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Microbial biomass and production associated with decaying leaf litter of the emergent macrophyte *Juncus effusus*

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Abstract

Emergent macrophytes are a major source of organic matter production in freshwater wetlands, and often represent the bulk of the plant material entering the detrital pool. We examined the decomposition and microbial dynamics associated with litter of the emergent macrophyte, *Juncus effusus* (soft rush), during its movement from an aerial standing dead to a submerged decay environment. Standing dead leaves of *J. effusus* were collected after an initial period of standing litter microbial decay, placed in 1-mm mesh litter bags, and submerged in a wetland. Litter bags were retrieved periodically over 268 d and analyzed for microbial (fungal and bacterial) biomass and production, ATP concentrations, litter mass loss, and quality (C : N : P and plant fiber). Submerged litter decay of *J. effusus* was slow ($k = 0.0010\ d^{-1}$), with only 23% weight loss after 268 d. Both fungal and bacterial biomass and production decreased significantly soon after standing plant litter was submerged in the wetland surface waters. Despite decreases in microbial biomass and production, fungal decomposers remained the dominant microbial assemblage associated with decaying plant litter, accounting for 99% and 91% of the total microbial biomass and production, respectively. Mean fungal production ranged from 73–2,836 $\mu g\ C\ g^{-1}$ AFDM d^{-1} (AFDM: ash-free dry mass remaining) during the study period, whereas attached bacterial production ranged from 4–32 $\mu g\ C\ g^{-1}$ AFDM d^{-1}. Patterns of litter ATP and nutrient concentrations (N and P) were similar to those observed for fungal and bacterial biomass, suggesting that at least a portion of the detrital N and P may have been incorporated into microbial biomass. Significant changes in microbial colonization and activity associated with emergent macrophyte litter can occur following the collapse of standing dead plant matter to the water or surface sediments. Furthermore, our findings suggest that fungi are significant contributors to the decay of coarse particulate plant matter in wetland ecosystems.

In freshwater wetlands, emergent macrophytes frequently constitute a major fraction of organic matter production, with annual net above ground primary production often exceeding 2,000 g m^{-2} yr^{-1} (Wetzel 1990; Gessner et al. 1996; Wetzel and Howe 1999). The utilization of living plant matter by animal consumers as a food resource is considered negligible in most wetlands, since much of the carbon within macrophyte tissues resides in the recalcitrant cell wall structural materials (i.e., lignocellulose) which is not easily digested and assimilated (e.g., Mann 1988). Most plant matter eventually enters the detrital pool, where microorganisms, both bacteria and fungi, play an important role in litter breakdown and mineralization. Therefore, understanding the fate of vascular plant detritus and the microbial processes involved during litter decay are important aspects in elucidating energy flow and nutrient cycling within these ecosystems (Wetzel 1990).

Important details to consider when examining the decomposition of emergent macrophytes are the spatial and temporal conditions under which plant litter decomposes. In the case of emergent macrophytes, abscission and collapse of shoot material to the sediment surface does not occur immediately following shoot senescence and death. This results in the accumulation of standing dead plant litter (Christian et al. 1990; Findlay et al. 1990; Wetzel and Howe 1999), which undergoes considerable initial microbial decay prior to its collapse into the aquatic environment (Newell 1993, 1996; Bärlocher and Biddiscombe 1996; Kuehn and Suberkropp 1998*a,b*; Kuehn et al. 1998, 1999).

Newell et al. (1995), by using methods to assess rates of fungal biomass production (Newell and Fallon 1991; Gessner and Newell 1997), reported that microbial biomass and production associated with naturally standing and fallen litter of the freshwater sedge, *Carex walteriana,* was dominated by fungal decomposers, with bacterial biomass and production increasing only slightly after standing litter fell to the sediment surface. These results contrast sharply with previous studies examining the microbial participation in emergent macrophyte decay (e.g., Benner et al. 1986; Moran et al. 1988; Mann 1988), and suggest that fungi may play a more important role in emergent macrophyte decomposition in wetland habitats than had been previously documented or recognized.

Juncus effusus is a rhizomatous perennial rush that forms large dense circular tussocks containing several thousands of emergent leaves, both living and dead, and in various stages

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of senescence and decay. In the wetland ecosystem examined in the present study, rates of annual above-ground production of *J. effusus* are quite high, ranging between 4.4–10.3 kg AFDM m^{-2} (mean 6.9) (AFDM: ash-free dry mass remaining), with maximum leaf shoot growth occurring in the late spring and early summer (Wetzel and Howe 1999). Observations reveal that very little (<1%) of the *J. effusus* plant biomass produced is consumed via herbivory (Wetzel and Howe 1999), indicating that the bulk of the biomass eventually enters the detrital pool following senescence and death. Following senescence, leaves initially remain in a standing dead position, eventually fragmenting and falling over to the overlying surface waters and surface sediments. Earlier studies reported that *J. effusus* litter is actively colonized and degraded by fungal decomposers in the standing dead position, suggesting that considerable microbial decomposition of plant litter can occur prior to its entry into the aquatic environment (Kuehn and Suberkropp 1998*a,b*). The present study was conducted to examine the additional phases in the decomposition of *J. effusus* litter. Specifically, we examined litter mass loss, nutrient contents, plant fiber composition, ATP concentrations, and microbial biomass and production associated with *J. effusus* litter after its movement from an aerial standing dead to a submerged decay phase.

