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Decomposition and significance of seagrass leaf litter (*Cymodocea nodosa*) for the microbial food web in coastal waters (Gulf of Trieste, Northern Adriatic Sea)

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ABSTRACT: Decomposition and microbial utilization of leaf material was investigated in micro- and mesocosm experiments using freshly sloughed leaves of a common Mediterranean seagrass species (*Cymodocea nodosa*). In the initial phase of decomposition, dissolved monomeric carbohydrates (MCHO) leached out of the material provoking a rapid (within 40 h) response of the free-living bacterial community. When abiotic leaching had ceased after ca 180 h, 13 mg MCHO-C g^{-1} (leaf dry wt) had been lost from the material, 92 % of which was taken up by microorganisms. At the advanced stage of decomposition, a rich nanoflagellate community had developed and after 14 d of incubation a free-living flagellate standing stock of 210 µg C per g (leaf dry wt) was still living at the expense of decomposing leaf material. In a long-term experiment using litter bags, the microbial colonization pattern and the breakdown of leaf tissue were followed under reduced influence of macrofaunal shredders and physical forces. The initially uniform bacterial coverage on leaf surfaces, as followed by SEM, changed within 14 d to a 7 times more abundant and heterogenous bacterial assemblage. After more than 3 wk, leaf surfaces began to break down as indicated by crevices densely surrounded by bacteria. At the same time a protozoan community, mainly consisting of monads and choanoflagellates, developed on leaf blades, reaching a maximum density of 2.4×10^5 cells cm^{-2} after 2 mo of degradation. Specific loss rates of weight, particulate organic carbon and nitrogen from litterbags were highest during the first 2 mo and declined thereafter to rates 5 times lower. Only 50 % of the original dry wt and organic carbon remained after 7 to 8 mo of incubation. Measurements of the O_2 consumption associated with decaying leaf material indicated that 40 % of the calculated C-mineralization rate can be attributed to the decreasing organic C-concentration in the plant litter during the first 2 mo. In a later stage of decomposition, only 4 % of the O_2 consumption could be matched by the organic C-loss of the decomposing material; dissolved organic material also in the surrounding water is proposed to be utilized by the attached microbes. It is concluded that the soluble fraction leaching out of the material in the early phase of decay is rapidly used by both the free-living and the attached bacterial community, thus supporting a microbial food web up to the protozoan level. It is suggested that most of the residual fraction of organic carbon of leaf debris is released more slowly into the water after hydrolysis by attached microbes thus indicating a loose hydrolysis-uptake coupling. Under exclusion of shredders and reduction of physical forces degradation was not complete after 231 d of incubation. Leaf debris of *C. nodosa* is suggested to have a small but significant impact on microbial secondary production in the study area largely via its leachates.

INTRODUCTION

Although vascular plants are thought to account for a significant fraction of the primary production in coastal marine waters (Mann 1982, Stevenson 1988) only a few aquatic macrophytes are extensively used by grazers (Mann 1988, and references therein). Therefore, litter and particulate detritus derived from vascular plants is reported to be abundant in many coastal ecosystems and is processed largely via detrital food webs based on bacterial decomposition (Mann 1972, Mann 1988, Valiela et al. 1985, Benner et al. 1988, Moran & Hodson 1989a).

Among the seagrass species present in the Mediterranean Sea, *Cymodocea nodosa* is one of the most important macrophytic primary producers (Den Hartog

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1970, Peduzzi & Vukovič 1990). In the Gulf of Trieste (Northern Adriatic Sea) *C. nodosa* is the most frequent phanerogam (Simonetti 1973) and, due to its high productivity, a significant contributor to the overall production of organic material in this area (Peduzzi & Vukovič 1990).

Bacteria are reported to utilize both the soluble fraction of plant debris, which leaches rapidly after plant death, and the more refractory compounds (Valiela et al. 1985, Benner et al. 1986, Findlay et al. 1986, Moran & Hodson 1989a, b). The conversion efficiency within the detritus-associated microbial community is one of the factors determining whether the degrading material can significantly support a microbial food web and/or higher trophic levels. Whereas only few specialized macrofaunal species might use this detritus with an efficiency up to 40% (Peduzzi 1987, Mann 1988), most studies dealing with bacterial growth on particulate detritus from a variety of aquatic macrophytes suggest low (around 10%) bacterial growth efficiencies (Stuart et al. 1981, Newell et al. 1983, Linley & Newell 1984). A more recent study reports carbon conversion efficiencies for bacterial growth on lignocellulose of up to 45%; these high values, however, were obtained – like many others – with ground and processed material (Benner et al. 1988).

In the present study it was asked whether *Cymodocea nodosa* leaf litter can support a microheterotrophic food web either via the soluble fraction or the utilization of the refractory structural components of unprocessed (i.e. unground) sloughed leaves. Furthermore, the significance of the leaf-attached microbial community for the utilization of this material is questioned. In the light of the high metabolic costs for the production of extracellular enzymes by bacteria (Gottschalk 1986) it was asked whether the material can be processed completely and utilized immediately by surface attached microbes alone. Finally, we tried to assess the significance of *C. nodosa* primary production for overall microbial secondary production within the Gulf of Trieste.

MATERIAL AND METHODS

Leaf material of the seagrass *Cymodocea nodosa* was collected in fall in the Gulf of Trieste off the Laboratorio di Biologia Marina at Trieste-Aurisina (Italy) in depths of 5 to 7 m. We selected leaves which were recently deposited within the seagrass bed, as indicated by a yellow-brownish, senescent stage with ca 50% yellow coloration (compare Newell et al. 1989). The leaf blades were used in all our different incubations as fresh, unground and undried material. In the study area *C. nodosa* is scarcely colonized by epiphytes (Peduzzi & Vukovič 1990); only leaves with no visible epigrowth were selected (Robertson et al. 1982). Several authors using dried and ground material report on shortcomings arising from this method such as the difficulties in extrapolating DOC-release – measured on leaves dried and powdered prior to incubation in seawater – to actual in situ rates (Robertson et al. 1982), or the immediate release of high quantities of soluble components after rehydration (Pellikaan 1984). To avoid such severe alterations when processing the material prior to the incubation, we established a dry weight-to-fresh weight ratio for *C. nodosa* leaves (Robertson et al. 1982, Benner et al. 1988, Robertson 1988). This allowed us to calculate the initial amount added to the incubation jars on a dry wt basis.

