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Annual secondary production of fungal and bacterial decomposers associated with standing and benthic litter of the freshwater emergent macrophyte, *typha angustifolia*

Brian Matthew Ohsowski

ANNUAL SECONDARY PRODUCTION OF FUNGAL AND BACTERIAL DECOMPOSERS ASSOCIATED WITH STANDING AND BENTHIC LITTER OF THE FRESHWATER EMERGENT MACROPHYTE, *TYPHA ANGUSTIFOLIA*

by

Brian Matthew Ohsowski

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ABSTRACT

Fungi and bacteria are significant decomposers of plants within wetlands, but annual secondary production investigations are lacking. Microbial carbon assimilation into biomass and CO_2 mineralization may contribute considerably to wetland biogeochemical cycling and energy flow. Naturally-occurring *Typha angustifolia* detritus was collected to determine annual areal carbon flow through secondary decomposers. Two decay phases, standing-dead and benthic litter, were analyzed concurrently to determine fungal (^{14}C-acetate incorporation) and bacterial (^{3}H-leucine incorporation) production estimates. Gradual collapse of the 2004 cohort standing-dead stocks resulted in lowest litter biomass at study's end while benthic detrital stocks displayed little biomass fluctuation. This study indicates significantly higher fungal production rates within standing litter compared to bacteria. A noteworthy increase in areal production and CO_2 mineralization occurred in benthic litter-associated fungi and bacteria. These results provide evidence indicating considerable annual carbon flow from emergent litter to heterotrophic decomposers within both decay phases.

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INTRODUCTION

Freshwater marshes are among the most biologically productive ecosystems on earth, with annual aboveground primary production typically exceeding > 2 kg dry mass m^{-2} yr^{-1} (Mitch & Gosselink 2007). Emergent vascular plants, such as *Typha sp.* (cattails) and *Phragmites sp.* (reed), are a distinctive vegetative feature within these habitats and will often comprise a significant portion of the plant biomass produced on an annual basis (Wetzel 1990). These emergent wetland plants typically form dense stands within freshwater marshes and as a consequence may often function as important atmospheric carbon sinks. Furthermore, emergent plants with a high potential for growth can also absorb and store large amounts of nutrient pollutants (e.g., nitrogen and phosphorus) such that direct-plant assimilation is usually a predominant term used in many wetland nutrient budgets (Mitsch & Gosselink 2007).

In wetlands, the utilization of living plant biomass as a food resource by animal consumers is negligible, since much of the recalcitrant plant tissues (i.e., lignocellulose) are not easily digested and assimilated. As a consequence, most of the plant biomass produced eventually enters the pool of decomposing plant matter, where microorganisms play an important role in its eventual breakdown and mineralization. In addition, microbial growth and biomass accumulation associated with plant detritus in turn provides an important food resource for animal consumers (Graça *et al.* 2000, Newell & Porter 2000, Silliman & Newell, 2003), thus forming a link in the flow of energy and nutrients to higher trophic (animal) levels of wetland food webs.

During vascular plant litter decomposition, heterotrophic microorganisms are

involved in a variety of processes that result in the conversion of plant litter carbon into fungal and bacterial biomass, mineralization of organic matter (CO_2 release) via microbial respiration, and export of fine particulate organic matter (FPOM) and dissolved organic matter (DOM). In many emergent macrophytes, fragmentation and collapse of plant organs (e.g., leaf-blades) to the sediments or surface waters do not typically occur immediately following plant shoot senescence and death. Consequently, standing-dead plant litter tends to accumulate in both freshwater and salt-marsh habitats (e.g., Asaeda *et al.* 2002), where it begins initial microbial decay in an upright aerial position without detachment from the parent plant (Gulis *et al.* 2006, Gessner *et al.* 2007, Kuehn 2008). For example, standing-dead litter of *Juncus effusus* in a subtropical marsh system (Alabama) ranged from 0.8 – 2.3 kg ash free dry mass (AFDM) m^{-2}, which at times was nearly equivalent to the amount of aboveground living plant biomass (Wetzel & Howe 1999). Thus, *natural* decomposition of emergent plants involves two distinct spatial phases separated in time: an initial phase resulting from litter decay processes that occur under aerial standing-dead conditions, followed by a benthic phase resulting from litter decay processes that occur under submerged or surface sediment conditions following the collapse of standing litter.

Diverse assemblages of microorganisms, principally fungi, are known to colonize both standing and collapsed litter of emergent macrophytes (Benner *et al.* 1986b, Thomaz & Esteves 1997, Newell 2001, Buchan *et al.* 2003, Schulz & Thormann 2005). However, despite abundant evidence of litter accumulation in freshwater marshes and extensive fungal colonization, our current understanding of quantitative fungal processes within these systems remains limited. To date, only a few studies have directly estimated the flow of plant litter

carbon into fungal decomposers (e.g., rates of secondary production) during plant litter decomposition or its potential contribution to overall plant litter carbon loss (Sinsabaugh & Findlay 1995, Newell *et al.* 1995, Komínková *et al.* 2000, Kuehn *et al.* 2000, Findlay *et al.* 2002, Verma *et al.* 2003, Newell 2003, Buesing & Gessner 2006, Su *et al.* 2007). Furthermore, only one published study has examined the contribution of fungal decomposers to ecosystem-level carbon flow (Buesing & Gessner 2006).

Studies by Buesing and Gessner (2006) suggest that litter-associated fungal and bacterial production in freshwater marshes can be potentially large at the ecosystem scale. However, the paucity of more comprehensive data limits our current understanding of litter-associated microbial processes, particularly fungal, in annual ecosystem level carbon flow. Specifically, in view of the quantitative importance of microbial decomposers to energy flow and biogeochemical cycles in wetlands dominated by emergent macrophytes, more detailed investigations of these processes clearly are needed. This study links the decomposition of the benthic litter phase to the aerial litter phase from the inception of macrophyte senescence over an annual period to understand microbial assemblage litter processing within distinctly different detrital pools. Furthermore, this research quantifies the key microbial decay processes (CO_2 mineralization, microbial biomass, and microbial production) associated with naturally-occurring *Typha* detritus as applied to biogeochemical cycling, nutrient retention, and energy flow in freshwater wetlands. Project goals of this research include (1) Determining quantified changes in biomass within standing-dead shoots and benthic litter of *Typha angustifolia,* a common emergent plant of freshwater wetlands throughout the Great Lakes region, over an annual cycle; (2) Simultaneous estimation of fungal and bacterial

biomass, production, and growth rates and total microbial respiration associated with collected plant litter in each detrital pool; and (3) Construction of a decomposition budget on an areal basis (g/m^2) to summarize carbon flows to and through the fungal and bacterial assemblages associated with detritial shoots and benthic litter.

MATERIALS AND METHODS

Study Site

The Paint Creek Wetland study site is a created retention basin wetland dominated by emergent macrophytes (*Typha angustifolia*, *Scirpus validus*, and *Phragmites australis*). The marsh, located in Ypsilanti, Michigan (42°12'N, 83°37'W), is within the Stony Creek watershed that drains sections of Washtenaw and Monroe counties. A permanent 50m transect with 1 meter increments was constructed through a monotypic stand of *Typha angustifolia*. Optic StowAway Temperature Loggers (Onset Computer Corp.) were placed at the surface sediments to continuously monitor benthic temperatures (Figure 1) throughout the study period. Likewise, ambient air temperature (Figure 2) and relative humidity (Figure 3) were also continuously monitored throughout the study period using two HOBO H8 Pro Series Loggers (Onset Computer Corp.) that were placed adjacent to the transect at approximately 1.5 meters above the surface sediments. Precipitation data were obtained from Willow Run Airport, located 4.21 miles NE of the wetland study site.

Data Collection and Experimental Design

Microbial assays for benthic and standing-dead litter were conducted from October 2004 to November 2005. Microbial assays were not conducted in the late fall and winter months as microbial production was assumed to be negligible. During each sampling period, standing stocks of both structurally intact benthic organic material (CPOM - course particulate organic matter) and standing-dead litter were collected from each designated subplot, thus allowing direct comparison of litter standing stocks and corresponding

microbial production between standing-dead and benthic environments. Six random sampling locations along the 50m transect were visited on each sampling period. No location was sampled more than once during the entire study. Wetland water (benthic assays) and ambient air (standing-dead assays) temperatures were recorded on each sampling date and employed as incubation temperatures during laboratory microbial assays. Wetland water was collected during each sampling period for use in microbial production and respiration assays. Water was collected in sterile bottles, returned to the laboratory, and vacuum filtered through a Glass Fiber Filter (0.7 µm, VWR) followed by filtration through a 0.22µm cellulose acetate membrane filter system (Corning Inc). Filtered wetland water was subsequently incubated at ambient wetland temperatures.

Live *Typha* shoots were collected in August and September 2004, separated into leaves and stems, and used to determine peak aboveground biomass of the 2004 cohort. All subsequent sampling dates disregarded living green shoots produced by the 2005 cohort. At each sampling location for all sampling dates, *Typha* shoots were clipped above the surface sediment within a 0.625m^2 subplot and transported to the laboratory in large paper bags. A representative sampling shoot from each subplot was selected for microbial assays. *Typha* sampling shoots were separated into stems and leaves and cut into 2cm (microbial assays) and 10cm (CO_2 evolution analysis) litter subsections. Litter subsections were placed into sterile Petri dishes and hydrated with filtered wetland water. Remaining shoots were counted, categorized into living and senescent stems and leaves, and dried at 105^oC in a forced-air drying oven (VWR Scientific Products) to a constant weight. The remainder of sampling shoot stem and leaf litter was dried, ground in a Wiley Mill (40 mesh, 425µm

particle size), and samples combusted at 500ºC to determine ash free dry mass (AFDM).

Structurally intact benthic organic matter (CPOM) was sampled within the same 0.625m^2 subplot (as above), placed into a clean plastic zip-lock bag, and transported to the laboratory on ice. In the laboratory, benthic CPOM was removed from the bag, cut in to 2 and 10 cm size litter subsections, placed in sterile Petri dishes, and hydrated with filtered wetland water (as above). Remaining organic material was frozen, lyophilized to dryness, and weighed. The benthic litter AFDM was determined as above. Carbon contents of both standing dead and benthic litter was assumed to be 50% carbon from ash free dry mass. (Gessner 2000)

Fungal Biomass and Production

Fungal biomass and production associated with collected plant litter was determined by rates of [^{14}C]-acetate incorporation into ergosterol (Newell & Fallon 1991, Gessner & Newell 2002). Subsections (2cm) of collected litter were placed into 20 mL autoclaved glass scintillation vials containing 4 ml of filtered (0.22 µm) wetland water and 5 mM Na[1-^{14}C]acetate (specific activity = 37 MBq $mmol^{-1}$) for 5-6 h at ambient wetland temperatures. Incorporation of [1-^{14}C]-acetate label was stopped by placing vials on ice and immediately filtering (0.7 µm, Whatman GF/F) the contents. Filters and litter pieces were washed twice with 4 ml of filtered wetland water, placed in scintillation vials, and stored frozen at -20 °C until analyzed. A killed-control was also included for each assay by adding formalin (2% final concentration) prior to the addition of the [^{14}C]-acetate radiolabel.

