. 2).

Measurement of Total Extracellular Organic Carbon

The percentage of photoassimilated ^{14}C recovered as total EOC ($PEOC_t$) differed significantly in treated and untreated plants ($F=15.472$; $p<0.001$). Mean $PEOC_t$ was 0.049% (SE=0.005) in treated plants (range 0.020-0.069) and 0.066% (SE=0.013) in untreated plants (range 0.022-0.152) (Fig. 3).

Measurement of Particulate Extracellular Organic Carbon

The percentage of photosynthetically-fixed ^{14}C recovered as particulate EOC ($PEOC_p$) was significantly less for treated plants than for untreated plants ($F=9.474$;

Table 5. Allocation of ^{14}C-labelled photosynthate in E. crassipes exposed to $^{14}CO_2$ during a 12-hour light period. Treated plants were exposed to sodium hypochlorite and chloramphenical to reduce the density of epiphytic bacteria.

	Treated Plants		Untreated Plants	
Tissue	Mean	SE	Mean	SE
Root	4.10	0.68	4.33	0.58
Rhizome	10.73	1.55	7.01	0.98
Ligule	2.49	0.42	3.51	0.88
Float	6.59	1.04	5.99	0.93
Leaf	20.40	1.67	17.59	1.88

Data are nCi mg^{-1} dry wt. N=10.

Fig. 2. Percent allocation of ^{14}C-labelled photosynthate in treated and untreated E. crassipes exposed to $^{14}CO_2$ for a 12-hour light period. Treated plants were exposed to sodium hypochlorite and chloramphenicol to reduce bacterial densities. Each bar represents the mean percent allocation for 10 replicate plants. One standard error for the mean is indicated for each bar.