Initial leaching phase experiments. To investigate leaching of *Cymodocea nodosa* leaf litter and to evaluate the significance of this process for the microbial dynamics of the surrounding water, incubation experiments were performed at 20 °C in the dark. The early course of dissolved monomeric carbohydrate carbon (MCHO-C) concentration and bacterial abundance was followed in an 80 h experiment; the development of the nanoflagellate population as well as of MCHO-C concentration was monitored in a 370 h experiment. In order to reduce bacterial predators, the seawater collected from the same site as the leaf material was filtered through 1.0 µm pore-size Nuclepore filters (Benner et al. 1986, Benner et al. 1988, Herndl 1988). To each precombusted (470 °C) glass jar containing 800 ml of water, approximately equal amounts of leaf material were added. Each experiment was established in triplicate. Two separate jars were incubated with leaves as described above but the incubation media were poisoned with $HgCl_2$ (20 mg l^{-1}) to inhibit microbial uptake of abiotically leaching compounds (Boto et al. 1989). A possible effect of poisoning on the condition of leaf tissue cannot be ruled out; however even at the end of the experiments the tissue was of the same coloration and appearance in both poisoned and unpoisoned treatments. Two chambers were prepared with only filtered seawater to serve as a control for endogenous microbial growth. All jars were covered with parafilm and gently aerated with 0.45 µm filtered air (Herndl & Peduzzi 1989). Water samples (12 ml) were taken at various time intervals. Subsamples were withdrawn for bacteria or nanoflagellate enumeration and for analyses of monomeric carbohydrates (MCHO). This type of compound was selected as a model component of the easily utilizable, dissolved organic matter (DOM) fraction of leaf material (compare also Pakulski 1986). Water samples for MCHO-analysis were filtered through precombusted glass fiber filters (Whatman GF/F) within 30 min after sampling and stored frozen (−20 °C). MCHO contents were determined

according to the spectrophotometric method of Johnson & Sieburth (1977) as described in more detail by Herndl (1988).

For microbial analyses, water samples were fixed with unbuffered formalin to a final concentration of 2 % formaldehyde (v/v). Bacterial and flagellate densities were determined using acridine orange direct counting (AODC) and epifluorescence microscopy (Hobbie et al. 1977). Bacterial biomass was calculated in terms of carbon using a factor of 20 fg C $cell^{-1}$ (Lee & Fuhrman 1987). Dimensions of flagellates were measured as described previously (Herndl 1988, Herndl & Peduzzi 1989); a cell-volume to cell-carbon conversion factor of 220 fg C μm^{-3} was used (Børsheim & Bratbak 1987).

Long-term degradation experiment. In order to follow microbial colonization and microbial breakdown of leaf tissue, an experiment was designed in which losses due to invertebrate shredders and physical means were minimized. Such loss of undegraded material may lead to an overestimation of decay rates (Valiela et al. 1985, Moran et al. 1989); the aim of the present work was to measure largely microbial mediated decomposition (compare also Robertson 1988).

Leaf material was split into equal amounts of fresh weight (370 mg each, which corresponds to 87.6 mg dry wt; the error of this fresh wt/dry wt conversion was < 4 %) and enclosed in 34 nylon mesh bags (10 cm by 10 cm; mesh size 1 mm). Care was taken to minimize the period of emersion in order to avoid damage to the leaves during this procedure. The litterbags were placed in a glass tank (100 l; precleaned and acid-rinsed) which was filled with 63 µm Nitex-screened seawater from the study site and gently aerated. To evaluate losses of dry wt, particulate organic carbon and nitrogen under these experimental conditions, 4 replicate bags were removed randomly on Days 0, 14, 25, 42, 62, 84, 153 and 231 of the experiment. From 2 separate bags, subsamples were taken at the same dates to obtain material for scanning electron microscopy (SEM) and for O_2 consumption measurements as described below. At each sampling date, 500 ml of seawater were withdrawn from the tank and analyzed for particulate material to ensure that the loss of material through physical means or possibly remaining shredders was efficiently minimized. Prior to sampling, the incubation water in the tank was gently stirred in order to homogenize the water body. Since the particulate content of the water remained at a low level (near the detection limit) and did not increase significantly (linear regression: $y = 1.4235 + 0.0021x$, x = incubation time in days, y = mg dry wt l^{-1}; $r^2 = 0.124$; $n = 8$; $p > 0.2$) throughout the entire experimental period, loss of particulate material from litterbags was assumed to be negligible.

For SEM leaf material was prepared according to the method described by Paerl & Shimp (1973) and Paerl (1975). Pieces of leaf blades were preserved in a filtered formalin-seawater solution (4 %, v/v). Before the SEM procedure, the samples were rinsed with decreasing concentrations of filtered seawater and distilled water to eliminate salts. Stepwise dehydration in a graded series of ethanol solutions and finally acetone was followed by critical-point drying. After sputter-coating with gold-palladium, the material was examined with a JEOL JSM-35 CF scanning electron microscope. Numbers of attached bacteria and flagellates were estimated following the procedure described by Novak (1984) and converted to microbial biomass as described above. We are aware of the problems arising due to the shrinkage of cells using this method and therefore biomass estimates are probably underestimates. However, the main purpose in this part of the study was not to exactly balance biomass transfers, but to gain an insight into microbial colonization and distribution patterns on the leaf surfaces, which other methods (e.g. Benner et al. 1988, Robertson 1988) cannot provide. Concurrently, the SEM technique allowed us to examine alterations in the structure of the leaf tissue.