Methods

Field procedures—This study was conducted in a small, freshwater wetland ecosystem located in Hale County, Alabama (32°54′30″ N, 87°26′30″ W). Prior to the collection of standing litter for submerged litter bags, studies were conducted to estimate the level of fungal biomass and rates of fungal and bacterial production associated with both standing and submerged plant litter of unknown age (*see below*). These studies were performed on a single sampling date (mid-June) from three randomly selected replicate plant tussocks. Following these initial studies, standing dead leaves of *J. effusus* were collected, returned to the laboratory, air dried, and cut into 18-cm long pieces. Air drying of leaves did not result in an increased loss of inhabitant fungal biomass (i.e., ergosterol) in standing litter (*see also* Kuehn and Suberkropp 1998*a*), which has also been reported by Newell (1995) for standing litter of salt marsh rush *J. roemerianus.* Leaf litter (2.5 g) was placed into 1-mm mesh (15 × 60 cm) fiberglass litter bags, fastened to rectangular PVC pipe supports, and submerged ca. 20–30 cm (early July) below the air–water interface in three randomly selected areas of the wetland. Six litter bags (two from each site) were collected on each sample date for determination of plant litter weight loss as %AFDM, microbial (bacteria and fungi) biomass and production, and nutrient contents (C:N:P). An additional three litter bags (one from each site) were collected at the beginning ($time_0$) and end ($time_{268}$ d) of the study for determination of plant litter fiber composition. Data analyses were conducted by SYSTAT (Wilkinson et al. 1992) and values were considered significant at $P < 0.05$ level.

Mass loss, nutrient content and litter composition—Three litter bags collected on each sample date (one from each site) were used for the determination of plant litter weight loss (AFDM) and litter nutrient contents. Intact plant material was removed from litter bags and dried at 60°C to a constant weight. Dried plant material was ground to 40 mesh (ca. 400 μm particle size) with a Wiley mill, and subsamples were combusted overnight at 550°C to determine the %AFDM remaining. Plant litter decay rates (k) were calculated by using a nonlinear ($M_t = M_o \cdot e^{-kt}$) regression model and linear regression model after log transformation ($\ln(M_t/M_o) = -kt + b$), where M_t is the AFDM at time t (d) and M_o is the initial AFDM in litter bags.

Additional subsamples of ground plant material were used for analysis of litter nutrients. Carbon and N concentrations were determined with a CHNS analyzer (model 1108, Carlo Erba). Phosphorus concentrations were determined with a Lachet flow injection analyzer (automated ascorbic acid method) following high temperature sulphuric acid digestion (Greenburg et al. 1992). Plant fiber components were analyzed based on methods described in Allen (1989) and Ryan et al. (1990). Briefly, plant material was removed from litter bags, dried (60°C), ground as above, and lyophilized overnight prior to analysis. Nonpolar organic components in plant litter samples were extracted with dichloromethane. Subsamples of ground litter (ca. 2 g) were placed into tared glass fiber extraction thimbles (200 ml), placed into a Soxhlet extraction apparatus, and dichloromethane (250 ml) cycled through samples for 3 h (4–5 extractions h^{-1}). After extraction, the dichloromethane was evaporated and the remaining sample residue was dried (105°C) and weighed (nonpolar extractives = sample wt. − residue wt.). A portion of the dichloromethane extracted residue was subsequently used for the analysis of water soluble components, holocellulose, α-cellulose, and acid insoluble lignin within plant litter.

For water soluble litter components, ca. 0.2 g of sample residue was extracted in 100 ml of hot (60°C) distilled water for 2 h with constant stirring (125 rpm). The resultant litter residue was collected on a preashed, preweighed GF/F filter, dried (105°C) and weighed (water solubles = sample wt. − residue wt.). For lignin contents, ca. 0.8 g was boiled in 400 ml distilled water for 3 h followed by addition of 22 ml 10% sulfuric acid and boiling for an additional hour. The remaining residue was collected on a GF/F filter and dried (40°C). Sulfuric acid was added to a portion of the dried residue (ca. 0.5 g) (72% H_2SO_4, 10 ml g^{-1} sample) and incubated at 30°C for 1 h followed by the addition of distilled water (28 ml g^{-1} acid-residue mixture) and autoclaving for an additional 1 h. The acid insoluble lignin remaining was collected on a preashed, preweighed GF/F filter, washed 2× with 100 ml distilled water, dried (105°C), weighed and ashed (500°C) (lignin = residue − ash). Holocellulose was determined after delignification by acid chlorite. A subsample of the dichloromethane extracted residue (ca. 0.6 g) was added to 30 ml of distilled water containing 0.12 ml of 10% acetic acid. Sodium chlorite was added (0.6 g g^{-1} sample) and the mixture heated (75°C) in a water bath for 3 h. The remaining residue was then collected on a preashed, preweighed GF/F filter, dried at 105°C, weighed, and a portion of the residue ashed (holocellulose = residue − ash). For determination of α-cellulose, a subsample (ca. 0.3–0.4 g) of holocellulose residue (above) was added to 20 ml of a 24% KOH solution

and incubated in a water bath (20°C) for 2 h. The remaining residue was collected on a preashed, preweighed GF/F filter, dried at 105°C, weighed and ashed (α-cellulose = residue − ash, hemicellulose = holocellulose − α-cellulose).