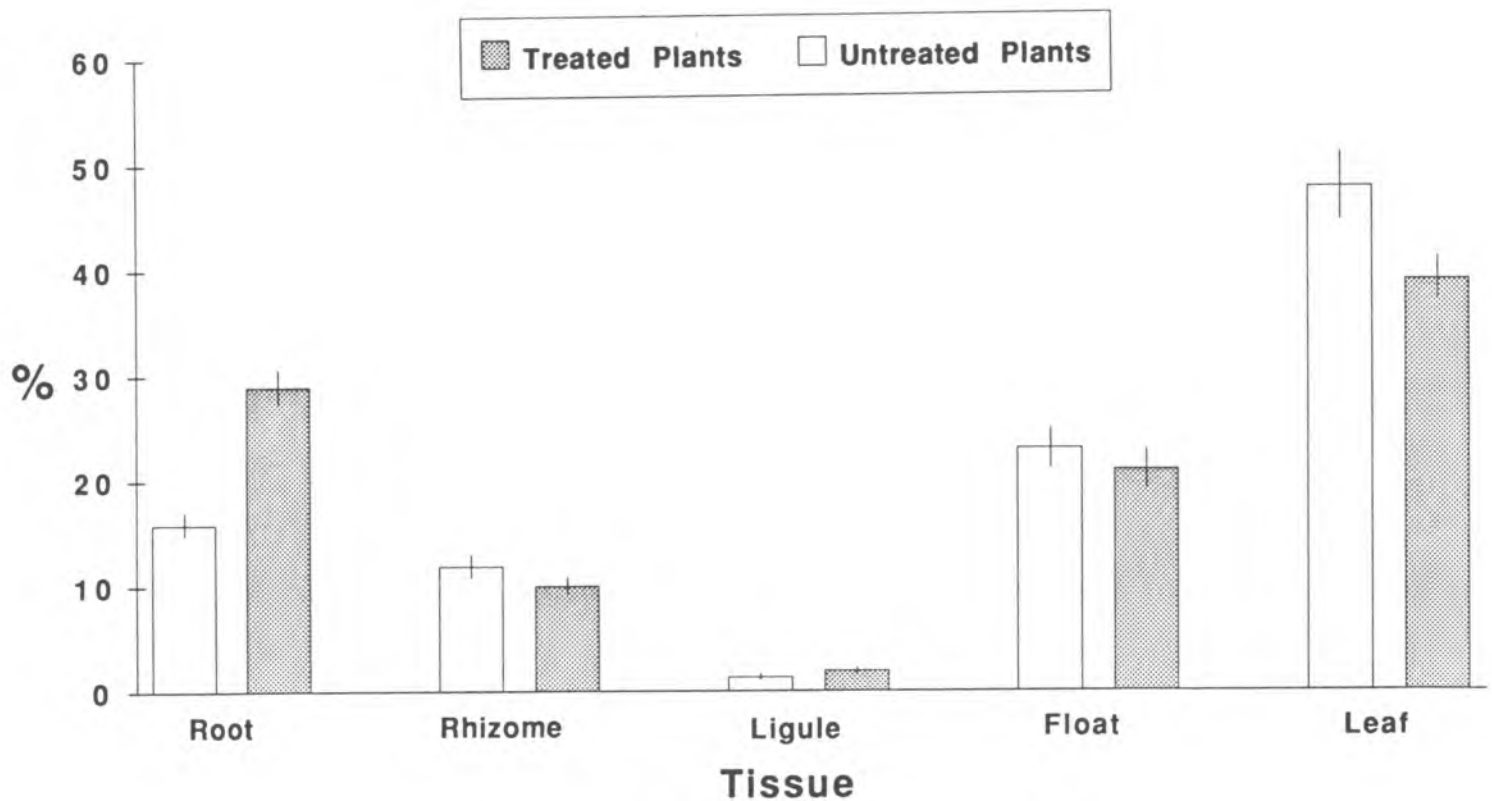
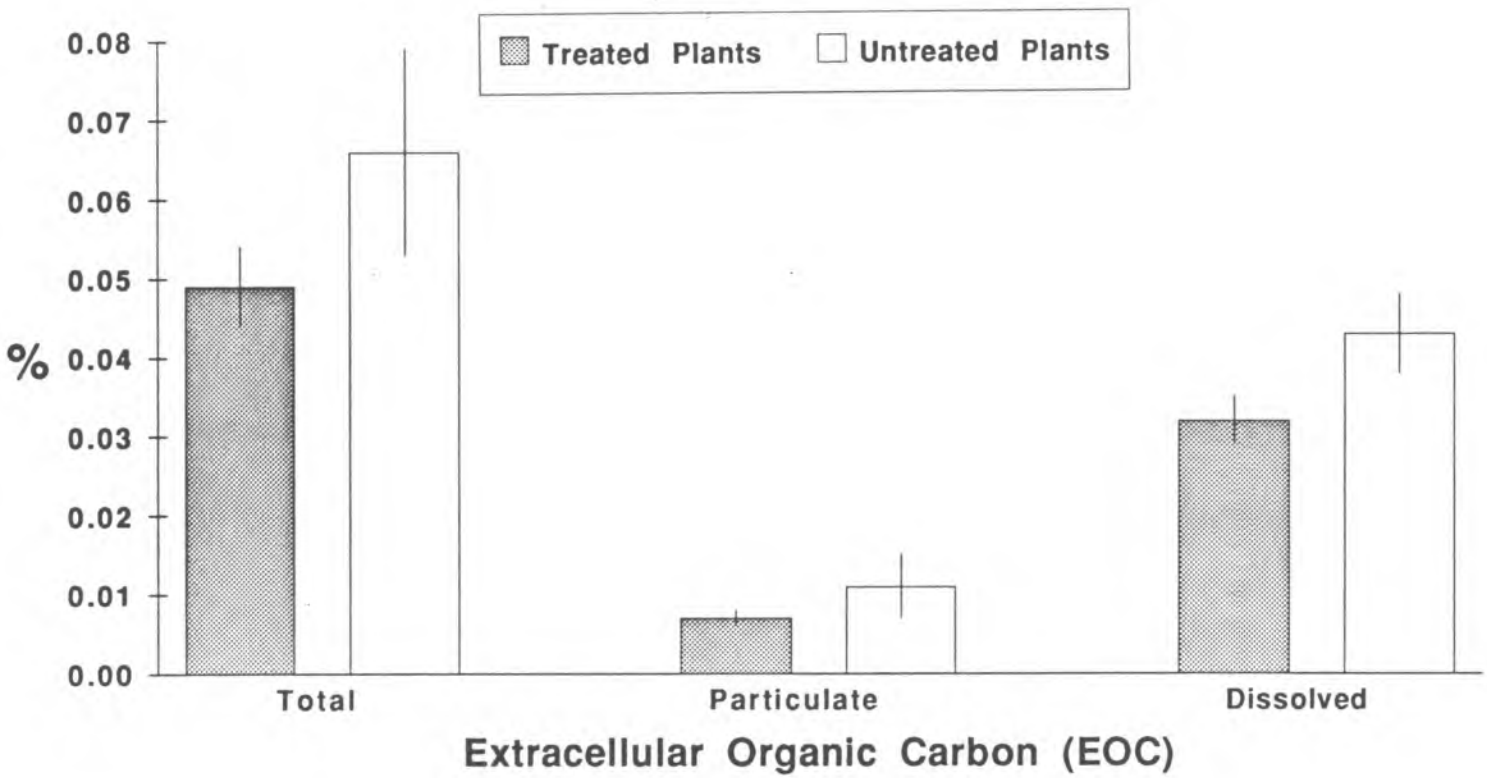