After removal from the tank, leaf material from the bags was rinsed shortly with deionized water to eliminate salts, oven-dried (75 °C), weighed and ground to a powder. Subsamples of this material were used to determine organic carbon and nitrogen content with a Carlo Erba ANA-1500-C/H/N-analyzer. Losses were expressed as percent of original remaining as described by Robertson (1988).

O_2 consumption measurements of the leaf surface-attached microbial community were conducted in 0.2 µm double-filtered seawater taken from the tank at each sampling date using the setup described by Peduzzi & Herndl (1986). To estimate mineralization rates of organic carbon, the oxygen-carbon equivalent given in Elliot & Davison (1975) assuming an R.Q. of 1.0 was used.

RESULTS

Initial leaching phase (0 to 400 h)

Early development of MCHO-C concentration and microbial abundance in incubation media. In the incubation chambers, each containing on average 167.8 mg dry wt (SE = 21.6) of *Cymodocea nodosa* leaf material, the initial development of the MCHO-C concentration and the bacterial biomass within the 1.0 µm filtered incubation media were followed over an 80 h period. The results are presented in Fig. 1. The mean MCHO-C concentration in the incubation medium started to deviate significantly from the background level in the

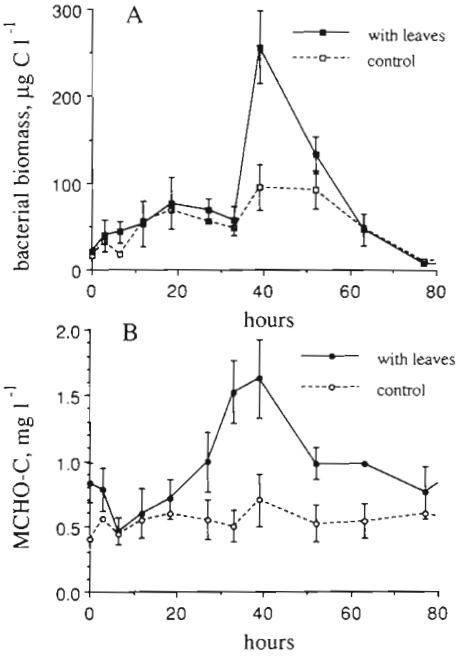


Fig. 1. Early development of (A) free-living bacterial biomass and (B) dissolved MCHO-C concentration in batch culture experiments with decomposing *Cymodocea nodosa* leaf material. Symbols represent means (± 1 SE) of 3 replicate incubations (control incubations without leaf material in duplicates)

control jars (no leaves) ca 20 h after starting the experiment, reaching a peak value at 39 h (Fig. 1B). Thereafter the bacterial biomass, supported by seagrass leaf litter, began to increase (Fig. 1A) and reached a maximum (256.8 µg C l^{-1}; corresponding to a bacterial density of 12.8 × 10^6 cells ml^{-1}) in the jar with leaf material at the same time as the MCHO-C concentration. Subsequently the bacterial biomass and MCHO-C concentration declined again reaching initial levels after ca 80 h.

The development of the nanoflagellate population in the incubation medium containing leaf material exhibited a clearly different pattern when compared to the control medium (Fig. 2A, B). In the chambers incubated with *Cymodocea nodosa* leaves, the mean flagellate biomass started to increase at 39 h and thereafter oscillated up to a mean maximum value of 103.1 µg Cl^{-1} (corresponding to a flagellate density of 53 × 10^3 cells ml^{-1}) 250 h after starting the experiment and