Microbial biomass and production—The remaining three litter bags collected during each sampling period were used for the determination of ATP concentrations and microbial biomass and production associated with leaf litter. ATP concentrations were determined by methods described in Suberkropp et al. (1983). Ten 2-cm leaf pieces from each litter bag were homogenized (Polytron, setting 7 for 15 s) in 5 ml of cold 1.2 N sulfuric acid containing oxalic acid (8 g L^{-1}) and 5 ml of 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) at pH 7.5. Samples were centrifuged (20 min @ 9,000 × *g*), filtered through glass wool, and 4 ml aliquots neutralized to pH 7.5 with NH_4OH. Neutralized samples were adjusted to 10 ml and stored at −20°C until assayed. Sample ATP concentrations were quantified with the luciferin-luciferase enzyme method with light emission measured by a Turner luminometer and compared to ATP standards. Extraction efficiencies were determined by adding known amounts of ATP to litter samples.

Fungal biomass was estimated by the extraction and quantification of ergosterol from decomposing plant litter (conversion factor: 10 μg ergosterol mg^{-1} living fungal C, and assuming a fungal C content of 50%) (Gessner and Newell 1997). Instantaneous growth rates of fungi associated with plant litter were determined *in situ* from rates of [^{14}C]acetate incorporation into ergosterol (conversion factor: 19.3 μg fungal biomass $nmole^{-1}$ acetate incorporated) (Weyers and Suberkropp 1996; Gessner and Newell 1997). Plant litter was removed from litter bags, cut into 2-cm leaf pieces, and three leaf pieces incubated in Teflon-lined screw cap tubes (20 × 150 mm) containing 4 ml of filtered (0.45 μm pore size membrane filters) wetland pond water and 5 mM Na[1-^{14}C]acetate (specific activity = 48.3 MBq $mmol^{-1}$) for 3 h at wetland pond temperatures. Additional leaf pieces from replicates were dried at 60°C and combusted overnight at 550°C to determine AFDM of leaf material in samples. Incorporation of [^{14}C]acetate was stopped by placing tubes on ice and immediately filtering the contents. Filters and leaf pieces were washed twice with 4 ml of filtered pond water, placed in 5 ml of methanol, transported on ice to the laboratory, and stored at −20°C until analyzed. Ergosterol was extracted from plant litter samples by refluxing in alcoholic KOH (4% KOH in 95% methanol, extraction efficiencies 95%) for 30 min at 80°C (Suberkropp and Weyers 1996). The resultant extract was partitioned into *n*-pentane and evaporated to dryness under a stream of nitrogen gas at 30°C. Ergosterol in dried samples was redissolved by bath ultrasonication in 1 ml of methanol, filtered (0.45 μm Acrodisc PTFE filters) and stored tightly capped in 4 ml screw cap vials at −20°C until analyzed. Ergosterol concentrations were quantified by high pressure liquid chromatography (HPLC). A Whatman partisphere C-18 reverse phase column (0.46 x 25 cm, 100 μl sample loop) (Whatman) connected to a Shimadzu LC-10A5 liquid chromatography system was used for separation and analysis (Shimadzu Scientific). The mobile phase was methanol (HPLC grade) at a flow rate of 1 ml min^{-1}. Ergosterol was detected at 282 nm (R_t = ca. 12.5 min) with a Shimadzu SPD-10A UV-VIS detector, and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical). Ergosterol fractions eluting from the HPLC were collected in 20 ml scintillation vials with an automated fraction collection system (Advantec). Collected fractions (2 injections per sample, total volume ca. 1 ml) were mixed with 10 ml of scintillation fluid (Ecolume) and dpm determined in a (Beckman) scintillation counter (H# method).

Bacterial biomass and production associated with submerged litter were also measured. Bacterial biomass was determined by epifluorescence direct count microscopy after staining with 4′,6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980). Three 2-cm leaf pieces were placed in 15 ml sterile polypropylene screw capped conical tubes (Corning) containing 10 ml of 10% (v/v) filtered (0.2 μm) phosphate buffered formalin. Samples were placed on ice, returned to the laboratory, and stored at 4°C until analyzed. Bacterial cells attached to leaf pieces were removed by bath ultrasonication (Branson, model 2210, power output −109 watts). Prior laboratory experiments were conducted to determine optimal sonication times for removal of attached bacteria [bacteria cells × 10^6 ml^{-1} ± 1 SE (N = 3), 1 min; 3.6 ± 1.7, 2 min; 2.93 ± 0.32, 3 min; 2.84 ± 0.28, 4 min; 1.84 ± 0.19, 5 min; 1.32 ± 0.23]. A treatment time of 3 min was chosen for subsequent analyses. After sonication, 1 ml samples were removed, stained with DAPI (1.0 μg ml^{-1}) for 5 min in darkness, and vacuum filtered (15 kPa) through 0.2 μm pore size black polycarbonate membrane filters (Poretics). Filters were then rinsed with 1 ml of 0.2 μm filtered distilled water and mounted on glass slides. Bacterial cells were counted in 10 random Whipple grids with a Nikon epiflourescence microscope (1,000×) and random fields photographed (Ektachrome 200) to determine average biovolumes of bacterial size classes. Bacterial cell volume and counts were converted to biomass by the conversion factor of 220 fg μm^{-3} (Bratbak 1985).

