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Criticism of the litterbag technique for the study of aquatic plant decay: Suppression of epiphytic algal biomass

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With 2 figures and 1 table

Abstract: Recent studies have suggested thiat autotrophs (cyanobacteria and eukaryotic algae) attached to emergent plant detritus are important to the decay of that detritus; however, the litterbag methodology commonly employed for study of plant decay may inhibit epiphytic autotrophs. Thus, the purpose of this study was to examine the change in epiphytic algal biomass on *Typha latifolia* litter in four types of litter enclosures. The enclosures were designed to provide various combinations of light intensity and invertebrate access to the surface of enclosed *Typha* litter. Within the enclosures, algal biomass (as chlorophyll-a) on the litter and rate of *Typha* decay were examined over 133 days (May–October, 1994). Over the entire study, average chlorophyll concentrations on litter incubated in enclosures constructed of fine mesh ranged from 0.41 ± 0.04 to $0.23 \pm 0.04\,\mu g\,cm^{-2}$ of detritus, relative to values of 0.63 ± 0.07 to $0.75 \pm 0.07\,\mu g\,cm^{-2}$ on detritus incubated in coarse-mesh enclosures or open, floating enclosures. The results clearly indicated that autotrophic biomass on *Typha* litter was inhibited by litter enclosures of fine mesh; consequently, previous studies of aquatic plant decay may have missed contributions to decay processes by this important component of the detrital community.

Introduction

The use of litterbags for the study of plant decay in both terrestrial and aquatic systems has been previously criticized for a variety of reasons (WIEDER & LANG 1982, BOULTON & BOON 1991, JACKSON et al. 1995). Enclosure of plant detritus in litterbags reduces microbial activity, affects invertebrate access to

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plant material, and modifies the conditions under which plant material decays by altering water flow regimes, chemical conditions, light intensity, and litter position. Thus, most comparisons of litterbag types show that plant tissues decay more quickly in litterbags constructed of larger mesh size as opposed to smaller mesh size (e.g., PIDGEON & CAIRNS 1981, MUTCH & DAVIES 1984, PETERSEN 1984). These comparisons, however, may be confounded by the presence of invertebrates, which may also increase rates of litter decay (e.g., COULSON & BUTTERFIELD 1978, ELWOOD et al. 1981).

Litterbags may also influence the development of epiphytic algal communities on enclosed plant detritus. In general, algae attached to plant detritus have been ignored as significant contributors to plant decay processes. Attached algae can stimulate bacterial productivity in periphyton (NEELY & WETZEL 1995) and may accelerate the decay of emergent plant material (NEELY 1994). However, algal roles may have been obscured in most decay studies by the use of litterbags. Thus, we employed four litterbag designs, varying in mesh size and construction, to test the hypothesis that confinement of litter in bags alters autotroph biomass in epiphyton attached to emergent plant detritus while controlling for the confounding effects of invertebrates.

Methods

Four types of litter enclosures, each containing approximately 2.1–2.2 gms of standing dead, *Typha latifolia* detritus collected during March, 1994, were used in this experiment. The enclosures consisted of: (1) rectangular litterbags constructed of 1 mm^2 fiberglass mesh (fine-mesh treatment); (2) rectangular litterbags with mesh openings of 19×25 mm (coarse-mesh treatment); (3) tube-shaped enclosures with fine fiberglass mesh covering the periphery of the tube and coarse-mesh covering the ends of the tube (described herein as the invertebrate-accessible treatment); and, (4) floating, fine mesh baskets in which litter was contained (open treatment, litter placed in the open basket). All litter enclosures were approximately 17.5×12 cm in dimension, with the exception of the open enclosures, which were 10.5 cm tall and circular with a diameter of 15 cm. The invertebrate-accessible enclosures were placed horizontally in the water column, allowing invertebrates to access the litter from the ends of the enclosure while suppressing algal productivity by sunlight attenuation.

In combination, these treatments represent a factorial arrangement of light suppression and invertebrate activity within the litterbags. The coarse mesh bags minimized attenuation of sunlight and allowed invertebrates to access the litter, in contrast to the fine mesh bags, which were intended to reduce both light intensity and invertebrate activity within the bags. The tube-shaped enclosures allowed invertebrate access from the ends of the tube, but the fine mesh around the length of the tube reduced light intensity. The open enclosures were intended to exclude swimming macroinvertebrates from the litter without attenuating sunlight. Buoys were attached to the sides of these

open enclosures, which allowed the litter to settle to the bottom of the enclosure but prevented the top of the enclosure from sinking below the water surface.