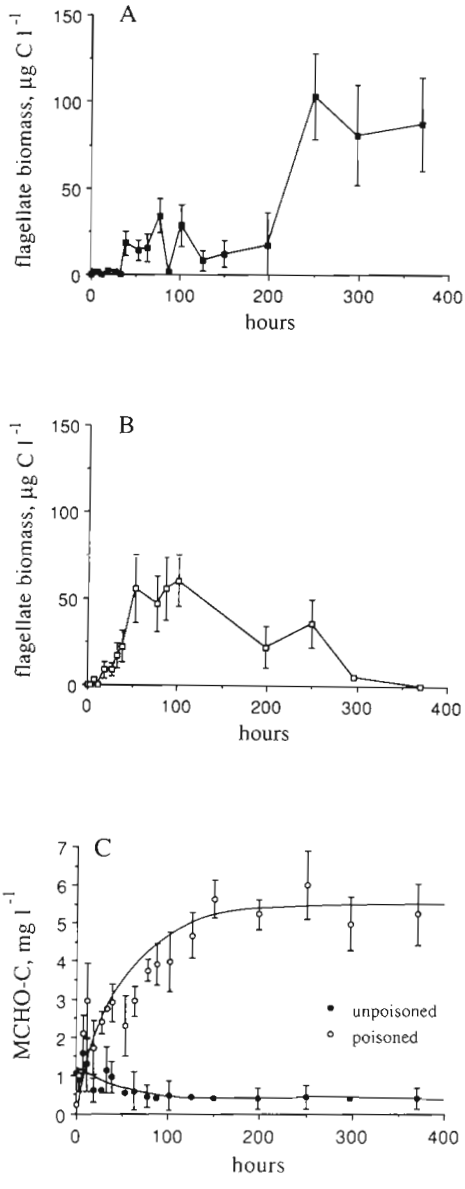
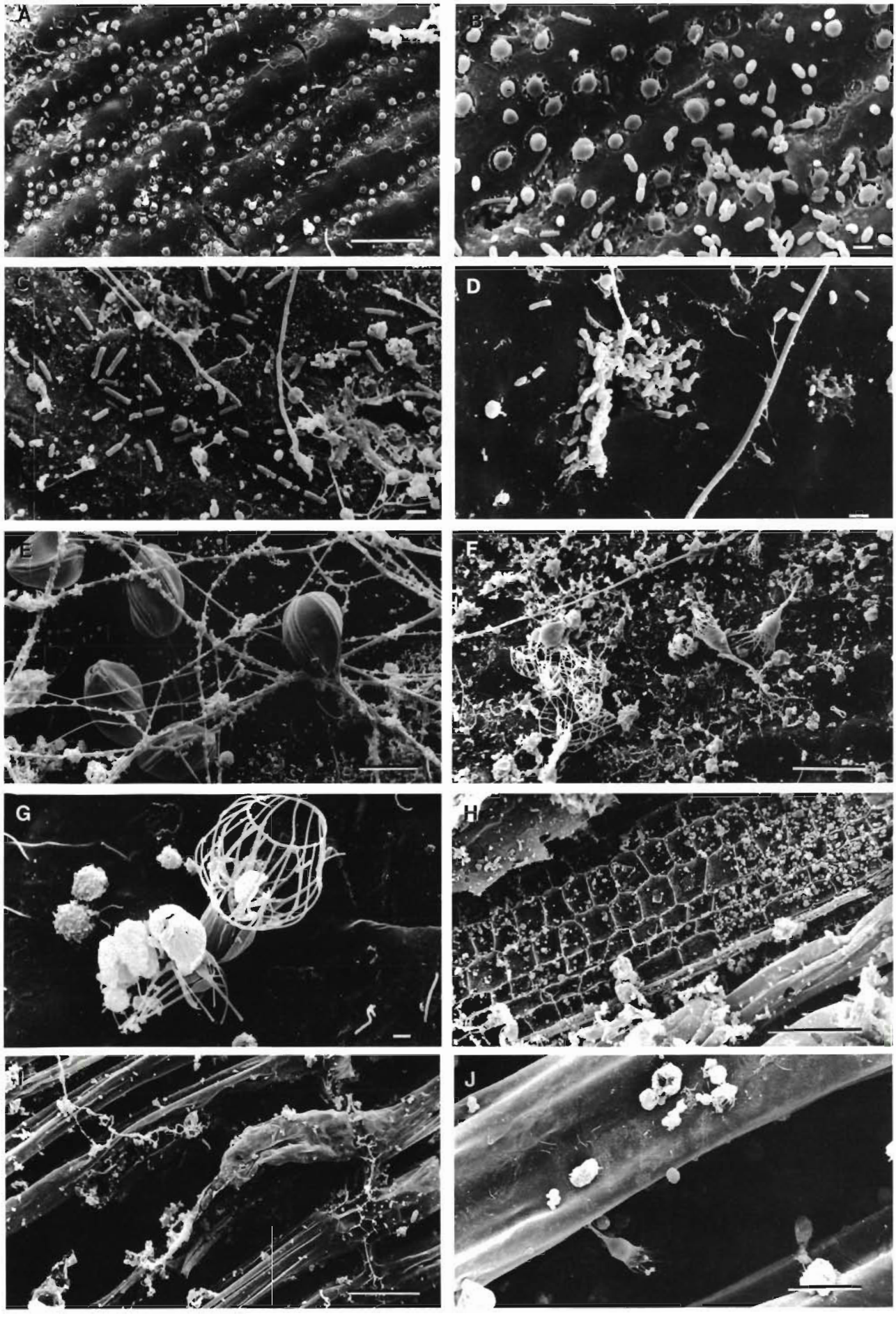


Fig. 2. Batch cultures with *Cymodocea nodosa* leaf litter. (A) Development of flagellate biomass in media incubated with leaf material and (B) in the control medium without leaves. (C) Course of dissolved MCHO-C concentration in media with leaf litter; (○) treatment poisoned with $HgCl_2$, (●) untreated microcosm conditions. All values are means (± 1 SE) of 3 replicate incubations except poisoned treatment (2 replicates). Line in (C) was fitted as described in the text; line for unpoisoned media fitted by eye. Error bars are shown wherever error exceeds the size of the symbol

Fig. 3. *Cymodocea nodosa*. Scanning electron micrographs of decomposing leaves. (A) Surface of recently sloughed leaves covered mainly with coccoid bacteria. (B) Few days sloughed leaf covered with an active bacterial assemblage as indicated by dividing rod shaped cells and lytic halos. (C) Leaf surface after 14 d of decomposition in litterbags. (D) 25 d incubated leaf material showing a rift in the surface densely covered with bacteria. (E) Surface-associated Protozoa (*Ploeotia sp.*; Euglenida) on leaf blades after 6 wk of decomposition. (F) Leaf surface on Day 62 with choanoflagellates (*Acanthoeca sp.*) prevailing. (G) *Diplotheca sp.* (Choanoflagellida) on Day 231. (H) Disintegrated leaf tissue with remaining cell walls and detrital material. (I) Leaf fibers left after 231 d of decomposition. (J) Close up of fibrous material scarcely colonized by microorganisms with no visible signs of disintegration. Bars indicate 1 µm in B, C, D and G, 10 µm in A, E, F and J, 100 µm in H and I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remained above 80 µg C l^{-1} until the end of the experiment (Fig. 2A). In the control medium (no addition of leaf litter), the flagellate biomass initially increased even more rapidly but reached a peak of only 60.3 µg C l^{-1} at 101 h and subsequently decreased slowly approaching 0 until 370 h after starting the experiment (Fig. 2B).

The per cell biovolumes of the 2 observed categories of nanoflagellates (monads and choanoflagellates) remained fairly constant during the course of the incubation in both the control and the leaf chamber. Mean choanoflagellate volume was 17.2 µm^3 (SD = 1.8; n = 80), average monad volume was 0.87 µm^3 (SD = 0.24, n = 95).