Bacterial production associated with plant litter was monitored by using rates of [^{3}H]leucine incorporation into bacterial protein (BPP) (Thomaz and Wetzel 1995; Suberkropp and Weyers 1996). Three 2-cm leaf pieces from each replicate (as above) were incubated in tubes with 4 ml of filtered pond water containing 400 nM [4,5-^{3}H]leucine (specific activity = 142 GBq $mmol^{-1}$) for 30 min at wetland pond temperatures. Experiments using the eukaryotic inhibitors cycloheximide (0.02%) and colchicine (0.01%) indicated that no significant eukaryotic uptake of the added leucine label occurs at the 400 nM concentration used (*see* Suberkropp and Weyers 1996). Incorporation of radiolabeled leucine was stopped by the addition of trichloroacetic acid (final conc. 5% v/v). Labeled protein from samples was extracted by bath ultrasonicating samples for 5 min and subsequent heating at 80°C for 30 min. Samples were removed and cooled to room temperature. The resultant precipitated extract was filtered through polycarbonate filters (0.22 μm, Millipore), washed three times each with 4 ml of cold 5% TCA, and then washed two times each with 2 ml of 80% ethanol. Filters were then washed two times each with 2 ml of distilled water and placed in 20 ml scintillation vials. Ten milliliters

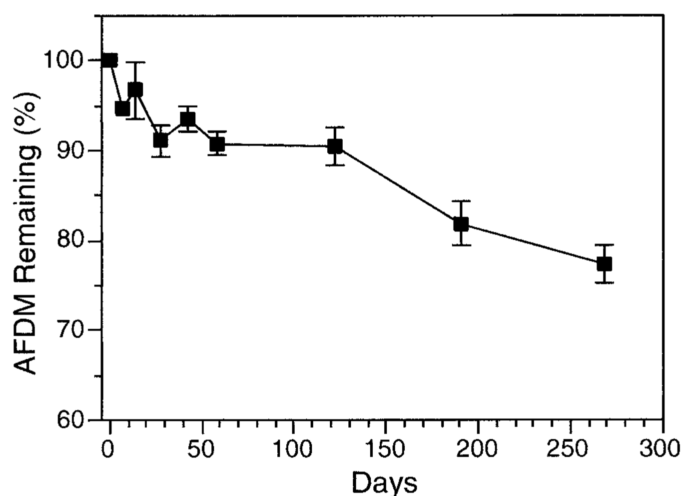

Fig. 1. Percent ash free dry mass remaining (AFDM) of *J. effusus* leaves in submerged litter bags. Symbols indicate the mean ± 1 SE ($N = 3$).

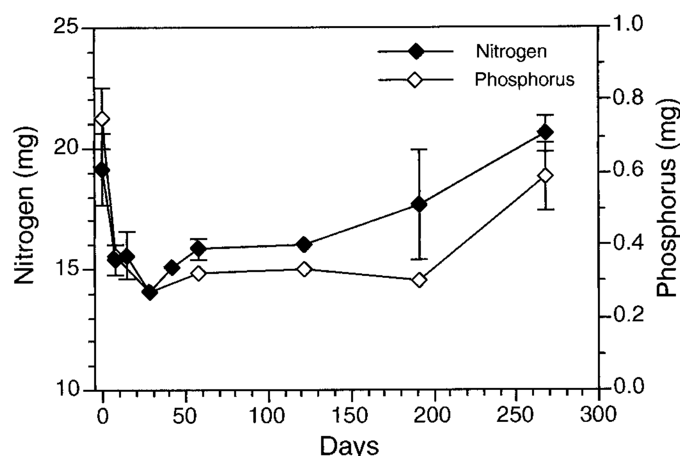

Fig. 2. Changes in total litter bag nitrogen and phosphorus contents of submerged plant litter. Symbols indicate the mean ± 1 SE ($N = 3$).

of scintillation fluid were added to each vial and dpm determined with a (Beckman) scintillation counter (H# method), corrected for quenching.

Results

Leaf decay and nutrient dynamics—Decomposition of *J. effusus* enclosed in litter bags was slow, with only 23% weight loss observed in 268 d (Fig. 1). Decay coefficients (k yr^{-1}), using a nonlinear regression model and linear regression model after log (ln) transformation, were -0.36 ± 0.03 ($r^2 = 0.99$) and -0.29 ± 0.03 yr^{-1} ($r^2 = 0.81$), respectively. Substantial decreases in litter N and P concentrations (%) were observed shortly after submergence of litter bags in the wetland (ANOVA, $P < 0.01$), resulting in corresponding increases in litter C:N, C:P and N:P ratios (Table 1). After this initial decrease, N and P concentrations gradually increased to values equal to or greater than initial litter concentrations. Total amounts of nitrogen and phosphorus (mg) in litter bags (Fig. 2) followed a similar pattern with significant losses in total litter bag N and P observed soon after submergence (ANOVA, $P < 0.01$), followed by gradual accumulation of litter N and P during the remaining study period. Structural plant polymers accounted for over 78% of the total organic mass in decaying *J. effusus* litter (Table 2). No significant changes in these constituents were observed during the study period ($P > 0.05$, Student's t-test). However, small but significant differences were found for ash, water soluble, and nonpolar litter components ($P < 0.05$, Students t-test) (Table 2).

Microbial biomass and production—Fungal and bacterial production rates associated with decomposing standing and submerged *J. effusus* litter collected immediately before initiation of this study were considerably higher than those observed during the first 191 d of litter decay within submerged litter bags (Table 3). Estimates of fungal and bacterial production associated with this material were similar on both standing and submerged litter, with fungal production accounting for greater than 94% of the total microbial production. In addition, rates of fungal production associated with standing dead litter were higher than those rates observed for submerged litter, but this difference was not significant ($P > 0.05$, Student's t-test) (Table 3).