The field study, conducted during the 1994 growing season (May–September) in Willow and Rash Ponds at The University of Michigan, consisted of 160 enclosures for each of the four treatments, i.e., 640 enclosures were made and placed in two field sites, each with a randomized block design of four subsites. *Typha latifolia* occurs naturally along the shoreline of each of these ponds; all enclosures were placed at the interface of the *Typha* stands and the open water. Water depth in each pond at the site of enclosure placement was approximately 0.5–0.8 m. Water clarity was high with turbidity levels consistently below 2.5 nephelometric turbidity units (SCHNITZER 1995). Because of the water clarity, dense mats of *Chara* sp. were present. Presumably as a result of the *Chara,* oxygen concentrations were often above saturation levels (SCHNITZER 1995).

On ten sampling dates, sixteen samples of each enclosure type were removed from the field (two of each enclosure type per subsite). From each subsite, one enclosure of each type was used for estimating algal biomass, while the second enclosure was used to estimate *T. latifolia* decay. Epiphytic algal biomass was assessed by the mass of chlorophyll-a per cm^2 of detrital surface. Chlorophyll-a was extracted by homogenizing two disks (13 mm diameter – extracted with a cork borer) in a 5 ml solution of 1 : 1 dimethyl sulfoxide (DMSO) and 90 % acetone, alkalized with 5 drops per liter of a saturated $MgCO_3$ solution according to the methods of FALLON et al. (1985) and PALUMBO et al. (1987). Chlorophyll-a was measured using a Sequoia-Turner Model 450-003 spectrofluorometer equipped with a NB440 excitation filter and a SC665 emission filter. Fluorescence measurements were calibrated to chlorophyll mass by using pure spinach chlorophyll-a (Sigma C-5753). Decomposition was quantified as the percentage of dry weight remaining on each sampling date relative to the initial *T. latifolia* litter weight. The enclosures of the open-treatment baskets ultimately could not be used for estimates of plant decay because of litter loss through the season. Litter samples were gently washed in deionized water to remove any epiphytes and other debris remaining on the litter, and dried at 105 °C for a minimum of 24 h.

A nested analysis of variance (ANOVA) was used to compare chlorophyll regimes and the mass of cattail material remaining in litter bags (SAS 1985). The ANOVA was modelled to compare differences among enclosure treatments, between the two wetlands, between subsites nested within the wetlands and the interactions of these parameters (Table 1). The percentage of *T. latifolia* detrital weight remaining was arcsin transformed prior to the analysis. Tukey's multiple range test was used for pairwise comparisons between the four enclosure treatments. Statistical significance was evaluated at the 0.05 confidence level.

Results and discussion

The type of litter enclosure significantly affected the average biomass of epiphytic autotrophs with greater colonization in the coarse-mesh and open bag types (Fig. 1). On the basis of the ANOVA (Table 1), significant differences in