Treated Plants
Untreated Plants
60
50
40
%
30
20
10
0
Root
Rhizome
Ligule
Float
Leaf
Tissue

Fig. 3. Percent of photosynthetically-fixed $^{14}CO_2$ recovered as particulate, dissolved and total EOC from treated and untreated E. crassipes at the end of a 12-hour light period. Treatment consisted of exposure to sodium hypochlorite and chloramphenicol to reduce bacterial densities. Each bar represents the mean percent release for 10 replicate plants. One standard error of the mean is indicated for each bar.


Treated Plants
Untreated Plants
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
%
Total
Particulate
Dissolved
Extracellular Organic Carbon (EOC)

p=0.004) (Fig. 3). Mean $PEOC_p$ for treated plants was 0.007% (SE=0.001). For untreated plants, $PEOC_p$ averaged 0.011% (SE=0.004). However the percentage of total leakage recovered as EOC_p in treated and untreated plants did not differ significantly (F=0.7634; p=0.388) (Fig. 4). Particulate EOC was 12.40% (SE=2.15) of EOC_t in treated plants and 13.61% (SE=2.24) of EOC_t in untreated plants.

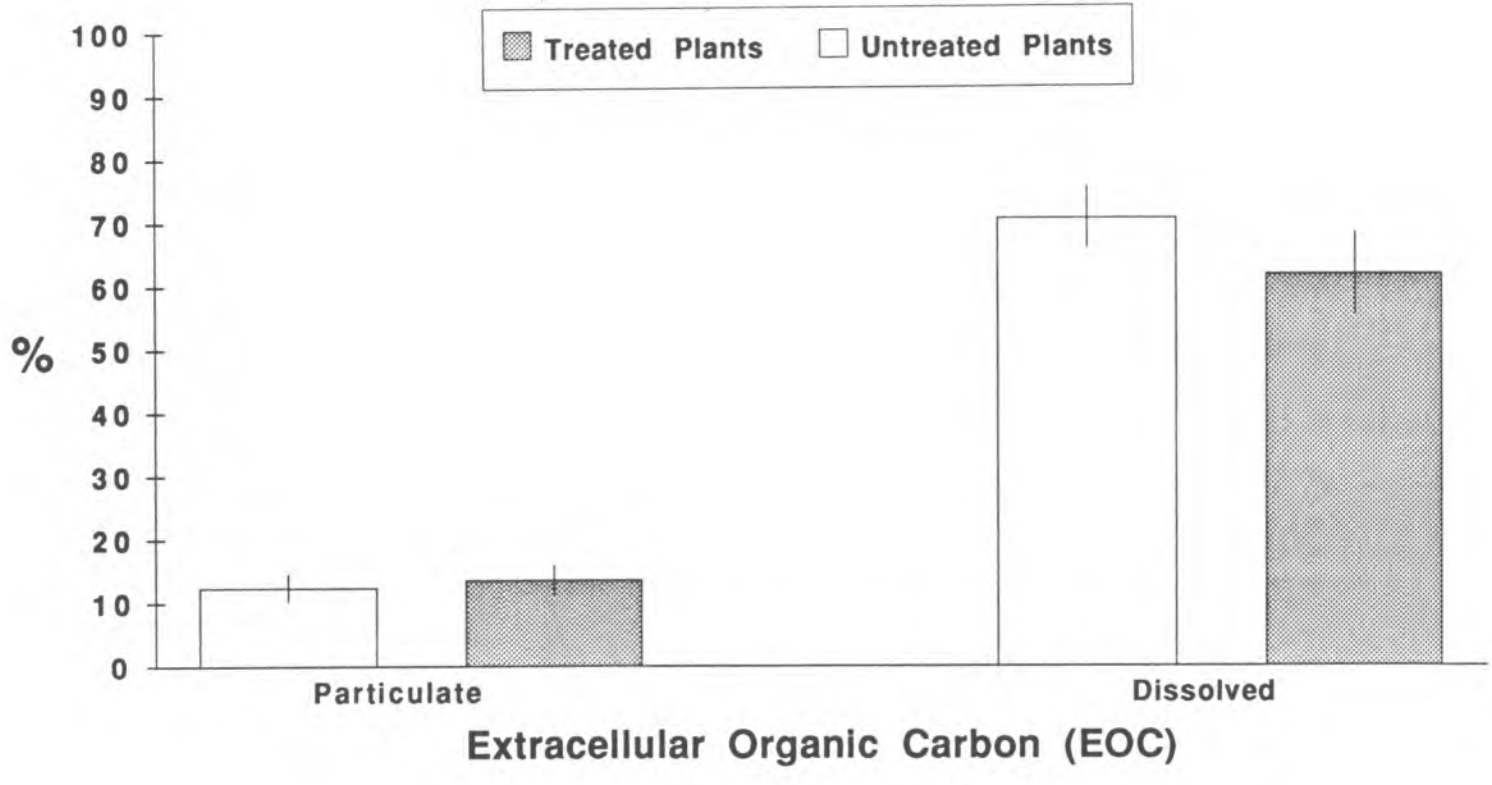
Measurement of Dissolved Extracellular Organic Carbon