Frozen samples were lyophilized and weighed, and the ergosterol was extracted in

alcoholic KOH (0.8% KOH in HPLC-grade methanol, total extraction volume 10 ml) for 30 min at 80°C in tightly capped thick-walled Pyrex digestion tubes with constant stirring. The resultant crude litter extract was cleaned by solid phase extraction (see Gessner & Schmitt 1996, Su *et al*. 2007), and the ergosterol was purified and quantified by High Pressure Liquid Chromatography (HPLC).

A LichroSpher 100 RP-18 column (0.46 x 25 cm, Merck Inc.) maintained in a Shimadzu column oven (CTO-10AS) at 40 °C and connected to a Shimadzu autosampler (SIL-10AD) and a Shimadzu liquid chromatograph system (Pumps LC-10AT, Controller SCL-10A) were used for separation and analysis. The mobile phase was HPLC grade methanol at a flow rate of 1.5 ml min^{-1}. Ergosterol was detected at 282 nm using a Shimadzu (SPD-10A) UV/VIS detector (retention time = ca. 12 min) and was identified and quantified on the basis of comparison with ergosterol standards (Fluka Chemical Co.).

Ergosterol fractions eluting from the HPLC column were collected in 20 ml scintillation vials using an automated Advantec (SF-3120) fraction collector system, mixed with 10 ml of scintillation fluid (Ecolume, MP Biomedicals Inc.), and radioactivity assayed by using a Beckman LS 6500 Scintillation Counter, corrected for quenching. For determination of litter-associated fungal biomass, litter ergosterol concentrations were converted to fungal carbon assuming conversion factors of 10 µg ergosterol mg^{-1} fungal C, and 50% C in fungal dry mass (Gessner & Newell 2002). For determination of fungal production, rates of acetate incorporation were converted to fungal growth rates, assuming 12.6 µg fungal biomass $nmol^{-1}$ acetate incorporated (Gessner & Newell 2002).

Bacterial Biomass

Bacterial biomass on litter samples was determined by epifluorescence microscopy after staining with SYBER Gold I (Molecular Probes, Inc). Subsections (2cm) of the detrital sampling shoot (leaves and stems) and benthic CPOM were preserved in 10mL of 2% (v/v) phosphate-buffered (0.1% sodium phosphate) formalin solution and refrigerated at 4^{o}C until analysis. Bacterial cells attached to litter samples were detached by ultrasonic probe sonication (Cole-Parmer, Inc., power output 50 W) for 3 min on ice. After sonication, samples were gently mixed and sample aliquots (30-5000 µL) were placed into a 25-mm glass vacuum filtering apparatus containing 2 ml of sterile distilled water (0.22 µm filtered and autoclaved). An additional 1 ml of sterile distilled water was then added to ensure a homogeneous suspension of bacterial cells before filtration. Samples were vacuum filtered (≤ 20 kPa) through 25-mm, supported Anodisc filters (0.2 µm pore size, Whatman), with a 0.8-µm mixed-ester membrane backing filter (Fisher Scientific Inc). Filtering apparatus was rinsed with 2 mL of sterile distilled water during filtration to improve sample recovery.

Filters were removed from the filtering apparatus and placed sample side up on separate drops of 2.5% (v/v diluted from stock) SYBR Gold I in clean plastic Petri dishes, and stained for 15 min in the dark. Residual moisture from filters was blotted away after staining by touching a Kimwipe to the backside of the filter. Filters were mounted on glass slides with 30 µL of antifade mounting solution containing 50% glycerol, 50% PBS (120mM NaCl, 10mM NaH_2PO_4 at pH 7.5), and 0.1% p-phenylenediamine. A 25-mm glass cover slip was then placed on the filter surface, and a drop of immersion oil was applied (Cargille, Type DF, Formula 1261). Bacterial cells were categorized to one of six classes (small / large

cocci; small / medium /large rods), and cells were enumerated at 1000x magnification (10 fields [minimum]; ≥350 cells) using a Leica DMRB epifluorescence microscope.

To determine biovolumes of bacterial size classes, fluorescent bacterial images were captured by Leica FireCam Software with a Leica Camera using equivalent image capture settings to minimize the halo-effect artifact surrounding the individual organisms. Bacterial organisms in the captured image were categorized into morphology type and size class as previously described. A series of filter types (Gauss filter, kernel 5x5; Laplace filter, kernel 5x5; 3x median filter, rank 3) was applied to the captured image (Massana *et al.* 1997). Once filtered, the digital bacterial images were calibrated using a captured image of a stage micrometer (Graticules, Ltd.) and analyzed to determine area and perimeter using CMEIAS Ver. 1.27 (Liu *et al.* 2001) operating in the UTHSCSA Image Tool Ver. 1.27 (Wilcox *et al.* 1997) . For bacteria with rod morphologies, the biovolume (μm^3) was estimated by employing a set of algorithms calculating an equivalent width ($W_e = [(P - \sqrt{(P^2 - 4\pi*A)}) / \pi]$) and length ($L_e = [P/2 + W_e (1 - \pi/2)]$) followed by a volume calculation ($V = [\pi/4 * W_e^2(L_e - (W_e/3))]$; P = bacterial perimeter and A = bacterial area (Massana *et al.* 1997). The application of equivalent width and length minimizes error resulting from curvature in the rod bacteria morphology. For bacteria with cocci morphology, the biovolume (μm^3) was calculated with a volume algorithm ($V = [4/3 * \sqrt{(A^3/\pi)}]$) (Massana *et al.* 1997). For each of the six size categories, the average bacterial biovolume was determined from a minimum of 50 individuals. Biovolume estimates were used to convert total sample bacterial biomass to total sample bacterial carbon (femtogram C) using a formula ($C = 89.5 * V^{0.59}$), which accounts for size-dependent differences in carbon density (Simon & Azam 1989).

Bacterial Production

Bacterial production associated with the detrital litter was assessed by determining incorporation rates of labeled L-[4,5-^{3}H] leucine into bacterial proteins. Litter subsections (2cm) were placed into sterile (autoclaved), 20 ml glass scintillation vials containing 4 ml of 0.22µm filtered wetland water, with fixed amounts of L-[4, 5-^{3}H] leucine (TRK683 Amersham, specific activity 5.6 TBq/mmol) and unlabeled L-leucine (Sigma Corp.) (Final concentration 2.5µM). Two replicates and 2 trichloroacetic acid (TCA) killed controls (5% v/v final concentration) were conducted on each sampling date. Incubations were conducted at *in situ* temperatures for 30 min.

After incubation, leucine incorporation was stopped by the addition of 50% TCA (5% v/v final concentration). Sample vials were placed in a circulating water bath (80^{o}C) for 30 minutes, cooled to room temperature, and then placed on ice to precipitate proteins. Precipitated proteins were then vacuum filtered through mixed nitrocellulose filters (0.22µm, Millipore Corp.), using a 0.8µm mixed nitrocellulose backing filter. Filters and litter pieces were washed 3 times each with 4 ml of cold 5% TCA, and then 2 times each with 4 ml of cold 80% ethanol. Filters and litter pieces were then washed 2 times each with 4 ml of cold sterile distilled water and placed into 15 ml polypropylene conical centrifuge tubes (Corning, Inc.).

Ten ml of alkaline extractant (0.3N NaOH, 0.1% SDS, 25mM EDTA) was added to each tube, and tubes were incubated at 80^{o}C in a dry-block heater for 60 min. Tubes were removed and cooled to room temperature. Sample aliquots (500µl) of extracts were placed into sterile 1.8 ml plastic microcentrifuge tubes and adjusted to a pH ~7 with the addition of

HCl. Sample aliquots (100µl) of this solution were placed into individual wells of a Microdialyser System 100 (Pierce Chemical, Inc., part # 0066315) and dialyzed for two hours against an ammonium bicarbonate buffer (0.2M NH_4HCO_3, 0.1% SDS, 25mM EDTA, 0.1M NaCl) through a 500 MWCO dialysis membrane (Spectrum Inc.). After dialysis, liquid samples were removed, mixed with 100µl of 50% H_2O_2 in 20 ml glass scintillation vials and incubated overnight to "clear" samples of humic coloration. Subsequently, 10 ml of scintillation fluid (Ecolume) were added to each vial, mixed, and allowed to sit for >24 h before radioactivity was determined by liquid scintillation (Beckman Inc.). For determination of bacterial production, rates of leucine incorporation were converted to rates of bacterial secondary production (kg), assuming 1.44 x mol leucine incorporated (Buesing & Marxsen 2005).

Microbial Community Respiration (Rates of CO_2 Evolution)

Rates of CO_2 evolution from sampling shoot subsections (leaves and stems) were determined using a LiCor LI-6400 Infra Red Gas Analyzer (IRGA) (see Kuehn *et al.* 2004). Subsections of plant litter (3-5 10cm leaves sections; 1 10cm stem section; analyzed separately) were placed into a Petri dish, lined with filter paper, and saturated with 25mL of filtered wetland water. After 2 and 24 hours of incubation in the dark, plant litter was placed into a custom made U-shaped Plexiglas chamber (total volume 135 cm^3), and rates of CO_2 evolution measured. Plant litter samples were allowed to equilibrate in the chamber (3-5 minutes) before rates of CO_2 evolution were recorded. Following respiration measurements, plant litter samples were frozen, lyophilized, and weighed.

Microbial Community Respiration (Rates of O_2 Consumption)

Rates of microbial respiration associated with submerged benthic litter were estimated from measurements of dissolved oxygen consumption (see Su *et al.* 2007). Subsections of litter were placed into small, custom-made glass chambers containing ca. 25ml of filtered (0.7 µm GF/F Whatman) wetland water. Changes in dissolved oxygen concentration within chambers were monitored using a YSI 5010L BOD oxygen probe connected to a YSI 5100 dissolved oxygen meter (Yellow Springs Instrument Co.). All measurements were conducted in darkness at *in situ* temperatures. Additional chambers (ca. 2-4) containing only filtered wetland water were also monitored as controls. Respiration rates were calculated as the slope of regression lines from samples minus the mean slope of the controls. Rates of oxygen consumption were converted to rates of CO_2 evolution with the assumption of a respiratory quotient (RQ) of 0.8.

Annual Production, Respiration, and Growth Efficiency Analysis

Because conditions for assessing fungal and bacterial production (i.e., rates of [1-^{14}C]acetate and ^{3}H-leucine incorporation, respectively) require submergence of plant litter samples in solution, estimates of production and respiration rates of microbial assemblages (fungi and bacteria) inhabiting standing-dead litter were determined using relative humidity data, which reveal time periods (i.e., high relative humidity) in which microbiota are likely to be metabolically active (see Kuehn & Suberkropp 1998a, Kuehn & Suberkropp 1998b, Kuehn *et al.* 2004, Kuehn 2008). Microbiota associated with collected samples were assumed to be metabolically active (i.e., released from water stress) during periods in which

100% relative humidity (dew formation) is reached. Corresponding estimates of production and respiration rates of microbial assemblages (fungi and bacteria) inhabiting submerged benthic litter were assumed to be free from water stress and hence continually active.