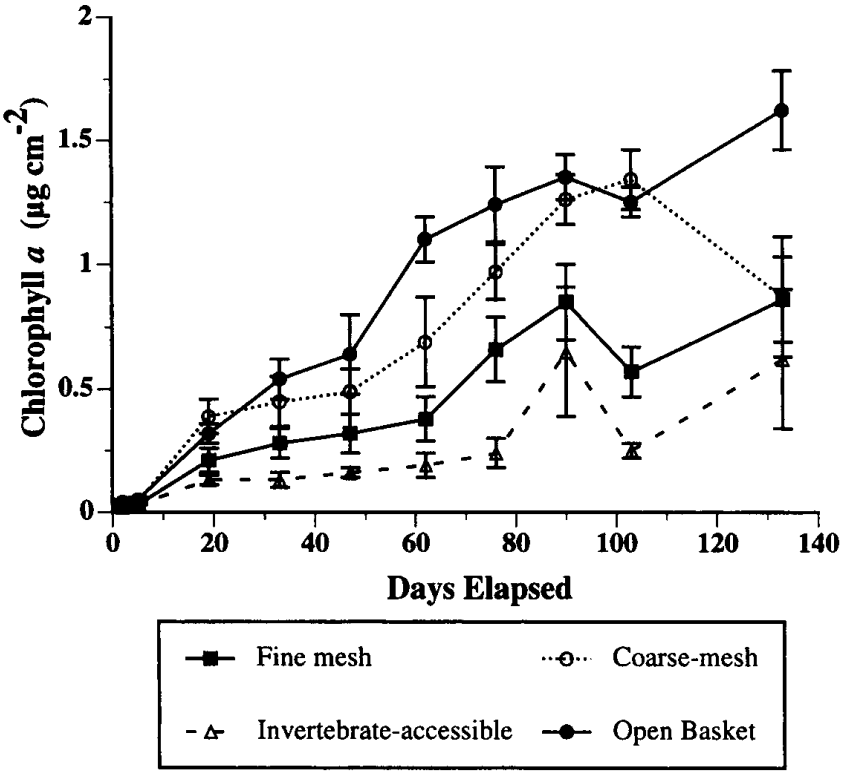

Fig. 1. Average chlorophyll-a concentration from epiphyton attached to *Typha latifolia* litter in each of four litter treatments from May to October, 1994 (± SE; n=8).

Table 1. ANOVA of chlorophyll in detrital epiphyton and percentage weight remaining (arcsin transformed) for decaying *T. latifolia* litter subjected to four litter enclosure treatments at two sample sites for 133 days.

Treatment	Chlorophyll-a		% Weight remaining	
	d.f	F	d.f	F
Time	9	47.9*	9	238.26*
Enclosure	3	53.4*	2	1.55
Wetland	1	0.24	1	5.40
Subsite (within Pond)	6	1.32	6	0.93
Time×Enclosure	27	3.65*	18	1.42
Time×Wetland	9	2.03*	9	1.35
Enclosure×Wetland	3	0.89	2	0.11
Time×Enclosure×Wetland	27	0.89	18	0.85

* $P<0.05$.

average chlorophyll-a mass occurred because of enclosure type, but did not vary between wetland sites or subsites. Because only differences between the treatments were statistically significant, data from the two field sites were pooled in Fig. 1. The long term pattern of difference in chlorophyll mass de-

veloped slowly among the treatments, requiring about 35 days (Fig. 1). SOBCZAK (1996) also reported similar results in a study of the effect of algal biomass and dissolved organic carbon on epilithic bacteria, in which shading reduced chlorophyll-a regimes, but only after sufficient time for development of treatment differences. Delayed patterns of difference are clearly to be expected in studies involving the development of periphyton communities under various treatment regimes.

The rank order of average seasonal chlorophyll mass from the detrital epiphyton among the treatments was open-basket enclosures > coarse-mesh enclosures > fine-mesh enclosures > invertebrate-accessible enclosures (Fig. 1). With the exception of the difference in chlorophyll regimes between the open-baskets and the coarse mesh enclosures, Tukey's multiple range test indicated that all other treatments were statistically different from one another. Thus, chlorophyll regimes were not affected by coarse mesh, relative to unenclosed litter in the baskets; however, the fine mesh enclosures clearly suppressed either the density, biovolume or chlorophyll production of the autotrophic component of the epiphyton.

In general, estimates of chlorophyll-a biomass in this study were within the range of values previously reported for epiphyton. In an analysis of the influence of light on epiphytes of *T. latifolia,* NEELY & WETZEL (1997) reported an average chlorophyll-a biomass of approximately 0.45 $\mu g/cm^2$, which is very close to the overall average of 0.51 $\mu g/cm^2$ found in this study. FALLON et al. (1985) reported a chlorophyll-a biomass approximately three times lower than values reported in this study, but BURKHOLDER & WETZEL (1989) found values more than twice those of this study. Although chlorophyll mass is an often used indicator of aquatic autotroph biomass (AXLER & OWEN 1994), the phenomenon of shade adaptation can affect the accuracy of this index. Low light intensity often results in increased chlorophyll concentrations (GARCIA & PURDIE 1992, VELDHUIS & KRAAY 1993), e.g., as much as 25-fold in cyanobacteria (VELDHUIS & KRAAY 1993). Adaptation to lower light intensities varies among taxa, but the response is common for marine and freshwater phytoplankton, periphyton, and submersed aquatic macrophytes. Thus, the effect of litter enclosure type on detrital algal biomass may even be larger than reported here because the fine mesh bags attenuated the most light, but still had the lowest chlorophyll regimes.

Although macroinvertebrate herbivores can reduce periphyton standing crop and community composition (ALLEN 1995), differences in chlorophyll among the four enclosure types in this study cannot be explained by differences in macroinvertebrate activity within each enclosure treatment. If grazing had been the primary determinant of chlorophyll regimes among the various enclosure treatments, then detrital chlorophyll regimes should have been similar between the coarse-mesh and the invertebrate-accessible enclosures.