The percentage of photosynthetically-fixed ^{14}C recovered as dissolved EOC ($PEOC_d$) differed significantly in treated versus untreated plants (F=36.430; p<0.001) (Fig. 3). The percentage recovered as EOC_d from treated plants (0.032%; SE=0.003) was significantly less than the percentage recovered as EOC_d from untreated plants (0.043%; SE=0.005). The percentage of total leakage recovered as EOC_d also differed significantly in treated and untreated plants (F=18.086; p<0.001) (Fig. 4). For treated plants, 70.90% (SE=4.81) of the total carbon leaked was dissolved. For untreated plants, 61.99% (SE=6.42) of the total carbon leaked was dissolved.

Measurement of Metabolically Active Bacteria

The mean number of active bacteria on treated and untreated roots differed significantly (z=-3.18; p<0.001)

Fig. 4. Percent of total EOC recovered as particulate and dissolved EOC from treated and untreated E. crassipes at the end of a 12-hour light period. Treated plants were exposed to sodium hypochlorite and chloramphenicol to reduce bacterial densities. Each bar represents the mean percent of total EOC for 10 replicate plants. One standard error of the mean is indicated for each bar.


Treated Plants
Untreated Plants
100
90
80
70
60
%
50
40
30
20
10
0
Particulate
Dissolved
Extracellular Organic Carbon (EOC)

at the beginning of the 12-hour light period. The mean density of active bacteria on roots of treated plants was 0.15×10^7 cm^{-2} (SE=0.01), whereas the mean density of active bacteria on roots of untreated plants was 0.34×10^7 cm^{-2} (SE=0.05) (Fig. 5).

At the end of the 12-hour light period, the mean density of active bacteria on roots of treated plants (0.15×10^7 cm^{-2}; SE=0.02) and on roots of untreated plants (0.30×10^7 cm^{-2}; SE=0.04) also differed significantly (t=-3.376; p=0.0034) (Fig. 5).

The mean densities of active bacteria on roots of treated plants and on roots of untreated plants did not change significantly during the experiment (Table 6).

Total Number and Percent Active Bacteria

At the beginning of the 12-hour light period, the density of total bacteria on the roots of treated versus untreated plants differed significantly (z=-3.781; $p<0.001$). The mean density of total bacteria on roots of treated plants was 0.27×10^7 cm^{-2} (SE=0.02), and the mean density on roots of untreated plants was 1.40×10^7 cm^{-2} (SE=0.08) (Fig. 5).

After 12 hours, the mean density of total bacteria on treated roots (0.47×10^7 cm^{-2}; SE=0.04) versus untreated

Fig. 5. Number of active, inactive and total bacteria on root caps of treated and untreated E. crassipes at the beginning (0 h) and end (12 h) of a 12-hour light period. Treated plants were exposed to sodium hypochlorite and chloramphenicol to reduce bacterial densities. The height of each bar represents the mean bacterial density for 10 replicate plants. One standard error for the mean for total bacteria is indicated for each bar.