In standing-dead litter samples, daily rates of microbial respiration were calculated by multiplying the hourly respiration rates (CO_2 evolution) by the time period (hours per day) in which standing-dead litter was exposed to ≥ 98% relative humidity. Likewise, daily growth rates (μ, $\%d^{-1}$) of fungal decomposers were also calculated by multiplying the hourly fungal growth rate (μ, $\%h^{-1}$) obtained via rates of [1- ^{14}C] acetate incorporation by the corresponding time periods (hours per day) in which standing litter was exposed to ≥ 98% relative humidity. Rates of daily fungal production (mgC g detrital C^{-1} d^{-1}) were subsequently calculated by multiplying litter-associated fungal biomass (B) by the adjusted daily growth rate (μ). Daily rates of bacterial secondary production were calculated by multiplying the hourly bacterial production rates obtain via rates of [^3H]-leucine incorporation by the time period (hours per day) in which standing litter was exposed to ≥ 98% relative humidity. Daily growth rates (μ) for bacterial microbial assemblages were calculated by dividing daily rates of bacterial secondary production by litter-associated bacterial biomass.

Areal daily microbial respiration and production rates were calculated by multiplying daily rates of respiration and microbial production (gC g detrital C^{-1} d^{-1}) by the litter standing stocks (standing and benthic mass - gC m^{-2}) for each plot. Microbial community growth efficiencies were calculated by: MGE = [(Σ fungal production + Σ bacterial production) / (Σ fungal production + Σ bacterial production + Σ microbial community respiration)].

An estimate of annual microbial respiration and production (bacteria and fungi) was

calculated by summing the average daily respiration and production rates (gC m^{-2} d^{-1}) over a 365-day period (Suberkropp 1997, Methvin & Suberkropp 2003, Carter & Suberkropp 2004). In order to obtain microbial respiration and production values for days between sampling dates, the following criteria were assumed and calculated: **1)** Average hourly microbial respiration rates, microbial growth rates (μ, % h^{-1}), and litter microbial biomass (gC g detrital C^{-1}) for ½ of the days between sampling dates were assumed to be equal to the respiration rates, growth rate, and microbial biomass on one sampling date while respiration rates, growth rates, and microbial biomass for the other ½ of days within the interval was assumed to be equal to the corresponding values observed on the next sampling date. Estimates assume no production during winter months due to cold temperatures (November 2004 – March 2005). **2)** To account for mean daily changes in benthic and standing-dead litter detrital mass between sampling dates, a slope and subsequent daily litter mass from the equation of the line was determined for each collection date interval. **3)** In standing dead litter, individual daily respiration and microbial growth rates (μ, % d^{-1}) for days within the sampling interval were calculated (as above) by multiplying the hourly respiration and microbial growth rate (μ, %h^{-1}) by the specific daily time period in hours in which conditions were $\geq$ 98% relative humidity. Microbial respiration and production for each day within the sampling interval was subsequently calculated by multiplying this "adjusted" daily growth rate (μ) by the litter associated microbial biomass. In benthic detritus, daily time period in hours for microbial respiration and growth rate calculations (as above) for all sampling dates was assumed to be 24. Daily microbial respiration and growth rates between sampling intervals were adjusted (Q_{10} = 2) to account for fluctuations in average air temperatures

(standing-dead) and sediment temperature (benthic litter). **4)** Microbial production on an areal basis for each day was then calculated by multiplying daily microbial production (gC g detrital C^{-1} d^{-1}) by the daily "mass adjusted" litter (gC m^{-2}).

Production to biomass ratios (P/B) were calculated by dividing the sum of annual microbial production (gC m^{-2} d^{-1}) by mean annual microbial biomass (gC m^{-2}). Calculated turnover time (d) = [1/(P/B)]*365. Total annual microbial community growth efficiencies (MGE) were calculated by: Annual MGE = [(Σ fungal annual production + Σ bacterial annual production) / (Σ fungal annual production + Σ bacterial annual production + Σ annual microbial community respiration)].

Statistical Analysis

Randomized block ANOVAs were employed to test for differences between litter type (benthic CPOM, stem, leaf). Factors incorporated in the ANOVA model were treatment (benthic CPOM, stem, leaf), time, time x treatment interaction, and blocks nested within time. Since the randomized block ANOVAs were mixed models including both fixed and random factors, appropriate mixed model F-tests were used (Zar 1999). Bonferroni pairwise comparisons were executed to detect differences among treatments. In the event of non-normality or heteroscedasticity, an appropriate data transformation was applied to maximize normality and homoscedasticity. All statistical calculations were performed using the GLM module of Systat 11 (Systat Software Inc. 2004).

RESULTS

Primary Production and Detrital Standing Stocks

Aboveground net primary production (NPP) of *T. angustifolia* shoots collected during August 2004 and 2005 averaged 1110 ± 140 gC m^{-2} $year^{-1}$ and 1001 ± 95 gC m^{-2} $year^{-1}$, respectively (mean ± 1 standard error). Stem detritus (Figure 4B) at the study site ranged from 393 ± 60 gC m^{-2} (October 2004) to 1186 ± 180 gC m^{-2} (May 2005). Low stem detrital mass in October 2004 may be an artifact due to inability to clearly distinguish between the 2003 and 2004 cohorts at the time of processing. Leaf detrital mass (Figure 4B) peaked at 240 ± 44 gC m^{-2} in October 2004 and subsequently decreased throughout the study to 16 ± 7 gC m^{-2} in November 2005 due to leaf fragmentation and collapse to the benthic sediments. Organic material (CPOM) collected from the benthic litter layer (Figure 4A) ranged from a low of 149 ± 9 gC m^{-2} in September 2005 to 277 ± 35 gC m^{-2} in August 2005. Detrital carbon stocks in the benthic layer did not fluctuate greatly throughout the study period.

Fungal Biomass and Production

Fungal biomass associated with aboveground stem detrital carbon ranged from 10.5 ± 1.3 mgC g detrital C^{-1} in April 2005 to 25.0 ± 4.6 mgC gC^{-1} in August 2005. Fungal biomass per g detrital C (Figure 5A) was greater on aboveground leaf detritus than stem detritus ($p < 0.001$), ranging between 55.7 ± 9.6 mgC gC^{-1} in April 2005 to 103.2 ± 21.3 mgC gC^{-1} in June 2005. As the study progressed, fungal biomass on a per m^2 basis (Figure 5C) was the greatest on stem detritus (range: 6.6 ± 1.9 gC m^{-2} [October 2004] to 18.7 ± 3.2 gC m^{-2} [June 2005]) compared to leaf detritus (range: 1.2 ± 0.7 gC m^{-2} [November 2005] to 13.2 ± 3.6 gC m^{-2}

[October 2004]) ($p < 0.001$). This finding corresponded to the greater detrital standing stocks of the stem litter compared to leaf litter within the wetland study site. Fungal biomass associated with benthic litter ranged from 44.3 ± 7.97 mgC gC^{-1} [July 2005] to 77.88 ± 10.67 mgC gC^{-1} [April 2005] (Figure 6A). Fungal biomass associated with benthic detritus on an areal basis ranged from 8.76 ± 1.59 gC m^{-2} in November 2005 to 20.20 ± 3.97 gC m^{-2} in April 2005. Benthic fungal biomass per m^2 was greater than standing leaf fungal biomass ($p < 0.001$) No differences were detected between benthic and stem detritus per m^2 ($p > 0.05$).

Standing-dead fungal production was greater on leaf litter (0.63 ± 0.18 mgC gC^{-1} day^{-1} [average]) compared to stem litter (0.19 ± 0.06 mgC gC^{-1} day^{-1} [average]) ($p < 0.001$) (Figure 7C). Fungal production in the aerial standing detritus was dominated by the fungal community associated with stem litter on a per m^2 basis ($p < 0.001$), which is due to greater stem detrital biomass within the wetland (Figure 7E). Benthic litter-associated fungal production rates (1.72 ± 0.20 mgC gC^{-1} day^{-1} [average]) (Figure 8C) greatly exceeded fungal production rates associated with standing-dead stem ($p < 0.001$) and leaf litter ($p < 0.001$).

Fungal growth rates (Figure 7A) associated with standing-dead stem and leaf detritus averaged 1.09 ± 0.30 % day^{-1} and 0.92 ± 0.26% day^{-1}, respectively, throughout the study period. Fungal growth rates associated with stem detritus ranged from 0.23 ± 0.03% day^{-1} (April 2005) to 3.18 ± 0.30% day^{-1} (November 2005). Fungal growth rates associated with leaf detritus ranged from 0.20 ± 0.02% day^{-1} (September 2005) to 2.68 ± 0.43% day^{-1} (November 2005). Benthic fungal growth rates averaged 2.83 ± 0.37% day^{-1} (Figure 8A), ranging from 1.40 ± 0.16% day^{-1} (October 2004) to 4.93 ± 0.98% day^{-1} (July 2005).

Bacterial Biomass and Production

Bacterial biomass associated with standing-dead stems (Figure 5B) ranged from 0.015 ± 0.004 mgC g detrital C^{-1} in April 2005 to 0.112 ± 0.086 mgC gC^{-1} in July 2005. Bacterial biomass associated with leaf aerial detritus (Figure 5B) ranged from 0.013 ± 0.003 mgC g detrital C^{-1} in April 2005 to 0.109 ± 0.083 mgC gC^{-1} in August 2005. Bacterial biomass per gC associated with standing detritus displayed a general pattern of increase during the months of June through October. No significant bacterial biomass per gC differences were detected between stem and leaf aerial detrital compartments ($p = 1.000$). On a per m^2 basis (Figure 5D), average bacterial biomass on stem detritus (0.051 ± 0.013 gC m^{-2}) exceeded leaf detritus (0.003 ± 0.001 gC m^{-2}) by an order of magnitude due to the greater availability of stem litter in the wetland ($p < 0.001$). Bacterial biomass associated with benthic detritus (Figure 6B) ranged from 1.20 ± 0.33 mgC g detrital C^{-1} in October 2004 to 2.11 ± 0.51 mgC gC^{-1} in April 2005. On a per m^2 basis (Figure 6D), average bacterial biomass associated with benthic detritus (0.33 ± 0.036 g m^{-2}) greatly exceeded bacterial biomass of stem ($p < 0.001$) and leaf ($p < 0.001$) detritus. As a whole, fungal biomass per g detrital C associated with stem ($p < 0.001$) and leaf ($p < 0.001$) standing detritus and benthic CPOM ($p < 0.001$) greatly exceeded bacterial biomass per m^2 in each respective compartment.