Patterns of fungal and bacterial biomass associated with *J. effusus* litter enclosed in submerged litter bags were similar to those observed in litter N and P contents (Fig. 3). Both fungal and bacterial biomass declined significantly after submergence of standing dead litter in the wetland ($P < 0.05$, ANOVA) (Fig. 3), decreasing by over 50% within the first 28 d. Changes in fungal biomass were significantly correlated with changes in bacterial biomass ($r = 0.79$, Pearson; $P < 0.01$). Fungal and bacterial biomass were also significantly correlated ($P < 0.05$) with decreasing pond water temperatures (Fig. 4); the resulting correlation coefficients were low (fungi $r = 0.49$; bacteria $r = 0.58$; Pearson; $P < 0.05$). Biomass levels remained low, increasing slightly only during the last part of the study. Living fungal biomass, as

Table 1. Changes in N and P concentrations within *J. effusus* litter during submerged decay. Atomic C:N, C:P, and N:P ratios are also given. Values are the means ± 1 SE ($N = 3$).

Day	%N	%P	C:N	C:P	N:P
0	0.79 ± 0.08	0.030 ± 0.003	71 ± 7	4,100 ± 212	59 ± 12
28	0.65 ± 0.004	0.012 ± 0.0004	85 ± 1	9,801 ± 175	114 ± 2
122	0.73 ± 0.03	0.015 ± 0.001	75 ± 3	7,935 ± 324	106 ± 3
268	1.00 ± 0.03	0.028 ± 0.004	51 ± 1	4,147 ± 709	81 ± 12

Table 2. Plant litter composition of *J. effusus* at the beginning (i.e., standing litter) and end of the study period. Values indicate the mean ± 1 SE (N = 3).

Plant litter composition (% of total)	Sample date	
	July (1994)	April (1995)
α-Cellulose	36.71 ± 2.47	41.39 ± 2.53
Hemicellulose	28.94 ± 0.56	30.73 ± 0.47
Lignin	13.12 ± 1.13	10.48 ± 0.98
Nonpolar extractives*	6.59 ± 0.26	12.02 ± 1.14
Water soluble extractives*	6.58 ± 0.88	2.53 ± 0.24
Ash*	2.34 ± 0.71	9.68 ± 1.57
Sum	94.3	106.8

* Significantly different on the two dates ($P < 0.05$, Students t-test).

measured by ergosterol contents, ranged from 2–42 mg C g^{-1} AFDM, and accounted for 98.7 ± 0.8% of the total microbial biomass during the study period. In contrast, bacterial biomass accounted for only 1.3 ± 0.8% of total microbial biomass, ranging from 0.042–0.28 mg C g^{-1} AFDM. Changes in microbial biomass, as determined by ATP concentrations, revealed a pattern similar to other independent measures of fungal (ergosterol) and bacterial (direct counts) biomass (Fig. 5), with litter ATP concentrations decreasing significantly after litter submergence (ANOVA, $P < 0.01$). However, the correlation between ATP concentrations and fungal biomass (ergosterol concentrations) was low (r = 0.43; Pearson, $P < 0.05$). Conversion of ATP concentrations to microbial biomass using a factor of 250 μg C μg^{-1} ATP (Findlay and Arsuffi 1989), revealed that estimates of total microbial biomass using litter ATP contents were one to nine times lower than those values estimated from ergosterol concentrations plus bacterial direct counts.

Microbial production rates associated with decaying litter were also dominated by fungi, ranging from 36–322 μg C g^{-1} AFDM d^{-1} (mean 131 ± 78) during the first 191 d (Fig. 6). Production estimates increased significantly (2,836 ± 700 μg C g^{-1} AFDM d^{-1}) on the last sampling date ($P <$ 0.01, ANOVA, Tukey). Fungal production was 2–522 times that of bacterial production, accounting for 91 ± 12% of the total microbial production associated with plant litter during the study period. Bacterial biomass production accounted for 9 ± 12% of total microbial production, ranging from 0–38 μg C g^{-1} AFDM d^{-1} (mean 12 ± 11). The potential contribution of fungal and bacterial decomposers to overall carbon loss of *J. effusus* litter was estimated for the first 191 d of the study, and indicated that fungal decomposers accounted for a substantial portion of the litter mass loss observed (Table 4).

Table 3. Studies examining fungal biomass, and fungal and bacterial production associated with standing and submerged litter of *J. effusus* collected in June 1994. Studies were conducted just prior to collection of standing leaf litter for submerged litter bags. Values indicate the mean ± 1 SE (N = 3).

Litter type	Fungal biomass (mg C g^{-1} AFDM)	Fungal production (μg C g^{-1} AFDM d^{-1})	Bacterial production (μg C g^{-1} AFDM d^{-1})
Standing	39.9 ± 4.1	1,000 ± 320	60 ± 20
Submerged	55.0 ± 6.5	760 ± 580	50 ± 10

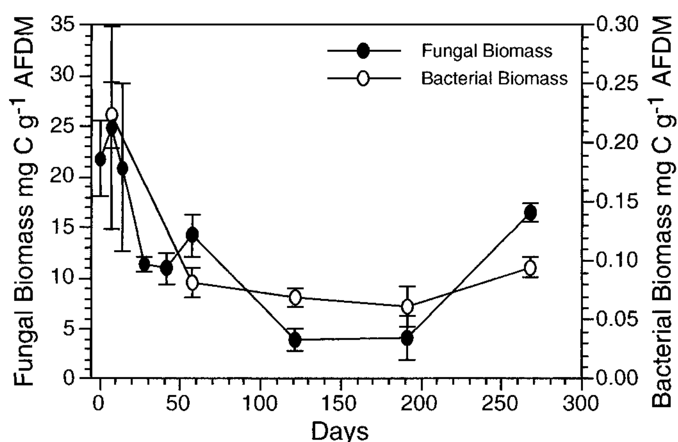

Fig. 3. Changes in fungal and bacterial biomass associated with submerged plant litter in litter bags. Symbols indicate the mean ± 1 SE (N = 3).