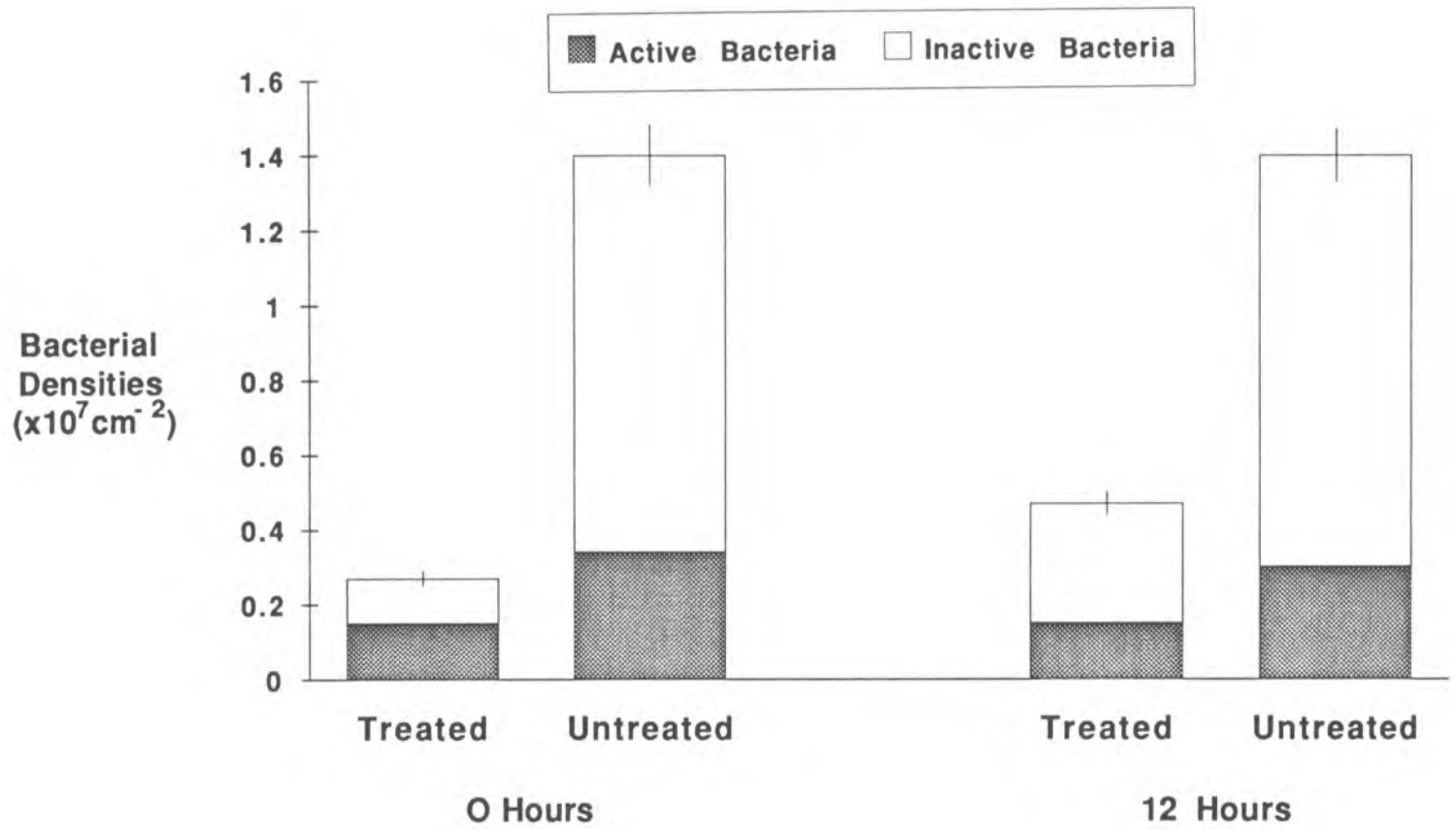

Active Bacteria
Inactive Bacteria
Bacterial Densities (x10^{7}cm^{-2})
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Treated
Untreated
Treated
Untreated
O Hours
12 Hours

Table 6. Active number ($x10^7$ cm^{-2}), total number ($x10^7$ cm^{-2}) and % active bacteria on roots of treated and untreated E. crassipes at the beginning and end of a 12-hour light period. Treated plants were exposed to sodium hypochlorite and chloramphenicol to reduce the density of epiphytic bacteria.