Average rates of bacterial production associated with standing-dead leaf litter (Figure 7D) (0.11 ± 0.05 mgC gC^{-1} day^{-1}) was greater than stem litter (0.04 ± 0.01 mgC gC^{-1} day^{-1}) throughout the study period ($p < 0.002$). Bacterial production in the aerial standing detritus was significantly greater in the bacterial community associated with stem litter on a per m^2 basis ($p < 0.001$) when compared to leaf litter (Figure 7F). Fungal production exceeded

bacterial production per g detrital C day [Stem: ($p < 0.003$); Leaf: ($p < 0.001$)] and per m^2 day [Stem: ($p < 0.001$); Leaf: ($p < 0.001$)]. Bacterial production rates in the benthic litter layer (Figure 8D)(2.76 ± 0.27 mgC gC^{-1} day^{-1}) greatly exceeded corresponding rates of bacterial production in standing-dead stem ($p < 0.001$) and leaf ($p < 0.001$) detritus (Figure 7D). Furthermore, in contrast to standing-dead detritus, rates of bacterial production per g detrital C also exceeded fungal production ($p < 0.001$).

Bacterial growth rates associated with standing-dead stem and leaf detritus averaged 199.7 ± 69.3 % day^{-1} and 328.1 ± 115.2 % day^{-1}, respectively (Figure 7B). Bacterial growth rates associated with stem detritus ranged from 36.3 ± 16.1 % day^{-1} (June 2005) to 695.4 ± 297.3% day^{-1} (November 2005). Growth rates associated with leaf detritus ranged from 1.3 ± 1.3% day^{-1} (June 2005) to 1106.2 ± 365.6% day^{-1} (November 2005). Bacterial growth rates associated with benthic detritus averaged 220.6 ± 20.1% day^{-1} (Figure 8B), ranging from 145.86 ± 27.4% day^{-1} (May 2005) to 313.7 ± 53.9% day^{-1} (September 2005).

Community Respiration

Average rates of microbial respiration associated with standing-dead leaf litter (755.6 ± 164.4 $\mu gC\text{-}CO_2$ gC $day^{-1)}$ exceeded stem litter (346.7 ± 140.4 $\mu gC\text{-}CO_2$ gC^{-1} day^{-1})($p < 0.001$). Respiration rates associated with standing-dead stem litter (Figure 9B) ranged from 21.7 ± 4.1 $\mu gC\text{-}CO_2$ gC^{-1} day^{-1} [April 2005] to 1439.7 ± 258.8 $\mu gC\text{-}CO_2$ gC^{-1} day^{-1} [November 2005]. Respiration rates associated with standing-dead leaf litter (Figure 9B) ranged from 85.9 ± 13.6 $\mu gC\text{-}CO_2$ gC^{-1} day^{-1} [April 2005] to 1515.2 ± 430.6 $\mu gC\text{-}CO_2$ gC^{-1} day^{-1} [November 2005]. On an areal basis, rates of microbial respiration associated with

stem litter (228.9 ± 71.3 mgC-CO_2 m^{-2} day^{-1}) exceeded leaf detritus (71.2 ± 35.2 mgC-CO_2 m^{-2} day^{-1})($p < 0.001$) due to greater amounts of stem detrital standing stocks (Figure 9D). Corresponding rates of microbial respiration associated with benthic detritus greatly exceeded rates observed from both standing-dead stem ($p < 0.001$) and leaf ($p < 0.001$) detritus, averaging 8225 ± 1260 µgC-CO_2 gC^{-1} day^{-1} (Figure 9A) (range of 2031 ± 448 µgC-CO_2 gC^{-1} day^{-1} in September 2005 to 15016 ± 2108 µgC-CO_2 gC^{-1} day^{-1} in November 2005).

Microbial Growth Efficiencies and Annual Microbial Production

Microbial growth efficiencies within the stem and leaf senescent standing stocks were 43.3 ± 3.4% and 48.8 ± 4.7%, respectively. Stem microbial growth efficiencies during the study period ranged from 29.8 ± 5.5% [October 2004] to 60.0 ± 5.9% [August 2005]. Leaf microbial growth efficiencies ranged from 20.0 ± 3.5% [October 2004] to 63.3 ± 7.3% [November 2005]. Microbial growth efficiencies in the benthic litter layer were 39.3 ± 3.4% with a range of 28.5 ± 2.6% [October 2005] to 63.6 ± 6.6% [November 2005]. Integrated throughout the study period, total annual fungal production associated with standing-dead shoots and benthic litter was 42.7 gC m^{-2} yr^{-1} and 113.3 gC m^{-2} yr^{-1}, respectively. Comparatively, total annual bacterial production associated with standing-dead shoots and benthic litter was 7.2 gC m^{-2} yr^{-1} and 244.4 gC m^{-2} yr^{-1}, respectively. Annual microbial production and respiration estimates for each decomposition compartment, P:B ratios, and microbial turnover times are described in Table 1.

DISCUSSION

Emergent Net Primary Production and Litter Dynamics

The majority of living *Typha angustifolia* biomass produced (NPP) within Paint Creek Wetland entered into the standing-dead detrital pool followed by fragmentation and collapse to the benthos. Aboveground shoot production (per m^2) was similar during the 2004 and 2005 growing season, suggesting a similar periodicity in detrital inputs to the Paint Creek Wetland site.

During the study period, total detrital mass per m^2 was largely composed of aerial standing-dead plant litter, with *Typha* stem litter constituting the largest bulk of standing detrital mass when compared to *Typha* leaf litter. Complete senescence of *Typha* shoots (stems and leaf-blades) did not occur until after the October 2004 sampling date, resulting in maximum estimates of standing-dead litter in Spring 2005 followed by gradual decline as the study progressed. In late Fall, most of *Typha* leaf-blades collapsed from standing shoots coincident with the senescence and translocation of nutrients from living shoots. As a consequence, very little standing-dead *Typha* leaf litter remained during the study period.

Although emergent macrophyte decomposition initially begins in the aerial standing position, a large proportion of the standing litter will eventually collapse to the sediment-water interface where commencement of benthic phase litter decomposition begins (Gulis *et al.* 2006, Gessner *et al.* 2007, Kuehn 2008). In addition to the large structurally intact benthic organic material (CPOM), the benthic litter layer also consists of plant detritus in various stages of decomposition including fine particulate organic matter (FPOM) and dissolved organic matter (DOM). Since only intact and recognizable stem and leaf litter

(CPOM) was sampled during this study, our partial microbial organic carbon budget within this freshwater wetland system can be considered conservative. Throughout the study period, benthic CPOM litter standing stocks per m^2 did not fluctuate greatly, suggesting that removal or transport of benthic detrital carbon were offset by inputs from the standing-dead litter.

In the present study, standing-dead and benthic *Typha* litter compartments are exposed to distinctly different environmental conditions, which ultimately influence the corresponding decay activities (i.e., production and respiration) of litter-associated microbial assemblages. Because of the harsh aerial environment, microbial decomposers associated with standing-dead litter are limited by the availability of water (Kuehn & Suberkropp 1998a, Kuehn *et al.* 1998, Kuehn *et al*. 2004). Prior laboratory-based studies reported that litter-inhabiting microbial communities respond rapidly to increased water availability, with significant increases in rates of microbial respiration (CO_2 evolution) occurring within 5 min after water addition (Kuehn & Suberkropp 1998a, Kuehn *et al.* 1998, Kuehn *et al.* 2004). Under *in situ* conditions, microbial assemblages inhabiting standing-dead litter display a diel periodicity in respiratory (metabolic) activities, with the highest rates of respiration observed at night following dew formation (i.e., increased water availability). As a consequence, total daily activity of microbial assemblages associated with standing-dead litter is restricted to time periods in which sufficient water is available.

In the present study, estimates of carbon flow (i.e., organic matter budget) into and through standing litter microbial assemblages is conservative, as I assumed that all decomposer activity was restricted to periods ≥ 98% relative humidity (i.e., dew formation). Microbial activities associated with standing-dead litter that might have occurred during

periods < 98% relative humidity were not included in this budget estimation. In contrast to the standing-dead decomposition phase, the presence of water-saturated sediments and periodic flooding of collapsed *Typha* resulted in benthic litter that remained continually hydrated. As a consequence, the lack of water stress resulted in higher daily rates of CO_2 evolution and microbial production in benthic litter layer when compared to standing-dead detritus.

Microbial Biomass and Rates of Production in Standing-Dead Detritus

Variations in microbial carbon flow dynamics within aerial emergent plant litter are dictated by environmental conditions and their concomitant effects on the decomposer activities of litter inhibiting microbial assemblages (bacteria and fungi). Prior studies have firmly established that filamentous fungi pervasively colonize standing-dead detritus within both salt marsh and freshwater marsh ecosystems (Newell & Porter 2000, Gulis *et al.* 2006, Gessner *et al.* 2007, Kuehn 2008). At the Paint Creek Wetland, standing litter-associated fungal biomass reached a maximum during the summer months when air temperatures and increased nightly dew formation were maximized. Mean fungal biomass and rates of production associated with standing-dead leaf detritus per gram detrital carbon were significantly greater than corresponding estimates from stem detritus, which is likely a result of structural quality (i.e., nutrient concentrations, lignocellulose) of the litter (Gessner *et al.* 2007). Throughout the study period, highest rates of standing litter fungal production per g detrital carbon occurred during maximal hours of activity (i.e., ≥ 98% relative humidity) coupled with fungal assemblages decomposing leaf litter.

Bacterial biomass and production associated with standing-dead litter per gram detrital carbon was trivial when directly compared to estimates of standing litter-inhabiting fungal decomposers. Similar to fungal biomass, peak bacterial biomass was observed during increased summer temperatures and periods of dew formation. However, bacterial biomass and rates of production per g detrital carbon in the standing-dead detritus were significantly less than corresponding fungal assemblage estimates.

The results of this research are consistent with prior studies that have simultaneously investigated bacterial and fungal biomass and production rates within standing-dead litter of *Juncus effusus* (Kuehn *et al.* 2000), as well as *Phragmites australis* and *Typha angustifolia* (Findlay *et al.* 2002). For example, Kuehn *et al.* (2000) observed a range of *J. effusus* standing litter-associated fungal biomass between 4-84 mgC gC^{-1}, compared to the standing litter bacterial biomass range between 0.042-0.56 mgC gC^{-1}. In June 1994 of the same study, Kuehn *et al.* (2000) found higher fungal production rates associated with *J. effusus* litter than bacterial production rates, 2.00 ± 0.64 mgC gC^{-1} d^{-1} and 0.12 ± 0.04 mgC gC^{-1} d^{-1} respectively. Similar to fungal decomposers, leaf-associated bacterial production rates per gram detritus were significantly greater than stem-associated production rates. Interestingly, no detectable differences were observed when comparing standing-dead leaf and stem bacterial biomass, possibly an artifact of the inability to distinguish between living and dead bacteria during enumeration.