Leaching and uptake of monomeric carbohydrates. In the media incubated with leaf material and treated with $HgCl_2$, the leaching dynamics of MCHO-C could be followed. The concentration of MCHO-C increased from < 1 mg MCHO-C l^{-1} to a level of 5 to 6 mg MCHO-C l^{-1} between 150 and 200 h after starting the incubation (Fig. 2C). An exponential saturation curve can be fitted to this pattern which is described by the following equation:

$$y = 5.542\ (1 + e^{-0.0195x})$$
$$(r = 0.45;\ F\text{-ratio} = 106;\ p < 0.05) \quad (1)$$

where y represents the MCHO-C concentration (in mg MCHO-C l^{-1}) within the incubation medium and x is the incubation time (h). From this equation it can be calculated that 12.9 mg MCHO-C g^{-1} (leaf dry wt) were lost from the material due to abiotic leaching within 180 h. The concentrations of MCHO-C in the unpoisoned jars (containing leaf material) declined from initial values around 1 mg MCHO-C l^{-1} and remained below 0.5 mg MCHO-C l^{-1} from 77 h until the end of the incubation period (Fig 2C).

In order to obtain an estimate of the minimum microbial uptake rate of MCHO-C during the period of leaching assuming no biotic release of MCHO-C in this experiment, the values from the unpoisoned treatment were subtracted from the corresponding MCHO-C concentrations in the $HgCl_2$-poisoned incubation media. For the first 6.25 d (150 h) of decomposing *Cymodocea nodosa* leaf material, a linear correlation model gave the best fit to these data (r = 0.972; *F*-ratio = 224; p < 0.001), from which a mean uptake rate by the free-living microbial community of 31.46 µg seagrass-derived MCHO-C l^{-1} h^{-1} can be calculated.

Long-term degradation phase (0 to 231 d)

Microbial colonization and breakdown of leaf tissue. The initial colonization pattern on the surface of sloughed leaf blades exhibited a uniform coverage by morphologically similar types of attached bacteria (Fig. 3A). Most of the bacteria appeared to be in an active stage as indicated by the presence of lytic halos around the cells (Fig. 3B). The initial density of 14.3 × 10^6 cells cm^{-2} (corresponding biomass: 2.85 µg C cm^{-2}) increased to > 100 × 10^6 cells cm^{-2} (corresponding biomass: > 20 µg C cm^{-2}) within 2 wk (Fig. 4). At this time the bacterial assemblage has changed to a more heterogenous community with rod-shaped and filamentous bacteria prevailing (Fig. 3C). After 25 d the average abundance of bacteria had declined to values close to initial densities (Fig. 4). When compared to the early stage of colonization, a much more patchy distribution of cells could be observed with copious clusters of bacteria around crevices in the leaf surfaces at this stage (Fig. 3D). Following a second much less pronounced peak 6 wk after starting the experiment, the abundance of attached bacteria dropped below the initial values and remained at this level until the end of the observation period.

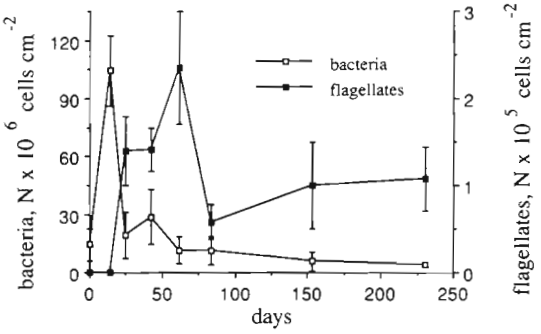

Fig. 4. Development of the microbial community attached to decomposing *Cymodocea nodosa* leaves. Data points represent means (± 1 SE) of 30 counted fields examined by SEM. Note different scales for *Y*-axes

Concurrently with the decline of bacteria between Day 14 and Day 62, the leaf-surface-associated protozoan community increased to a mean cell density of 2.35 × 10^5 cm^{-2} (corresponding biomass: 1.55 µg C cm^{-2}) (Fig. 4). In the early phase of protozoan colonization, monads of various sizes prevailed (Fig. 3E), whereas in the later stage (ca 60 d after starting the experiment to the end of the observation period) choanoflagellates appeared to clearly dominate the scenario (Fig. 3F, G). Following the peak density 2 mo after starting the incubation, protozoan abundance dropped and remained at a level of around 10^5 cells cm^{-2}.

The second part of this 8 mo decomposition experiment was characterized by the refractory nature of the remaining plant material. The non-structural leaf tissue was largely degraded, leaving only cell walls, cuticular remnants and fibers as slowly degradable structural polymers (Fig. 3H). Whereas cell walls started to break down after 5 mo of decomposition, the fibrous material appeared to be almost unaffected by microbial degra-

dation even after 7 to 8 mo (Fig. 3I, J). The surface of these fibers was only moderately colonized by microbes.

Loss of dry weight, organic carbon and nitrogen. The time courses of dry weight, organic carbon and nitrogen loss from litterbags filled with *Cymodocea nodosa* leaf material are presented in Fig. 5. The first part of the degradation process was characterized by a rapid loss of dry weight and organic C, lasting for ca 2 mo. The second part can be described as a phase of slower decomposition of more refractory material (Fig. 5A, B). The observed N loss differed from this pattern since a slight increase in percent remaining N was observed between Day 14 and Day 25 (Fig. 5C). After a period of 231 d around 50 % of the original dry weight and organic C was already degraded or disappeared from litterbags whereas 67 % of the original N was remaining. Estimates of the decay rates obtained by simple linear models, established separately for the initial and the advanced phase (all regressions are highly significant, $p < 0.0025$), revealed that during the first 62 d 0.48 % of dry wt and 0.53 % of the original organic C were lost per day. Between Day 62 and Day 231 only 0.11 % dry wt d^{-1} (0.10 % original organic C d^{-1}) were lost from leaf litter.