	0 Hours		12 Hours			
	Mean	SE	Mean	SE	t	p
Bacteria on Treated Roots:						
Active	0.15	0.01	0.15	0.02	-0.20	0.8499
Total	0.27	0.02	0.47	0.04	-7.69	0.0001
% Active	56.20	5.65	31.37	3.94	3.37	0.0082
Bacteria on Untreated Roots:						
Active	0.34	0.05	0.30	0.04	1.67	0.1293
Total	1.40	0.08	1.40	0.07	-0.08	0.9380
% Active	25.80	4.72	22.07	3.09	1.56	0.1541

T is the result of a paired sample t-test, and p is the significance level of t. N=10.

roots (1.40×10^7 cm^{-2}; SE=0.07) also differed significantly ($t=-12.055$; $p<0.0001$).

Total bacterial densities on treated roots increased significantly ($p=0.0001$) during the 12-hour light period (Table 6). Bacterial densities on the roots of untreated plants did not change during that time ($p=0.9380$) (Table 4).

Percent active bacteria on the roots of treated versus untreated plants differed significantly at the start of the 12-hour light period ($t=4.127$; $p=0.0006$). On the roots of treated plants, 56.2% (SE=5.6) of the bacteria were active. On the roots of untreated plants, 25.8% (SE=3.1) of the cells were active.

At the end of the 12-hour light period, percent active bacteria on the roots of treated versus untreated plants was not different ($t=1.869$; $p=0.078$). At that time, the percentage of active cells on the roots of treated plants was 31.4% (SE=3.9). On untreated plants, 22.1% (SE=3.1) of the bacteria were active.

The percent active bacteria on the roots of treated plants decreased significantly during the 12-hour light period ($p=0.0082$) (Table 6). Percent active bacteria on the roots of untreated plants did not change during that time ($p=0.1541$).

DISCUSSION

Bacterial Reduction Treatment

The bacterial reduction treatment used in this study did not damage water hyacinth visibly. The treatment did not affect plant growth as measured by the production of new leaves and increase in leaf length (Table 3). Leaf dynamics are good estimates of a plant's health because sequential leaf production plays an important role in keeping water hyacinth afloat and may aid in the transport of nutrients throughout a growing plant (Center and Van 1989). Also, the allocation of labelled photosynthate did not differ in treated and untreated water hyacinth (Fig. 2). If the treatment had damaged the plants, more photosynthate might have been allocated to the damaged tissue. Finally, treated plants did not produce higher EOC estimates than untreated plants (Fig. 3). Other researchers have found that damaged plants yield misleadingly high EOC estimates (Godmaire and Nalewajko 1986).

The bacterial reduction treatment effectively reduced the number of bacterial cells on water hyacinth roots (Table 2). However 12 hours after treated plants were removed from the antibiotic solution, the number of

bacterial epiphytes had increased significantly (Table 6). Because chloramphenicol is a bacteriostatic antibiotic, the increase in cell numbers verified the removal of the antibiotic solution from the roots. Although the bacterial population on treated roots almost doubled after 12 hours, treated roots still harbored significantly fewer bacteria than untreated roots. Thus the bacterial reduction treatment enabled me to compare leakage of organic carbon from water hyacinth having intact versus reduced epiphytic communities.

Extracellular Organic Carbon from Water Hyacinth

Water hyacinth leaked only a small proportion of photosynthetically-fixed carbon as EOC. Total leakage in a 12-hour period ranged from 0.02 to 0.15% of photoassimilated carbon (Fig. 3). These results are consistent with reports of other macrophytes losing less than 1% of fixed carbon as EOC (Hough and Wetzel 1975; Sondergaard 1981a; Godmaire and Nalewajko 1986). In fact, Wetzel and Manny (1972) found that another floating macrophyte, Lemna perpusilla, released only 0.02% of fixed carbon when exposed to $^{14}CO_2$. However water hyacinth EOC production was much less than the 0.3-35% of photoassimilated carbon reported as typical for algae (see reviews by Fogg 1966, 1971; Nalewajko 1977; Sharp 1977).