To date, no published studies have estimated standing litter-associated microbial biomass and rates of production in freshwater wetlands on an areal basis (Kuehn 2008). In contrast to findings of this study on a per gram detritial carbon basis, estimates of microbial

(fungal and bacterial) biomass and rates of production per m^2 of wetland were significantly greater on standing-dead stem litter than leaf litter, reflecting the scarcity of aerial *T. angustifolia* detrital leaves due to its early fragmentation and collapse. Since standing-dead stem litter resists fragmentation and collapse to the benthic sediments, retention of detrital stem mass in the aerial position allows for microbial community biomass accumulation over extended periods of time. When directly compared, rates of bacterial secondary production m^{-2} day^{-1} associated with both standing-dead stem and leaf litter were significantly lower than corresponding rates of fungal secondary production m^{-2} day^{-1}. Overall, the results of this study suggest that fungal organisms are uniquely adapted to the harsh environmental conditions characteristic of the standing-dead litter environment and that appreciable amounts of plant litter carbon flow through litter associated fungal decomposers.

Microbial Biomass and Rates of Production in Benthic Litter

Although microbial dynamics associated with submerged leaf litter in lotic systems have been intensely researched (Gulis *et al.* 2006, Gessner *et al.* 2007), there remains a paucity of data concerning fungal and bacterial dynamics in benthic litter of freshwater wetlands. This study provides critical data of litter processing within the microbial "black box" (i.e., production and respiration rates) of freshwater wetlands by directly comparing aerial and benthic decomposition phases. Per g detrital C, the flow of carbon from benthic litter into microbial decomposers was significantly greater than from aerial detritus, leading to accelerated microbial biomass accumulation and carbon mineralization within benthic litter compartment. Overall, the increase in microbial production rates that occurred within

the benthic litter layer would be expected, as benthic litter-associated assemblages were assumed to be continually free of water stress on a daily basis. In contrast, decomposer activities within standing-litter were restricted to time periods of increased water availability (i.e., nighttime dew formation). Furthermore, benthic microbial production estimates obtained in the present study may be underestimates, since only microbial activities associated with CPOM were quantified. Thus, total microbial secondary production is likely much larger.

Within Paint Creek Wetland, daily fungal biomass per gram detrital carbon in the benthic litter layer exceeded fungal biomass in standing-dead stems, but no significant differences were detected when compared to leaf litter. Fungal production rates per g detrital C^{-1} day^{-1} in the benthos were significantly greater than corresponding rates observed in standing-dead stem and leaf litter, indicating that environmental conditions (i.e., water availability) in benthic litter increase daily conversion rates of detrital carbon into fungal biomass. Fungal biomass and production rates observed in the present study are within the range reported in other studies examining fungal dynamics of submerged litter in freshwater marshes (Komínková *et al.* 2000, Kuehn *et al.* 2000, Findlay *et al.* 2002, Buesing & Gessner 2006, Su *et al.* 2007). For example, Su *et al.* reported *T. angustifolia* litter-associated fungal biomass ranging from 20 to 125 mgC g detrital C^{-1}. Buesing and Gessner (2006) found fungal production rates associated within submerged *P. australis* in a freshwater wetland to average 0.7 mgC gC^{-1} day^{-1} with a maxima of 2.4 mgC gC^{-1} day^{-1}. Thus, significant increases in fungal biomass and production rates associated with saturated benthic litter highlight the importance of water stress removal as it pertains to plant litter processing in

natural wetland environments.

Compared to the scant bacterial colonization of aerial litter, a significant increase in bacterial biomass and production occurred within benthic detritus litter, resulting in a heightened contribution of bacterial decomposers to submerged litter breakdown. Bacterial biomass and rates of production per gram of detritus were significantly greater than corresponding values observed in standing-dead stem and leaf litter. Constant hydration and increased surface area accessibility for bacterial colonization of litter within the benthic zone likely caused these observed increases in bacterial biomass and production.

In the present study, bacterial biomass values per gram detrital carbon were directly comparable to the ranges in previous submerged litter bag studies (Kuehn *et al.* 2000, Su *et al.* 2007). For example, Su *et al.* (2007) found bacterial biomass on *T. angustifolia* ranging from 0.10-2.20 mg C g detrital C^{-1}. Compared to previous bacterial production studies per gram detritus (Kuehn *et al.* 2000, Findley *et al.* 2002, Buesing & Gessner 2006, Su *et al.* 2007), bacterial production rates of this research were between the results published by studies. For example, Kuehn *et al.* (2000) reported submerged *J. effusus* litter-associated bacterial production rates ranging from 0.00 - 0.08 mgC gC^{-1} d^{-1}. In contrast, Buesing and Gessner (2006) reported bacterial production rates associated with *P. australis* submerged litter ranging between 2.6 – 18.8 mgC gC^{-1} d^{-1}.

On an areal perspective, very few studies have investigated rates of microbial production per m^2. Buesing and Gessner (2006) were the first to provide estimates of fungal and bacterial production associated with submerged plant litter. Other areal studies have reported estimates of leaf litter-associated fungal production (i.e., aquatic hyphomycetes) in

streams systems (Suberkropp 1997, Carter & Suberkropp 2004, Methvin & Suberkropp 2003). In this areal study, the data suggested significantly higher benthic litter-associated fungal production rates per m^2 compared to standing-dead stem and leaf litter.

Litter-associated bacterial biomass and rates of production per m^2 were significantly greater than those observed on standing-dead stem and leaf litter. Total bacterial production associated within benthic litter in Paint Creek Wetland was 611.8 mgC m^{-2} d^{-1}. In contrast, Buesing and Gessner (2006) observed extremely high rates of bacterial production (e.g., average 1.9 gC m^{-2} d^{-1} in benthic litter). The high bacterial production rates reported by Buesing and Gessner (2006) may be a result of the high final concentration (50µmol) of radio-labeled leucine employed in the leucine incorporation technique (Buesing & Gessner 2003, 2006, see also Kuehn 2008). In the present study, rates of bacterial production were estimated using a modified procedure that employed a final concentration of 2.5µmol radio-labeled leucine (Gillies *et al.* 2006), which may have resulted in a more realistic estimate of carbon flow into litter-associated bacterial decomposers.

Microbial Community Respiration

The CO_2 flux respired from litter-associated heterotrophic decomposers is an important component of the total carbon flow within freshwater wetlands (Gessner *et al.* 2007, Kuehn 2008). As expected with higher microbial production rates in benthic detritus, microbial community respiration associated with benthic decomposition m^{-2} day^{-1} was significantly greater than corresponding CO_2 mineralization from aerial standing-dead detritus. Increased fungal production as well as the dramatic increase of bacterial production

generated a significant increase of carbon mineralization into the atmosphere from benthic litter. During summer periods, CO_2 flux m^{-2} day^{-1} was nearly an order of magnitude greater within the benthic litter compartment as compared to aerial detritus, despite a peak in aerial microbial assemblage production. Although carbon mineralization was lower in the aerial litter, the annual contribution of CO_2 flux from stem and leaf aerial senescent litter composed 9.4% of the total annual carbon mineralized per m^2 from Paint Creek Wetland. Respiration rates observed in the present study (per g detrital C) concur with microbial respiration rates reported in previous benthic (Kominkova *et al.* 2000, Buesing & Gessner 2006, Su *et al.* 2007) and standing dead litter studies (Kuehn & Suberkropp 1998a, Kuehn *et al.* 1998, Kuehn *et al.* 1999, Kuehn *et al.* 2004). For example, Su *et al*. (2006) reported microbial respiration rates associated with submerged *T. angustifolia* litter ranging between 150 and 750 $\mu gC\text{-}CO_2\ gC^{-1}\ hr^{-1}$. Similarly, Kuehn *et al.* (2004) reported carbon mineralization from *P. australis* standing-dead plant detritus ranging between 20 $\mu gC\text{-}CO_2\ gC^{-1}\ hr^{-1}$ (dry periods) to 240 $\mu gC\text{-}CO_2\ gC^{-1}\ hr^{-1}$ during periods > 95% relative humidity.

Annual Microbial Production and Carbon Mineralization

The implication of this study impacts our current understanding of **1)** total annual carbon flow from standing-dead and benthic plant litter into secondary microbial decomposers in temperate freshwater marshes and **2)** contribution of microbial carbon mineralization to the atmosphere from freshwater wetlands. Of the peak *T. angustifolia* 2004 cohort NPP, ~3.8% and ~0.6% of living aerial shoots were assimilated into fungal and bacterial biomass, respectively. In addition, ~5.1% of 2004 *Typha* NPP was respired as CO_2

from the standing-dead microbial community. When analyzing annual standing-dead carbon flow into microbial biomass, total production of fungal biomass accounted for ~86% of the total microbial detritial carbon accumulation, indicating the importance of fungal assemblages in this decomposition phase. As expected, total carbon flow into microbial decomposers in the benthic litter exceeded estimates of detrital carbon flow within the standing-dead litter as a result of the prevailing environmental conditions (i.e., water availability) and concomitant increase in overall rates of bacterial and fungal production. The influence of bacterial assemblages increased in the benthic litter decomposition phase, accounting for ~68% of the total detrital carbon flow into microbial biomass. Since benthic microbial activity dominates plant litter processing in the Paint Creek Wetland, the greatest contribution of CO_2 mineralization from the *Typha* litter was respired from benthic litter-associated decomposers. Considering annual areal CO_2 flux from *T. angustifolia* litter in Paint Creek Wetland, the benthic litter-associated microbial community mineralized ~91% of the total 607.9 gC-CO_2 m^{-2} $year^{-1}$.

Microbial Growth Efficiencies

In addition to the determination of an organic carbon budget, microbial heterotrophic respiration data are essential when elucidating microbial growth efficiencies. Microbial growth efficiency (MGE) is defined as the quantity of microbial biomass produced divided by the quantity of organic substrate assimilated (del Giorgio & Cole 1998). Microbial growth efficiencies of secondary decomposers within the aerial and benthic litter from this study suggest a range of 20% - 64%, which is typical of microbial communities processing

litter in streams and wetlands (Suberkropp 1991, Komikova *et al.* 2000, Buesing & Gessner 2006). For example, Buesing and Gessner (2006) determined MGE to range between 29% and 65% during benthic decomposition of *P. australis* in a freshwater marsh.

Conclusion

In Paint Creek Wetland, microbial secondary decomposers assimilate and mineralize appreciable amounts of carbon from detrital substrates in standing-dead and benthic litter over an annual cycle. Within standing-dead litter stocks, fungal microassemblages dominate plant litter assimilation into biomass when compared to bacterial microassemblages. From an annual areal perspective, microbial production in standing-dead stem litter significantly exceeds standing-dead leaf litter even though higher rates of production were measured in standing-dead leaf litter per g detrital carbon. This finding corresponds to the greater detrital standing stocks of the stem litter compared to leaf litter within the wetland study site.