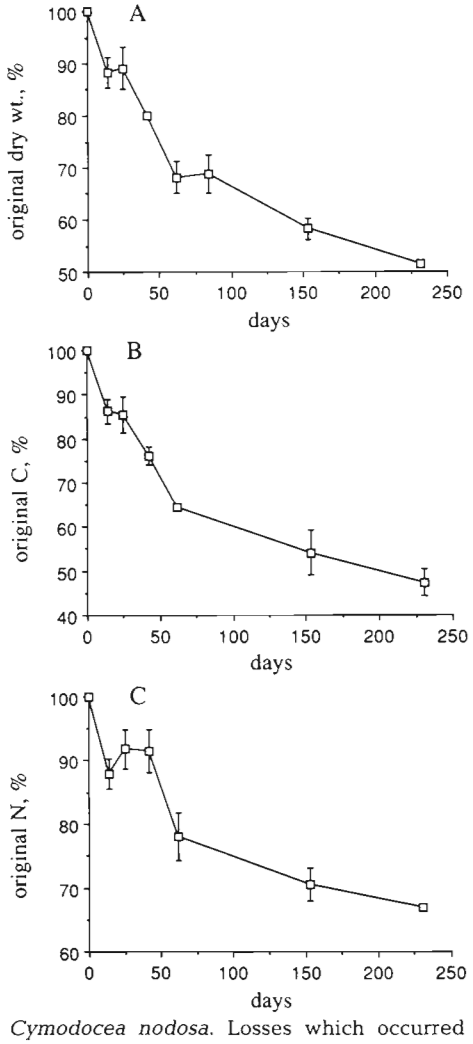

Fig. 5. *Cymodocea nodosa*. Losses which occurred during decomposition of leaf material. Percent of initial (A) weight, (B) organic carbon and (C) nitrogen remaining in litterbags. Data are means (± 1 SE, n = 4); error bars shown wherever exceeding size of symbols

Changes in leaf carbon and nitrogen. The organic C and N contents of the leaf detritus changed predictably during the degradation process (Fig. 6). The percent organic C in leaves decreased rapidly within the first 25 d, thereafter declining at a much lower rate to a final concentration of 46.7 % of dry wt after 231 d. In contrast, the N content increased with time from 4.25 to 5.54 % of dry wt (Fig. 6A). During the first 42 d the N increase was most pronounced. Correspondingly, the C:N ratio of remaining plant tissue decreased as decomposition proceeded (Fig. 6B).

O_2 consumption and C remineralization of leaf material. The course of the respiration rates associated with decaying *Cymodocea nodosa* leaf material in 0.2 µm filtered seawater is shown in Fig. 7. The hourly O_2 consumption and the corresponding C remineralization rates per g dry wt of leaf tissue increased until Day 25 from 0.23 to 0.39 mg O_2 (in terms of C from 70 to 119 µg C). Subsequently the rates decreased continuously approaching the initial level 150 d after starting the experiment and declining even below this initial level towards the end of the incubation.

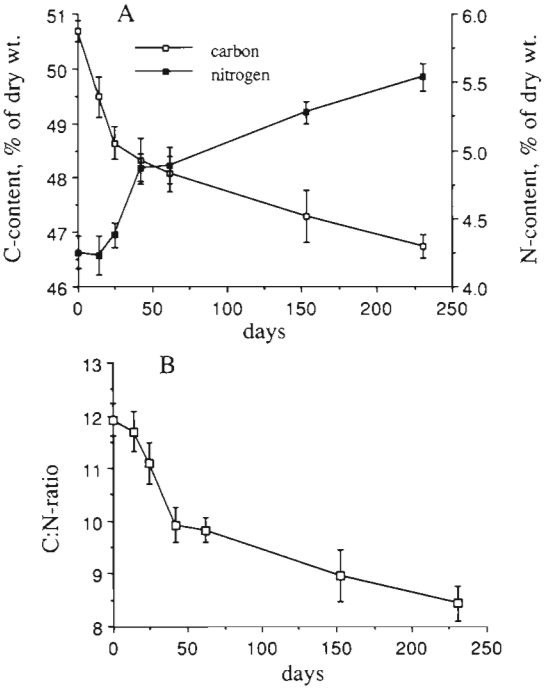

Fig. 6. *Cymodocea nodosa*. (A) Organic carbon and nitrogen concentration (% of dry wt) of decaying leaves; (B) changes in C:N ratios during decomposition. Values represent means (± 1 SE) of 6 to 10 determinations

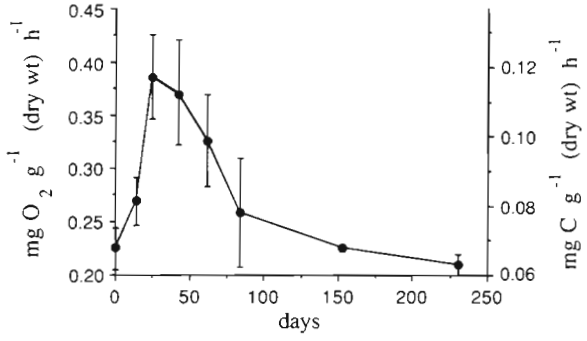

Fig. 7. *Cymodocea nodosa*. Oxygen consumption and carbon remineralization rates of decomposing leaf litter incubated in 0.2 µm double-filtered seawater. Each data point represents the mean (± 1 SE) of 4 replicate measurements

DISCUSSION

Initial phase of decomposition

Our short-term incubation experiments (duration: 3.2 and 15.4 d) clearly demonstrated that senescent and sloughed *Cymodocea nodosa* leaf material supports a microheterotrophic food web in the initial phase of leaching and decomposition. Since the concentrations of soluble carbohydrates in *C. nodosa* leaves are reported to be at least 8 % of dry wt (Pirc 1989) or even higher (Drew 1983), a substantial transfer of readily utilizable plant leachates to the ambient microbial community (i.e. surface-attached and free-living) can be expected. The observed decline of MCHO-C in the incubation medium associated with the peak in bacterial biomass strongly indicates a rapid uptake of this carbon source. This is in agreement with e.g. Benner & Hodson (1985) who stated that the bulk of the organic matter leaching from leaf material is rapidly utilized by microbes. Despite the fact that bacterial secondary production (BSP) based on biomass increments in batch cultures might be underestimated and not reflect in situ conditions, BSP at the expense of leaf-derived material within the incubation jars was calculated as 97.22 µg of bacterial C l^{-1} d^{-1} for the early phase of plant degradation (0 to 39 h). This value is well within the range of BSP supported by vascular plant detritus as reported recently by Moran & Hodson (1989a).