Discussion

The present study investigated the microbial decay and nutrient dynamics associated with *J. effusus* litter after movement of plant litter from a terrestrial standing-dead to submerged aquatic decay phase. Analysis of standing *J. effusus* litter prior to harvesting for submerged litter bags indicated appreciable colonization and production of microbial decomposers within plant litter. Levels of fungal biomass associated with standing litter (40 ± 4 mg C g^{-1} AFDM) were similar to those reported in earlier studies on *J. effusus* (Kuehn and Suberkropp 1998*a,b*). In addition, results of these initial studies revealed that fungi were a metabolically active component of the microbial assemblage within standing litter. On the basis of rates of acetate incorporation, fun-

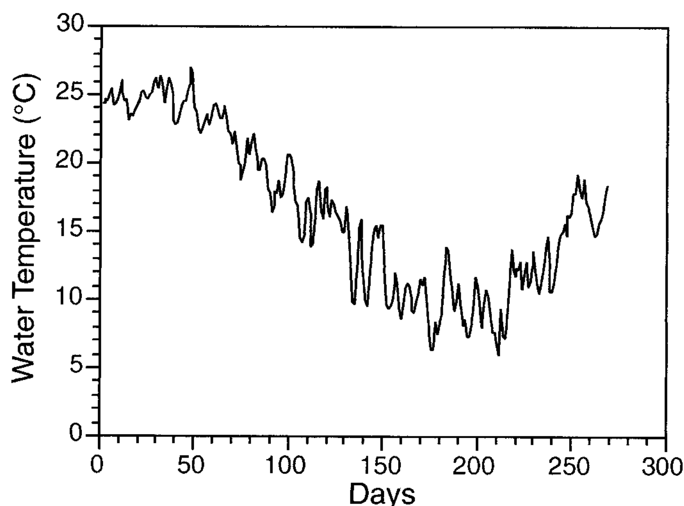

Fig. 4. Changes in wetland pond water temperatures during the study period.

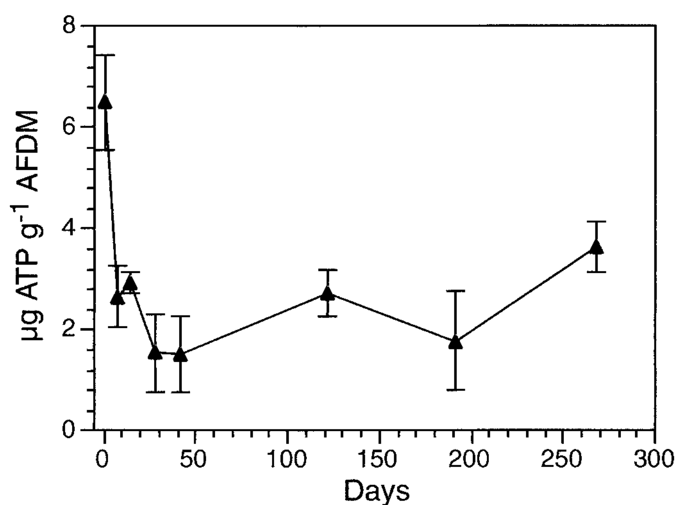

Fig. 5. Changes in ATP concentrations associated with submerged plant litter in litter bags. Symbols indicate the mean ± 1 SE (N = 3).

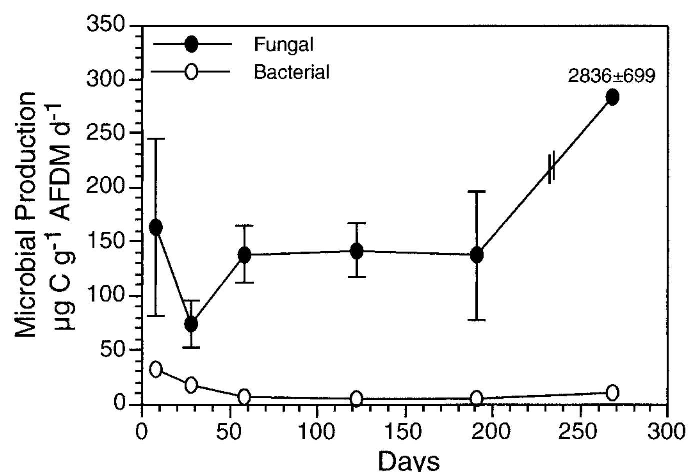

Fig. 6. Changes in fungal and bacterial production associated with submerged plant litter in litter bags. Symbols indicate the mean ± 1 SE (N = 3).

gal production accounted for a major portion of the total microbial production. These results concur with previous studies examining the contribution of fungal and bacterial decomposers to standing litter decay (Newell 1993; Newell et al. 1989, 1995). Newell et al. (1995) reported rates of fungal production associated with standing *Carex walteriana* litter ranging from 116–665 μg C g^{-1} AFDM d^{-1}, accounting for nearly all (>99%) of the microbial production within and on decaying litter.