Benthic litter-associated microbial assemblage production rates greatly exceeded microbial production rates in standing-dead stem and leaf litter. Standing-dead litter-associated microbial activity was likely reliant upon dew formation (periods $\geq$ 98% relative humidity) resulting in constrained activity hours during the year. In contrast, benthic microbial activity was not limited by water availability, thus microbial production rates are elevated in this phase of decomposition. Opposite to the pattern observed in standing-dead litter, bacterial production rates in benthic litter were greater than fungal production rates. Furthermore, annual mineralized carbon flux respired per m^2 from benthic litter was significantly greater than standing-dead stem and leaf carbon mineralization per m^2. Higher

rates of microbial respiration in this litter decay phase correspond to higher rates of fungal and bacterial production in this submerged litter environment.

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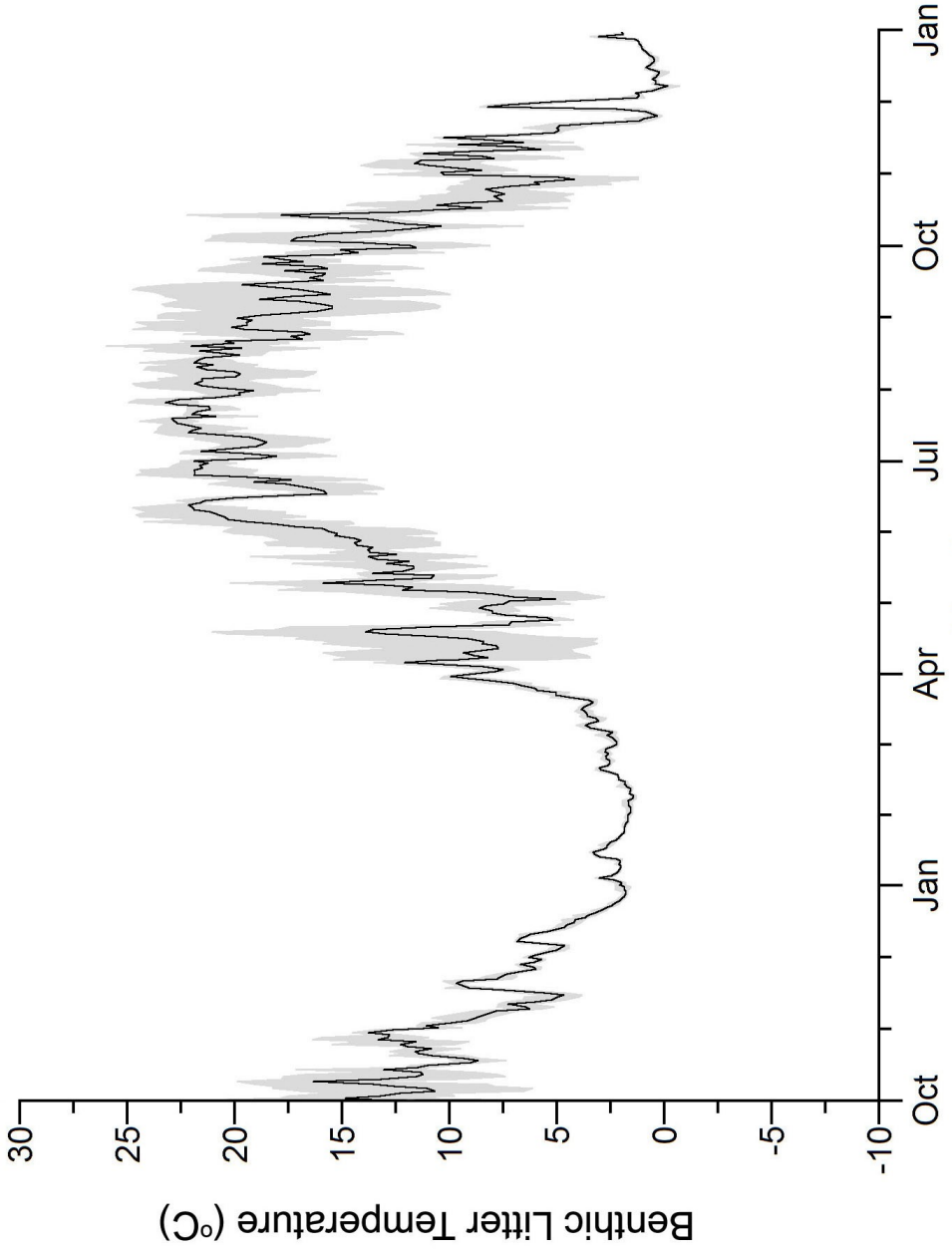

Figure 1: Daily range (gray area) and average (black line) of benthic litter layer temperature within the *Typha* stand during the study period.

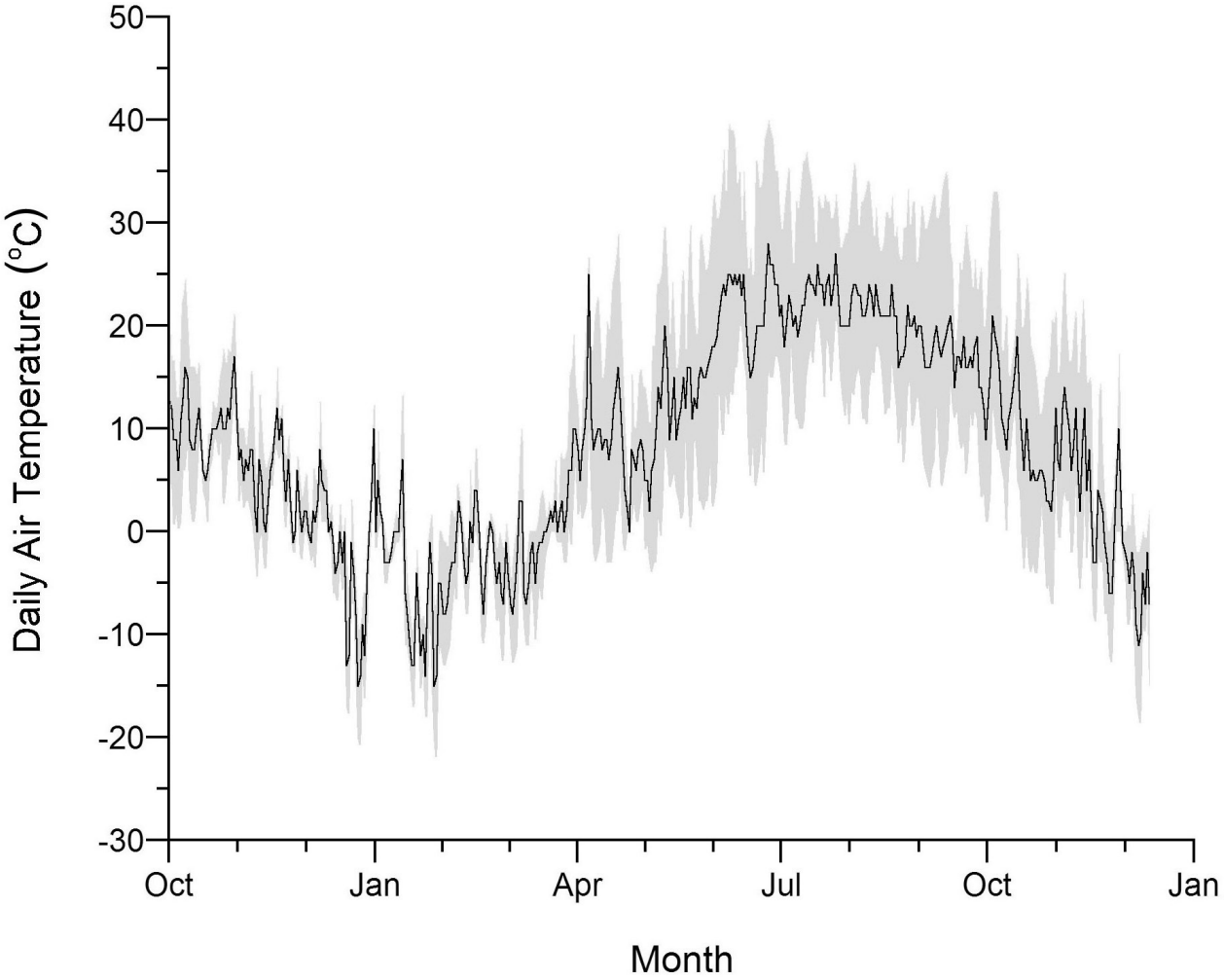

Figure 2: Daily range (gray area) and average (black line) temperature within aerial detritus of the *Typha* stand during the study period.

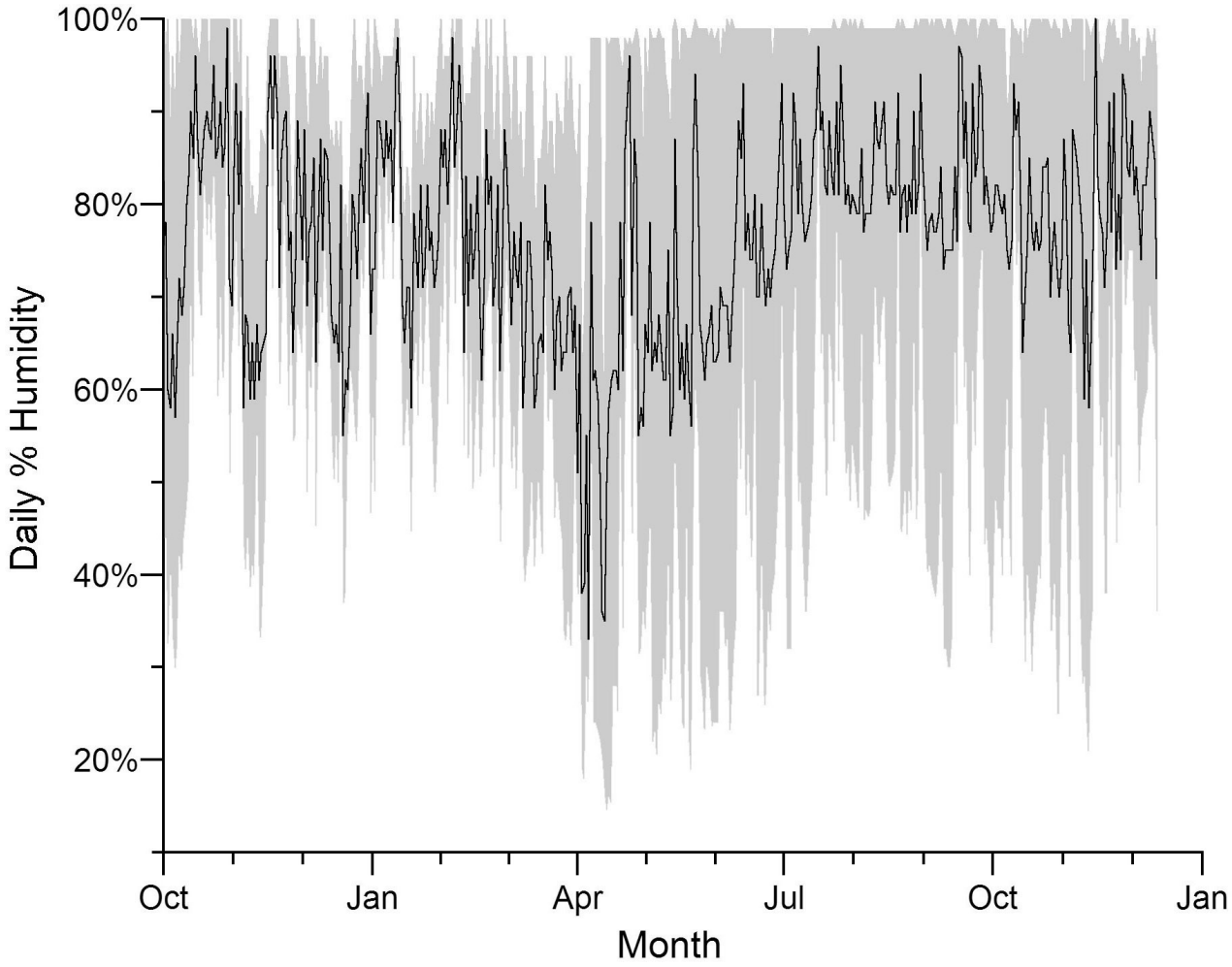

Figure 3: Daily range (gray area) and average (black line) % relative humidity in aerial detritus within the *Typha* stand during the study period.