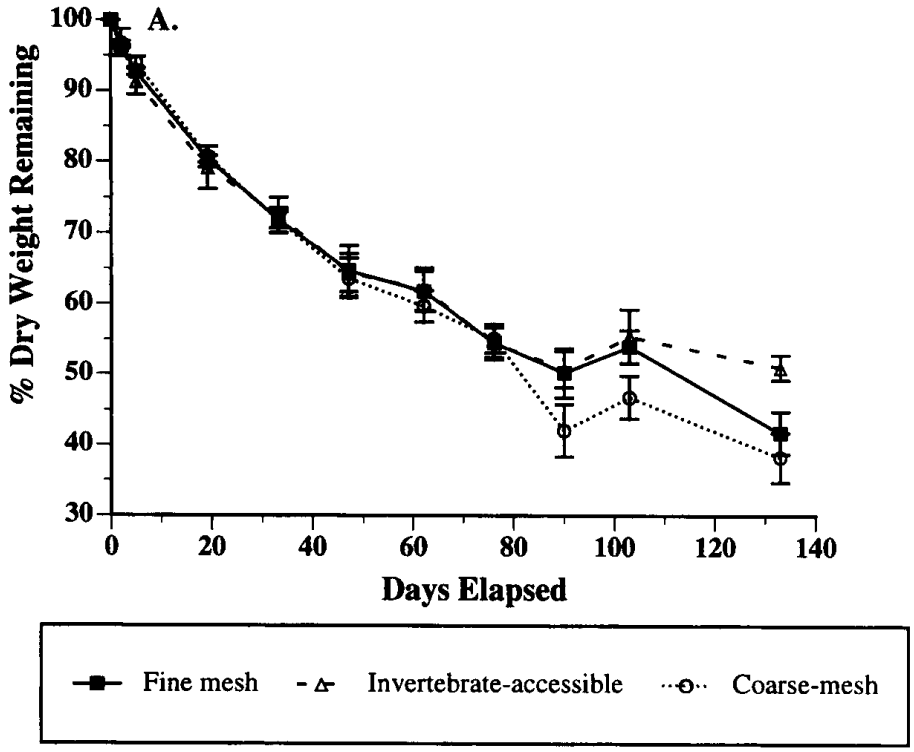

Fig. 2. Average percentage of *Typha latifolia* litter weight remaining in each of three litter enclosures from May to October, 1994 (± SE; n=8).

With the exception of the invertebrate-accessible enclosures, plant decomposition in this study was quite rapid and in marked contrast to rates reported in other studies. Half-lives for *Typha* decay are typically about 300 days or more (e.g., DAVIS & VAN DER VALK 1978, MORRIS & LAJTHA 1986, NEELY & BAKER 1989). In this study, however, 38.21 ± 3.53, 41.70 ± 3.04, and 50.95 ± 1.78 % of *Typha* dry weight remained in the coarse-mesh, fine-mesh, and invertebrate-accessible enclosures, respectively, after 133 days, (Fig. 2). The relatively high rates of decay may have been related to two factors: (1) high initial concentrations of nitrogen (1.42 %) and phosphorus (0.15 %) in the *T. latifolia* litter and (2) high oxygen regimes at the study sites (often supersaturated – see SCHNITZER 1995). Numerous studies have related rates of aquatic plant decay to both tissue concentrations of N and P, as well as dissolved oxygen concentrations in the bulk water (e.g., NEELY & BAKER 1989).

Despite the difference in chlorophyll regimes among the enclosure treatments, no clear relationship was observed between enclosure type and the rate of plant decay, or enclosure type and the biomass of attached epiphytic algae. Fig. 2 does indicate that the rate of decay among the treatments was beginning to diverge after 80 days of incubation, with the coarse mesh showing the greatest final rate of decay; however, these final differences were not signifi-

cant (Table 1). Under the duration and field conditions of this experiment, increased algal biomass evidently did not stimulate decay of *T. latifolia*.

In conclusion, litterbag type obviously alters the biomass of epiphytic algae attached to decaying emergent plant detritus. This alteration likely results from both changes in algal density and taxonomic composition of epiphytic algal assemblage. The extent to which these algal assemblages affect plant decay is not clear. Regardless, it is evident that detritus represents an important site for epiphyton development.

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