Movement of *J. effusus* litter from a standing dead to a submerged decay environment resulted in significant changes in both the nutrient and microbial dynamics associated with decomposing plant litter. Fungal and bacterial biomass and production decreased rapidly following litter submergence, indicating that the resident microbiota associated with decaying standing litter could not survive the abrupt changes in the environmental conditions from a standing to an aquatic decay phase. Similar findings have been reported for fungal communities inhabiting decomposing litter of the salt marsh grass *Spartina alterniflora* (Newell et al. 1989) and the freshwater emergent macrophyte *Phragmites australis* (Tanaka 1991, 1993; Gessner unpubl. data). Newell et al. (1989) found a sharp decrease (71%) in fungal biomass (ergosterol and immunosorbent assay methods) when standing dead leaves of *S. alterniflora* fell over onto the salt marsh sediment surface. After coming in contact with the surface sediments, bacterial biomass associated with decomposing *S. alterniflora* litter increased, but only represented a maximum of 7% of the total microbial biomass (Newell et al. 1989). Similar findings were observed in the present study, with litter associated fungal biomass (ergosterol) decreasing by 82% within 122 d after movement of standing litter to a submerged decay environment (note also: 58% decrease using litter ATP concentrations). However, fungal biomass associated with *J. effusus* litter increased after 191 d of litter submergence, with fungal biomass recovering to 73% of the value observed at the start of the study. Furthermore, rates of fungal biomass production increased nearly 22 times compared to those observed during the early stages of submerged litter decay. These results suggest a possible succession of microbiota associated with *J. effusus* plant matter during the standing to submerged decay sequence.

The distinct changes in biomass and production rates of fungi associated with *J. effusus* litter during its transition from a standing to submerged decay state should not be considered unusual, since the predominant taxa associated with standing litter prior to litter submergence consisted of terrestrial fungal species (including white-rot basidiomycetes; *see* Kuehn and Suberkropp 1998*a*). Consequently, the change in environmental conditions between the standing dead and aquatic decay phase could conceivably lead to a shift in the dominance of particular fungal taxa, since terrestrial fungi may not be well adapted to an aquatic environment. Previous studies have reported a distinct succession in fungal taxa between standing and submerged plant matter of both *P. australis* (Apinis et al. 1972; Taligoola et al 1972)

Table 4. Net production, biomass, P/B ratio, turnover times and the calculated potential contribution of fungal and bacterial decomposers to *J. effusus* litter breakdown during the initial period of submerged litter decay (0–191 days). The total net production is the sum of daily production rates during this period, assuming a mean daily rate between sampling intervals. Percentage of initial leaf carbon assimilated was calculated by dividing the total net fungal or bacterial production by previously reported growth efficiencies for fungi (0.35, Suberkropp 1991) and bacteria (0.17, Mann and Wetzel 1996). The contribution of fungal and bacterial assimilation to overall loss of leaf C was determined by dividing fungal and bacterial assimilation by the loss of leaf C observed (19%).

Parameter	Fungal	Bacterial
Total net production (mg C g^{-1} initial leaf C)	44	3
Mean biomass (mg C g^{-1} initial leaf C)	16	0.2
P/B ratio	2.8	15
Turnover time (d)	68	13
% of initial leaf C assimilated	13	2
% contribution to overall leaf C loss	68	11

and *Typha latifolia* (Pugh and Mulder 1971). Tanaka (1991) reported a rapid decrease in fungal populations (plate count method) and fungal biomass (hyphal lengths) within a few weeks after submerging standing dead *P. australis* leaves in a saline lake. These decreases were followed by a gradual increase during the remaining study period. Tanaka (1991) suggested that the initial decrease in fungal populations may have been due to the death of terrestrial fungi inhabiting standing leaf litter, in that these fungal populations may not survive a change to a saline environment. Additional evidence supporting the replacement of microbiota on submerged *J. effusus* litter is presented in Table 3, in which fallen litter of unknown age and presumably a longer period of submergence supported fungal biomass and production rates which were significantly higher than those initially observed from submerged litter enclosed in litter bags.

Despite submergence of *J. effusus* litter and decreases in litter associated microbial biomass, fungal decomposers continued to constitute the major microbial assemblage associated with *J. effusus* plant matter. Throughout this initial period of submerged litter decay, estimated fungal biomass and production greatly exceeded estimates of bacterial biomass and production. Similar findings in other freshwater habitats (Findlay and Arsuffi 1989; Findlay et al. 1990; Newell et al. 1995; Baldy et al. 1995; Sinsabaugh and Findlay 1995; Weyers and Suberkropp 1996; Baldy and Gessner 1997; Gessner 1997), add generality to the idea that fungi are an important microbial assemblage involved in the early stages of plant litter decay in aquatic environments. Newell et al. (1995) reported an increase in bacterial biomass and production once plant litter of *C. walteriana* fragmented and fell to the sediment surface. However, despite the change in the decay conditions, fungi continued to be the dominant microbial assemblage associated with decaying litter, accounting for 97% and 92% of the total microbial biomass and production, respectively (Newell et al. 1995).