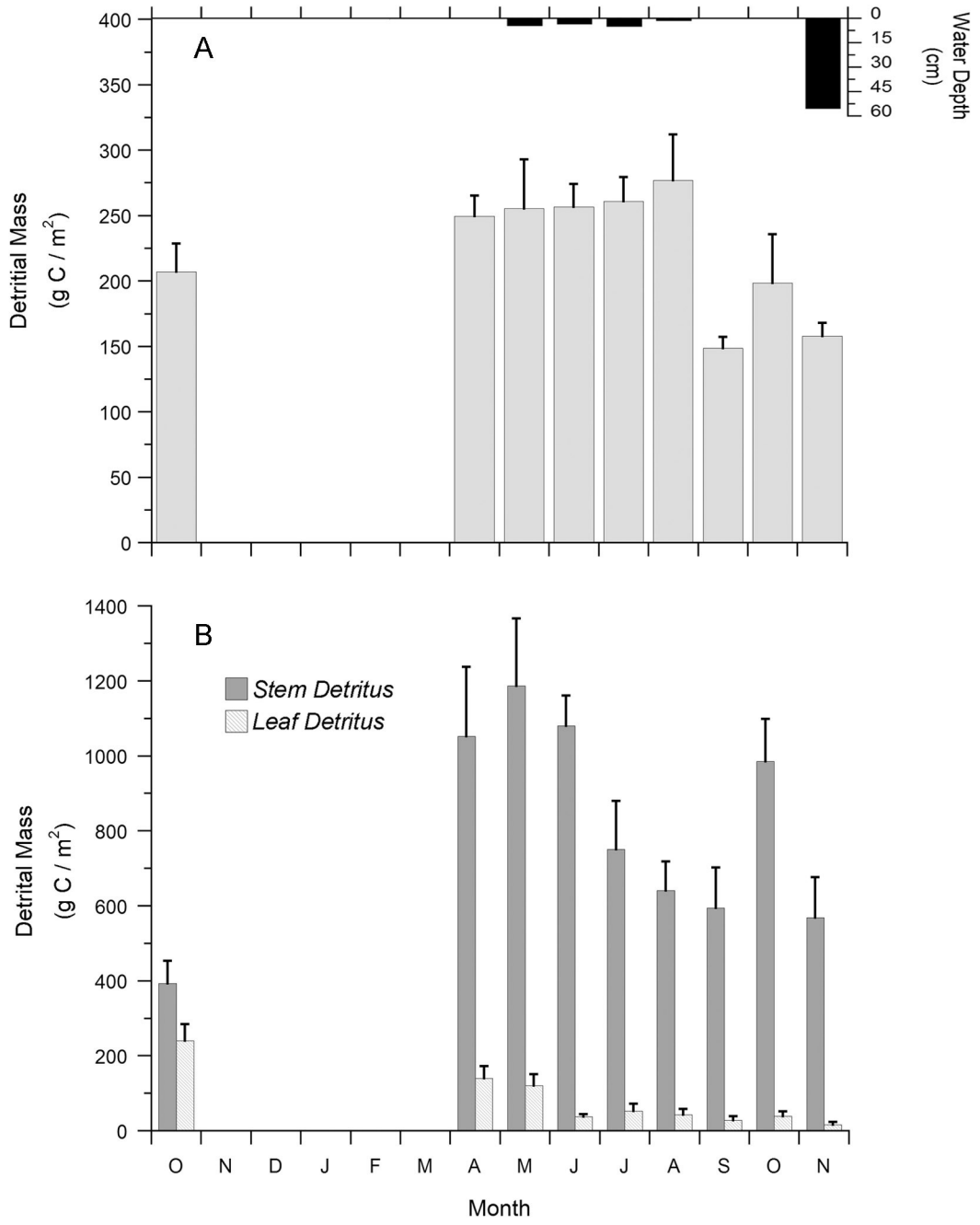

Figure 4: Detrital mass of the benthic litter (A) and stem/leaf aerial detritus (B) per square meter in the investigated *Typha* stand. The black bar graph (A) represents mean water depth on the sampling date in the benthic litter layer. Values indicate means ± 1 SE, n = 6.

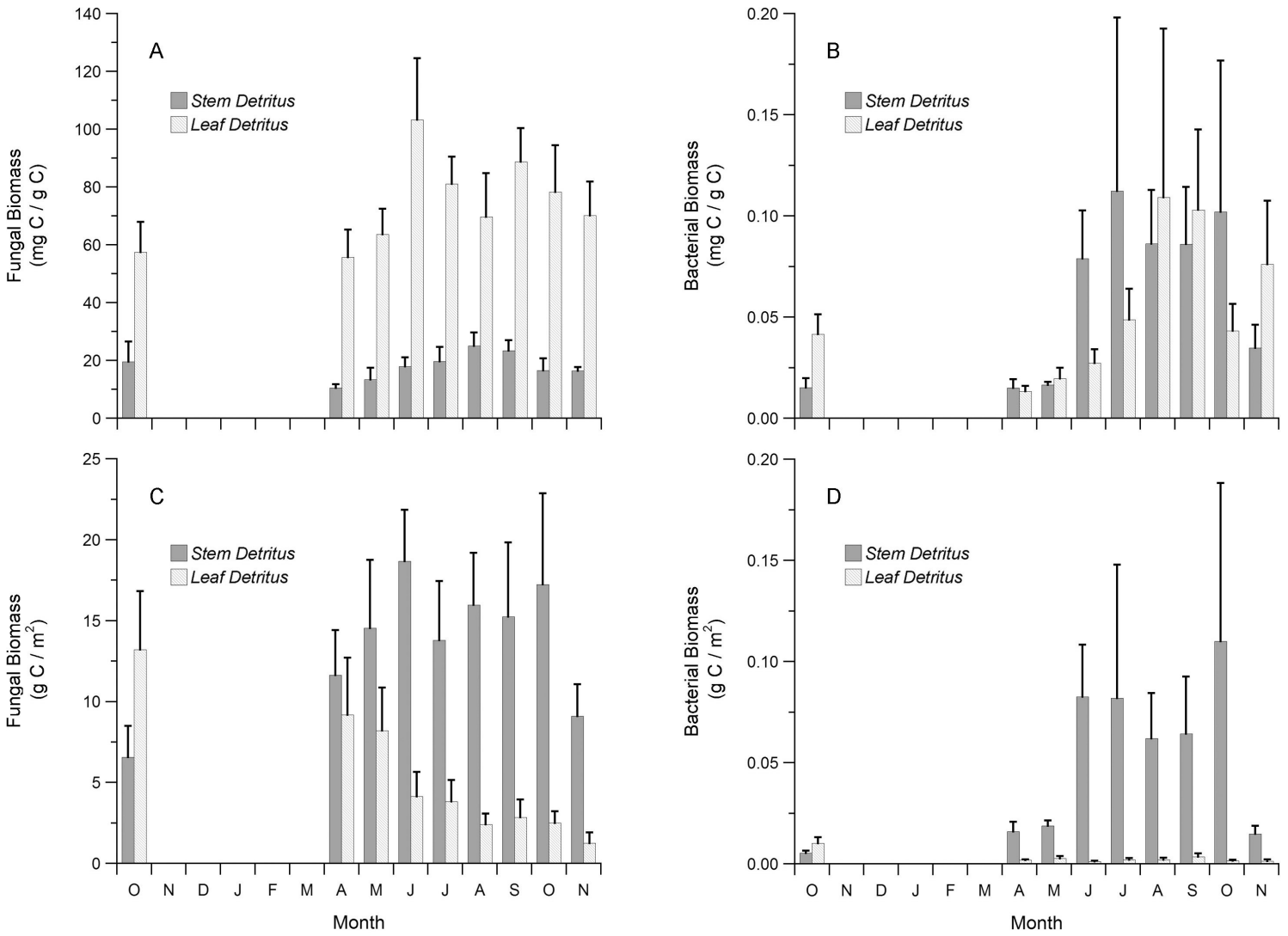

Figure 5: Fungal (A) and bacterial (B) biomass per gram detrital carbon in aerial detritus. Fungal (C) and bacterial (D) biomass per square meter of aerial detritus in the *Typha* stand. During analysis, aerial detritus was separated into stems and leaves. Values indicate means ± 1 SE, n = 6.