During the first 33 h, bacterial predators were reduced efficiently by 1.0 µm filtration (see Fig. 2A). The decline of the free-living bacterial population in our experiments after 39 h seems to be most likely due to enhanced nanoflagellate predation as indicated by the concurrent increase in flagellate biomass (see Figs. 1A & 2A). In the later stage of leaching the organic substrate appeared to be limited. After 100 h flagellate biomass started to decline in the control medium dropping back to values close to the initial level. Flagellate predators were never observed in the control medium, therefore the decrease in flagellate biomass could not be caused by grazing pressure. On the other hand, in the media enriched with leaf material there was an pronounced increase in the heterotrophic nanoflagellate community in the surrounding water. From this data it can be estimated that after a 14 d incubation period a free-living flagellate standing stock of 210 µg C per g (leaf dry wt) was still living at the expense of decomposing *Cymodocea nodosa* leaf material (either via bacterial grazing or direct utilization of leaf derived matter).

Soluble, non-lignocellulosic components of vascular plant detritus such as monomeric carbohydrates are thought to be lost largely through abiotic processes (Moran et al. 1989). Losses of these substances are reported to occur during the first 2 wk of incubation (Valiela et al. 1985, Moran et al. 1989) which was consistent with our observations regarding MCHO-dynamics. From our leaching equation (Eq. 1), and from the calculated microbial uptake rate of MCHO-C, it can be calculated that 92.3 % of this organic nutrient species is taken up within 176 h. The remaining 7.7 % either cannot be taken up or, more likely, might be due to release of monomeric carbohydrates by microorganisms. Monomeric carbohydrates could be due to the activity of extracellular enzymes from attached bacteria in the unpoisoned treatment (Melillo et al. 1984), thus maintaining a certain level of dissolved MCHO-C in the incubation media originating from insoluble fractions of the seagrass detritus rather than the leachable portion.

Microbial colonization and degradation of leaf litter

Bacterial abundance on decaying *Cymodocea nodosa* leaf material was similar to values reported for other seagrass and mangrove leaf debris (Newell 1981, Kirchman et al. 1984, Benner et al. 1988). Our observed high initial density of bacteria on recently sloughed leaves suggests that a basic stock of microorganisms is present also on senescent but still plant-attached leaves; this is in agreement with results reported by Novak (1984), who determined up to 20×10^6 cells cm^{-2} on living *Posidonia oceanica* leaf blades in the Mediterranean Sea. The increase in bacterial density on decomposing *C. nodosa* leaves within the first 2 wk might be explained by both the attachment of free-living bacteria to the surface and by cell division of attached bacteria on leaf blades. However, in our experiments, the observed shift to a more heterogenous bacterial assemblage at peak density might be indicative also for enhanced colonization. Moreover, Benner

et al. (1988) suggested that bacterial colonization can be important to the overall bacterial abundance on leaf surfaces during the early stages of decomposition.

The formation of bacterial clusters near crevices after more than 3 wk of decomposition, as observed by SEM, strongly indicates invasion of microbes into the plant tissue via mechanically or enzymatically created holes (note lytic halos in Fig. 3b). Therefore our observed bacterial abundances from Day 25 until the end of the experiment are probably underestimates, since they refer to cells attached to the outer surface only. The decrease in surface-attached bacterial biomass concurrently with the increase of protozoan abundance cannot simply be interpreted as a direct trophic interaction between the 2 groups. These fluctuations took place during a period of ca 2 mo and are hence more likely the result of a complex pattern of colonization, production, grazing and detachment processes. Therefore it is impossible to calculate grazing rates and conversion efficiencies from these data. Nevertheless our observations indicate that a rich and diverse microbial community is present on *Cymodocea nodosa* leaf surfaces during the first 2 mo of decomposition. The breakdown of the attached microbial biomass coincides with a shift to slower loss rates of weight and organic carbon from the litter bags (see Figs. 4 & 5). After leaching of water-soluble material (such as monomeric carbohydrates) during the first 2 wk and following a phase of high biomass of attached microbes, rates of losses from the remaining fibrous portion between Day 62 and Day 231 are ca 5 times lower than the initial rates. This is in agreement with weight loss dynamics reported by other authors investigating degradation of vascular plant material (Benner & Hodson 1985 and references therein, Valiela et al. 1985, Robertson 1988, Moran et al. 1989).

The changes in the percentage of the original content of nitrogen remaining in leaves as observed in our study followed a pattern previously found for various other decomposing macrophyte materials (Rice & Tenore 1981, Valiela et al. 1985, Robertson 1988, Moran & Hodson 1989a, Moran et al. 1989). Even at high bacterial densities on leaf material, bacterial nitrogen usually contributes $< 5\%$ of the nitrogen concentration of decomposing leaves (Christian & Wetzel 1978, Marsh & Odum 1979, Rice & Hanson 1984, Robertson 1988). There is some evidence in the literature that at least a part of the frequently observed increase in total detrital nitrogen during the decay of marine macrophytes might be due to the production of bacterial mucopolysaccharide exudates (Hobbie & Lee 1980) which may be incorporated in humic macromolecules associated with the detritus (Rice & Hanson 1984). The relatively low C:N ratios of *Cymodocea nodosa* leaf material as determined in the present work are confirmed by Pirc & Wollenweber (1988), who also measured low C:N ratios in *C. nodosa* and *Zostera noltii* leaf material. This high nitrogen concentration suggests that *C. nodosa* leaf material should be more susceptible to microbial degradation than most other aquatic vascular plants (Robertson 1988, Moran & Hodson 1989a). Our results support this idea since the decomposition rates of *C. nodosa* in this study can be considered as high, as they were obtained by excluding mechanical degradation due to physical forces or shredding activity.