Similar findings were observed in the present study, with fungal biomass and production representing over 98% and 91%, respectively, of the total microbial biomass and production associated with submerged *J. effusus* litter. In comparison, bacterial biomass and rates of bacterial biomass production associated with *J. effusus* litter were much lower, but within the range of estimates reported previously for decomposing litter of freshwater emergent macrophytes (e.g., Moran and Hodson 1992; Newell et al. 1995; Sinsabaugh and Findlay 1995). Estimates of microbially mediated carbon flow based on cummulative biomass production revealed that fungal assimilation of *J. effusus* litter could account for a major portion of the observed litter mass loss. These findings suggest that fungi can play an important role in litter breakdown during the initial stages of plant decay. However, note that in an earlier study, Moran and Hodson (1989) reported considerably higher bacterial productivities associated with *J. effusus* litter during submerged litter decay in a thermally impacted lake (range: ca. 50–810 μg C g^{-1} dry wt d^{-1}). Similar production estimates of bacteria were also reported by Thomaz and Wetzel (1995) for epiphytic communities (i.e., bacterial and algal biofilms) on submerged living and dead *J. effusus* plant matter (unknown age) from the present study site (10–760 μg C g^{-1} dry wt d^{-1}), which suggests the potential for significant bacterial participation in the decay of *J. effusus* litter or litter derived dissolved organic matter.

The decay of submerged *J. effusus* litter observed in the present study was slow ($k = 0.36 \pm 0.03$ yr^{-1}), but is consistent with previous studies examining submerged or sediment litter decay of freshwater emergent macrophytes (e.g., Davis and van der Valk 1978; Hietz 1992). Hietz (1992), who used both coarse and fine mesh litter bags, observed between ca. 20 and 30% weight loss in submerged leaf litter of *P. australis* over a 9-month period. In contrast to the present study, Moran and Hodson (1989) reported extremely rapid rates of mass loss of *J. effusus* in a thermally impacted lake. In their studies, *J. effusus* plant material enclosed in litter bags was completely degraded within 22 weeks after litter submergence. However, note that the starting plant material used in their studies consisted of living leaves that had been oven dried (55°C) prior to the construction and submergence of litter bags (Moran and Hodson 1989). These initial litter conditions (*see* Newell 1993), along with the higher temperatures encountered during their study may have contributed to the faster litter breakdown and higher bacterial productivities observed during their investigation.

Nutrient analysis of *J. effusus* litter revealed a sharp decline in both total litter bag nitrogen and phosphorus following submergence of standing litter in the wetland surface waters. This decline was followed by an increase in the total amounts of litter bag N and P between the last two sampling periods of the study. Similar results were reported in previous studies examining the decomposition and nutrient dynamics of standing to fallen litter of freshwater emergent macrophytes (e.g., Davis and van der Valk 1978; Hietz 1992). Hietz (1992) reported an initial loss of nitrogen (5–10%) and phosphorus (40–60%) from *P. australis* litter during the first month following litter submergence. This was followed by an increase in N and P over the 3-yr study period, presumably due to increased external nutrient accumulation by litter associated microbial assemblages (Hietz 1992).

The successive decreases and increases in litter associated nutrients (N and P) and microbial biomass observed in the present study with *J. effusus* imply that at least a portion of the nitrogen and phosphorus within plant litter may have been contained in the biomass of inhabitant microbial assemblages. Previous investigators have suggested that microbial biomass associated with litter contribute very little to the total nutrient contents in decomposing plant litter (e.g., Mann 1988). However, Newell (1993, 1996), by using improved methodology for the quantification of fungal biomass, determined that fungal decomposers in decaying salt marsh *S. alterniflora* litter can potentially retain most of the available nitrogen (99%) in decomposing plant matter. In the present study, the calculated contribution of fungal biomass to total detrital nitrogen at the beginning of the decay study averaged 36 ± 8% (assuming 4% N in fungal mass; *see* Newell and Statzell-Tallman 1982), but decreased during the remaining study period concomitant with decreases in fungal biomass. However, the percent fungal contribution to total detrital nitrogen reported here indicates only the contribution of living fungal biomass within *J. effusus* litter. Residual

nonliving fungal biomass (i.e., *N*-acetyl-glucosamine) within plant litter may represent a substantial portion of the total detrital N, since Newell et al. (1995) reported that nonliving fungal mass within decaying litter may be three to four times that of living fungal biomass. Further investigation is needed to assess the quantitative contribution of both bacterial and fungal biomass (living and dead) to N and P within decomposing plant litter.

As Newell et al. (1995) pointed out, the determination of a logical starting point in the study of decomposition of some emergent macrophytes is not a simple matter, since complex changes in mass loss, nutrient concentrations and microbial colonization, and production may occur during the progression of plant matter through senescence and litter decomposition. Results obtained in the present study support their contention as evident from the distinct changes in bacterial and fungal dynamics and nutrient concentrations associated with *J. effusus* litter as it proceeds along a course of initial standing dead to submerged decay environments. Thus, as Newell et al. (1995) proposed, a common decay sequence of emergent macrophytes may involve principally fungal decomposition of plant material during standing and early submerged litter decay, followed by a trend towards increased bacterial decomposition during later stages of litter decay. This view is supported, in part, by a study by Sinsabaugh and Findlay (1995) which examined microbial biomass and production associated with naturally derived particulate organic plant matter of various particle size classes from a freshwater tidal marsh. Results of their study indicated that fungal biomass and production were positively correlated with the particle size of decaying plant litter, whereas bacterial biomass and production were negatively correlated with size, indicating that bacteria assume a more significant role in plant litter decay as litter falls to the sediments and undergoes greater decomposition and fragmentation.

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