Higher release rates may be linked to environmental stress. Greater EOC production was found in response to low light intensity (Wetzel 1969), low CO_2 concentrations (Wetzel 1969; Baker and Farr 1987) and changes in the ionic composition of the medium (Wetzel 1969). For example, EOC from Najas flexilis exposed to media of different salinities ranged from 0.05-25.3% of fixed carbon in one study (Wetzel and Manny 1972) and 2.3-98.9% in another (Wetzel 1986). Baker and Farr (1987) found that duckweed released 4.3% of photoassimilated carbon when carbon was limited, as opposed to 1.1% when carbon was plentiful. Because environmental factors change seasonally, leakage rates also may change throughout the year (Hough and Wetzel 1975).

Loss of organic carbon from water hyacinth may have been underestimated for several reasons. First, due to the close association between water hyacinth and bacterial epiphytes, EOC production and uptake could have occurred almost simultaneously. This study detected only two types of EOC: organic carbon not utilized by epiphytes (EOC_d) and organic carbon incorporated into bacteria that subsequently detached from plants (EOC_p). Organic carbon consumed by epiphytes that remained attached to plants was not measured directly. However, EOC_p may be a reasonable estimate of heterotrophic uptake if bacterial detachment

rates are high, as reported by Hossell and Baker (1979a). Organic carbon consumed and then respired by bacteria also was not measured and represents an inherent error.

Epiphytic Heterotrophy of Water Hyacinth EOC

To investigate the magnitude of EOC assimilation by epiphytic bacteria, I compared EOC release in plants having intact epiphytic communities with EOC release in plants having reduced (66-81% fewer cells) epiphytic communities. I hypothesized that if epiphytic bacteria utilized water hyacinth EOC, the percentage of photoassimilated carbon released from the plant-epiphyte complex would be inversely related to the density of the epiphytes.

Unexpectedly, total leakage was greater in untreated plants (0.07%) than in treated plants (0.05%) (Fig. 3). When more bacteria were present on water hyacinth roots, more particulate and dissolved organic carbon was recovered from the water. However EOC_d as a percentage of total leakage was greater in treated than in untreated plants, as expected (Fig. 4). When fewer bacteria were present to utilize EOC, a greater proportion of total leakage consisted of dissolved carbon.

Bacteria in the water incorporated only a small percentage of water hyacinth EOC. Particulate EOC accounted for approximately 14% of total leakage from

untreated plants and 12% of total leakage from treated plants (Fig. 4). The difference between treated and untreated plants was not significant, perhaps due to high sample variability. However other researchers have reported that dissolved organic carbon can form particles abiotically (Sondergaard 1981a; Sondergaard 1981b; Jensen and Sondergaard 1982). Small organic molecules can form aggregates that are retained on filters. Thus, as filter-retained organic carbon represented bacterial heterotrophy of EOC, abiotic particle formation may have resulted in overestimates of bacterial EOC assimilation that masked true differences between treated and untreated plants.

The percentage of photoassimilated carbon released from the plant-epiphyte complex was not related inversely to the density of epiphytes. Rather, when more bacteria were present on water hyacinth roots, more EOC was recovered. One explanation for this result is that bacteria on treated plants were more active metabolically and therefore consumed more EOC than bacteria on untreated plants. Bacterial numbers on treated roots increased significantly during the EOC experiment, whereas bacterial numbers on untreated plants did not change (Table 6). Also, the mean percentage of active bacteria on treated plants was 44% --- significantly higher than the percentage of active bacteria on untreated roots. However treated

roots supported 52% fewer active cells than did untreated roots. Thus, to assume that the lower EOC values from treated plants were due to increased EOC assimilation, one would have to assume that individual bacteria on treated plants consumed more than twice as much organic carbon as individual bacteria on untreated plants, which is an unlikely scenario.

An alternate explanation is that bacteria on water hyacinth roots enhanced EOC release. Bacteria living in the rhizosphere of terrestrial plants release plant growth hormones that affect photosynthate release (Curl and Truelove 1986). Similarly, Patil and Iswaran (1980) isolated bacteria that produced growth hormone-like substances from water hyacinth roots. Growth hormones may change root membrane permeability resulting in greater leakage of photosynthate. Root membrane integrity is a major factor in leakage from terrestrial plants (Curl and Truelove 1986) and probably plays a similar role in macrophyte production of EOC. Thus untreated plants may have leaked more than treated plants because untreated roots harbored more hormone-producing bacteria. In addition, as epiphytic bacteria utilized EOC, an EOC concentration gradient might have developed. Because untreated plants harbored more heterotrophic bacteria than treated plants, the EOC concentration gradient at the root-

epiphyte boundary of untreated plants would have been steeper than that of treated plants. As a result, epiphytic heterotrophy of EOC may have caused EOC to diffuse more rapidly from water hyacinth having intact epiphytic communities.