The observed fluctuations in O_2 consumption associated with decomposing *Cymodocea nodosa* leaf litter indicate elevated activity of the attached microbial community between Day 25 and Day 62 of the incubation. After reaching a peak at Day 25, mineralization rates started to decline thereafter. Concurrently with the decline in mineralization rates the rate of decrease in organic C-content of the leaf material is retarded (see Figs. 6A & 7). The observed decrease in particulate organic carbon content during the first 25 d of decay accounts at maximum for 40% of the measured mineralization rate due to the respiratory activity of surface-attached microbes. In the later period of decomposition (Day 25 to Day 231) only about 4% of the respiratory remineralization can be attributed to the decreasing organic C concentration in leaf material. Therefore the total loss of originally available leaf-derived particulate organic carbon within the experimental system could match the observed remineralization rates by the attached microbes only via an additional pathway. It is suggested that a substantial fraction of the initially available carbon is released into the water column instead of being taken up directly by the attached microorganisms. There is evidence for the release of solubilized material even during latter stages of plant decay (Moran & Hodson 1989b) and it can be assumed that attached bacteria hydrolyse more organic matter than they are able to take up immediately thus exhibiting a loose hydrolysis-uptake coupling (Azam & Cho 1987, Hoppe 1989). The hydrolysed organic matter then becomes available to free-living bacteria as demonstrated recently with animal-derived detritus (Peduzzi & Herndl unpubl.); this DOM may be further hydrolysed by free-living bacteria and finally taken up by both free-living and attached microbes. On the other hand Brophy & Carlson (1989) have shown that free-living bacteria can transform DOM in more refractory components which they are unable to degrade. Attached bacteria might be suited better to utilize this transformed DOM due to differences in the enzyme expression compared to free-living bacteria (Hoppe 1989, Karner & Herndl unpubl.).

In summary the following conclusions can be drawn from our experiments:

(1) During the initial phase of decomposition of *Cymodocea nodosa* leaf litter, free-living bacterial growth is enhanced at the expense of easily utilizable material leaching out of the leaves. Based on the estimated uptake rate and the increase in free-living bacterial carbon over the initial 39 h growth phase of bacteria, a net conversion efficiency of seagrass leaf-derived MCHO-C into bacterial carbon of 12.9% was calculated assuming only the free-living population is significantly taking up this compound. Benner & Hodson (1985) report microbial conversions of the leachable portion of mangrove leaves of about 30 to 36%; our calculated conversion seems to be somewhat low and would be even lower if other fractions of the leachate are used, as can be expected. Therefore, even if the calculated bacterial cell production based on changes in numbers and consequently the conversion efficiency might be conservative, our results from the leaching phase strongly suggest that at least a part of the leaf-attached bacterial fraction contributes significantly to the overall utilization of low molecular weight leachates during the initial course of decomposition. Although we have no information about the productivity of attached bacteria during the first 40 h, the high initial bacterial biomass on leaf surfaces (2 to 3 times the entire free-living fraction within the jars; compare also Robertson et al. 1982) supports the above explanation. Moreover, the composition of the bacterial community on recently sloughed leaves can be assumed to be similar to the epiphytic bacterial population on leaves that are senescent but still plant-attached and living. These bacteria are reported to be capable of utilizing seagrass exudates and leachates (Kirchman et al. 1984).

After 2 wk of incubation, a free-living protozoan community is still living at the expense of seagrass leaf debris via grazing on bacteria or even direct utilization of high molecular weight dissolved organic matter (Sherr 1988).

(2) Following the initial leaching phase, we have shown that it is unlikely that the more residual fraction of the seagrass material is utilized exclusively by attached microbes via a tight hydrolysis-uptake coupling. The observed C remineralization rates by the attached microbial community, as determined by O_2 consumption measurements, imply an additional utilization of DOM released previously from the plant material into the surrounding water body. Furthermore, in the final phase of decomposition, most of the observed respiratory activity might be mediated by microorganisms using this material mainly as an edaphic substrate.

(3) Exclusion of shredders did not prevent the substantial weight loss and decay of *Cymodocea nodosa* leaf debris during the experimental period. This is consistent with reports by Valiela et al. (1985) who stated that activity of invertebrates produces only a small increase in the decay rate. Nevertheless, these organisms might be of substantial importance for further fragmentation and decomposition of the remaining fibrous fraction.

(4) Based on production measurements conducted by Peduzzi & Vukovič (1990) in the Gulf of Trieste, an average of 250 g C of *Cymodocea nodosa* leaf material is produced per m^2 (seagrass meadow) per year. Pelagic primary production in this area has been reported to amount to ca 42 g C m^{-2} yr^{-1} (Faganeli et al. 1981). As an example, in the Bay of Piran – which is a part of the Gulf of Trieste with an area of ca 25 km^2, 1 km^2 covered with *C. nodosa* (Peduzzi & Vukovič 1990) – seagrass leaf material would roughly account for 20% of the total (i.e. benthic and pelagic) annual primary production. Therefore we suggest that in the early phase of decomposition leaf debris can have a moderate, albeit significant, influence on the overall microbial secondary production in this area. The role of the more recalcitrant residual portion of the material for the microbial food web seems to be small, which was also found quite recently for mangrove litter (Alongi et al. 1989), but may be of some importance over a longer period of time. From our study, the importance of a more detailed investigation of the components of DOM, derived from various decomposing macrophyte detritus, and its role in short-term dynamics and small scale patchiness of microbial utilization becomes evident.

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