Epiphytic Bacteria on Water Hyacinth Roots

Water hyacinth roots supported a large and active microbial community. The mean density of epiphytic bacteria was 1.4×10^7 cm^{-2} in the spring and 1.8×10^7 cm^{-2} in late summer. These population densities are similar to those reported for the submerged portions of other aquatic macrophytes including duckweed (Baker and Orr 1986; Baker and Farr 1987). Wahbeh and Mahasneh (1984) reported similar seasonal differences in bacterial densities and suggested that seasonal distributions may be related to the availability of macrophyte EOC.

Although determination of bacterial numbers provides information about the structure of the epiphytic community, determination of metabolically active cells yields information about epiphytic community dynamics. Approximately 24% of total bacteria on roots of untreated water hyacinth were metabolically active. Estimates of active cells ranged from 11-49% of total bacteria. Similarly, 38% of epiphytes on Elodea sp. were

metabolically active (Quinn 1984) as were 8-45% of epiphytic bacteria on Elodea canadensis (Ramsay 1974). The proportion of respiring bacteria on water hyacinth roots was greater than that reported for some planktonic bacteria. For example, the active fraction of bacterioplankton in the Baltic Sea ranged from 6-12% in one study (Zimmermann et al. 1978) and 4-6% in a second study (Meyer-Reil et al. 1979). The proportion of respiring bacteria in the Pacific Ocean was estimated at 5-10% (Kogure et al. 1979). Estimates of active bacteria in freshwater ponds ranged from 5-9% (Zimmermann et al. 1978). However the active fraction of bacteria on water hyacinth roots was similar to that reported for bacterioplankton (21-29%) and benthic bacteria (16-32%) from a eutrophic lake in Ireland (Quinn 1984).

Carbon Cycling in the Macrophyte-Epiphyte Complex

The release of photoassimilated carbon from macrophytes appears to be a wasteful process. As a result, researchers have sought ways to explain its occurrence. Because aquatic plants evolved from terrestrial plants, some researchers (Wetzel and Hough 1973; Wetzel 1983) have suggested that leakage represents an incomplete adaptation to life in water. Others have suggested that EOC may be beneficial to bacteria that in turn benefit macrophytes

(Hough and Wetzel 1975; Sondergaard 1983; Zuberer 1984; Wetzel 1983). Thus "leaky" plants may have a selective advantage. The symbiosis hypothesis has been expanded recently to include a mutualistic association between macrophytes and grazers (Thomas 1982; Bronmark 1985). The release of photoassimilated carbon from macrophytes may be an inevitable metabolic process, however. Membrane permeability is affected by many environmental factors and probably plays a major role in leakage of organic carbon. Thus macrophytes may leak organic carbon simply because the energetic costs of not leaking are too high (Carpenter and Lodge 1986).

Leakage of organic carbon from water hyacinth probably was an insignificant portion of the plant's carbon budget. When water hyacinth roots harbored a reduced epiphytic community, EOC represented a small portion of photoassimilated carbon. Thus, even though epiphytic bacteria were situated ideally for the assimilation of macrophyte EOC, heterotrophic uptake did not appear to mask true EOC release in water hyacinth.

Leakage from water hyacinth also is unlikely to represent a significant portion of an aquatic system's total carbon budget. Organic interactions in the rhizosphere certainly are more intense than those in the more dilute open waters, however. Although water hyacinth

EOC may not be an important carbon source for aquatic bacteria in general, it may enhance the development of a large and active epiphytic community. Bacteria epiphytic on water hyacinth roots in turn may enhance EOC production.

The associations between macrophytes, epiphytes and other members of the aquatic food web are complex. In aquatic ecosystems having extensive littoral zones, the macrophyte-epiphyte complex probably plays a fundamental role in community dynamics. Thus, although water hyacinth is not very leaky, water hyacinth EOC and uptake of that EOC by bacteria may form an important link to other trophic levels in the aquatic food web.

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