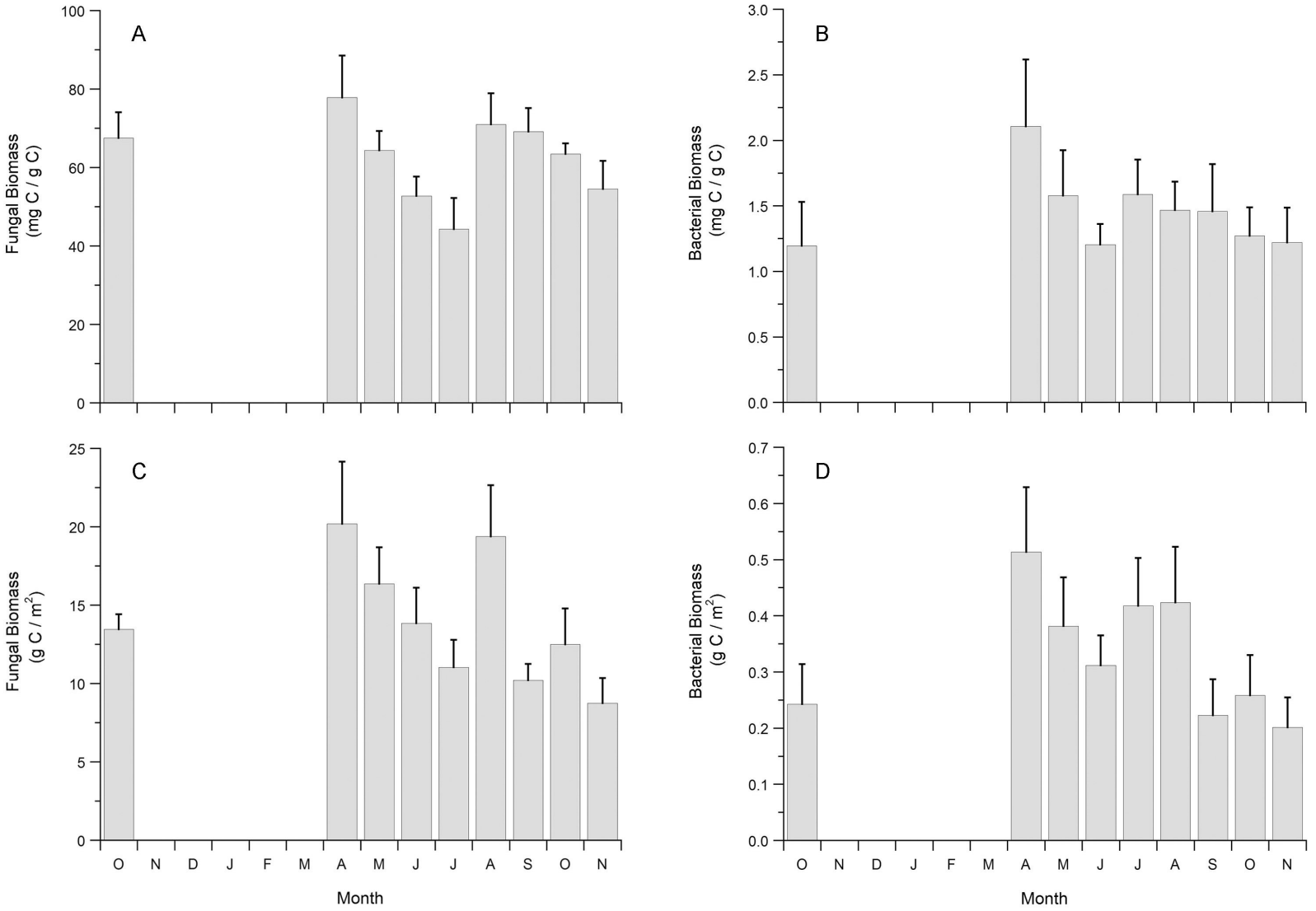

Figure 6: Fungal (A) and bacterial (B) biomass per gram detrital carbon in the benthic litter layer. Fungal (C) and bacterial (D) biomass per square meter of benthic litter in the *Typha* stand. Values indicate means ± 1 SE, n = 6.

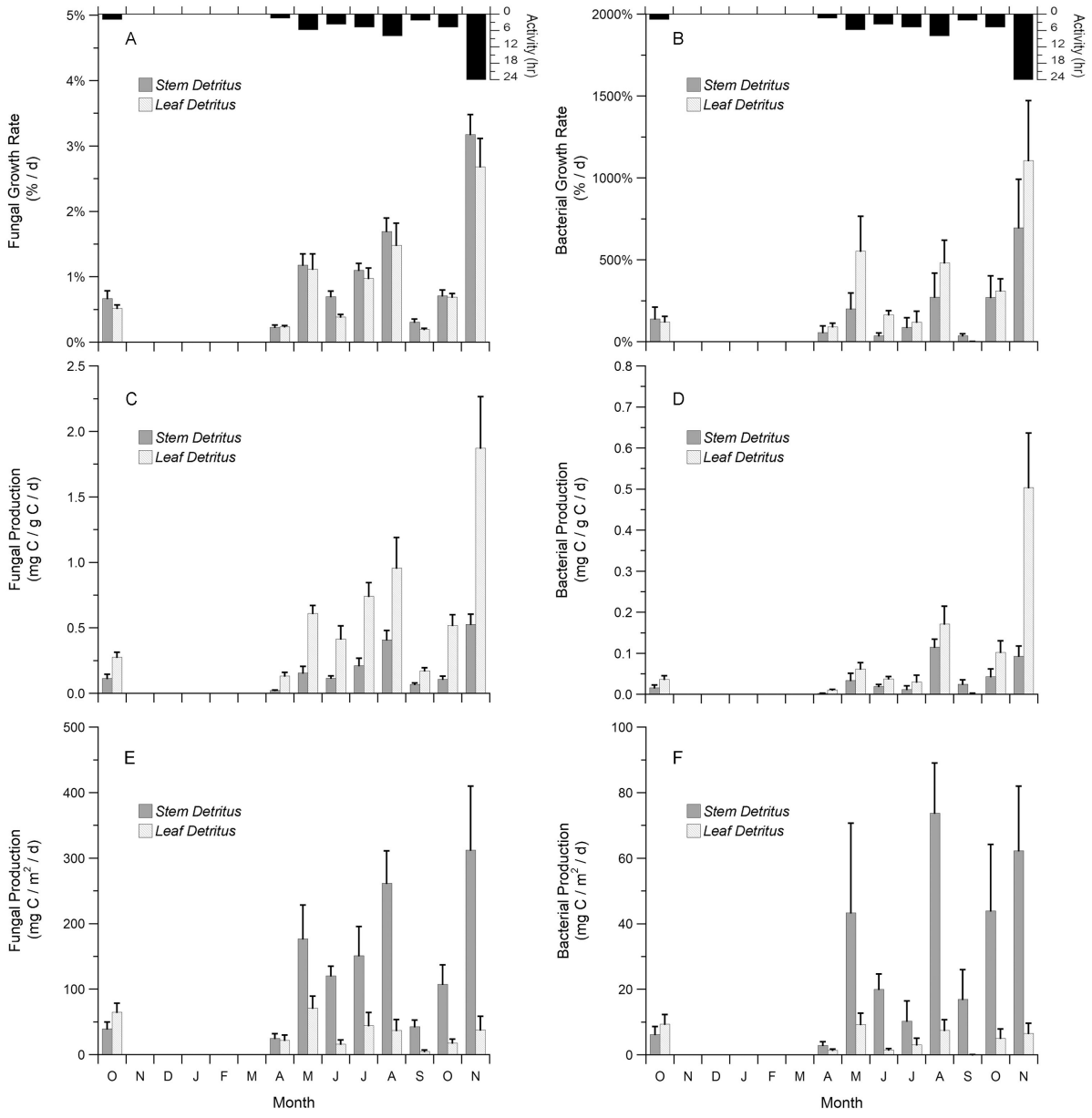

Figure 7: Fungal (A) and bacterial (B) growth rate per day in aerial detritus. The black bar graph (A,B) represents the hours of activity on the sampling date dictated by % relative humidity. Fungal (C) and bacterial (D) production per gram detrital carbon in aerial detritus. Fungal (E) and bacterial (F) production per square meter of aerial detritus in the *Typha* stand. During analysis, aerial detritus was separated into stems and leaves. Values indicate means ± 1 SE, n = 6.

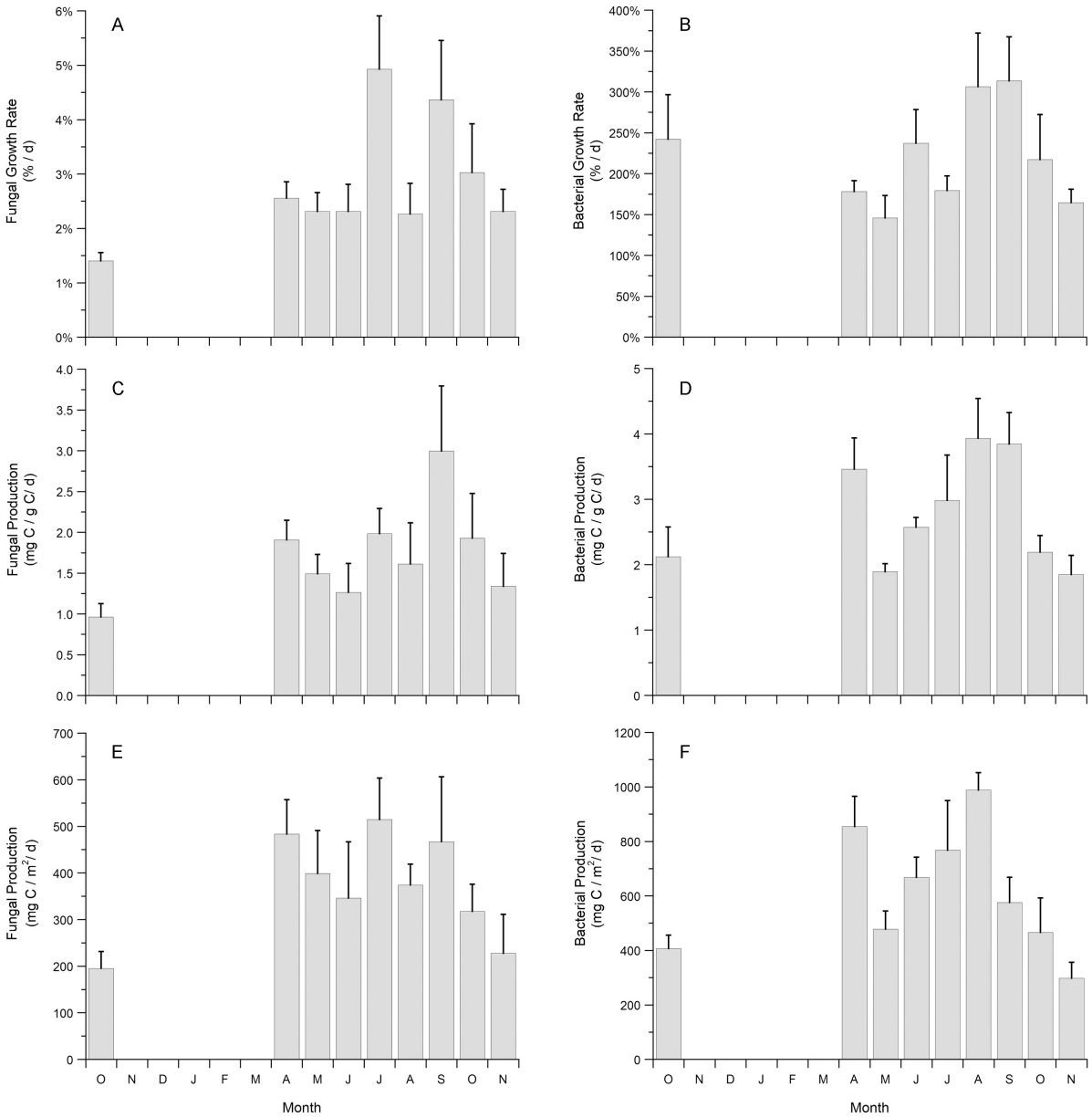

Figure 8: Fungal (A) and bacterial (B) growth rate per day in benthic litter layer. Fungal (C) and bacterial (D) production per gram detrital carbon in benthic litter layer. Fungal (E) and bacterial (F) production per square meter of benthic litter in the *Typha* stand. Values indicate means ± 1 SE, n = 6.

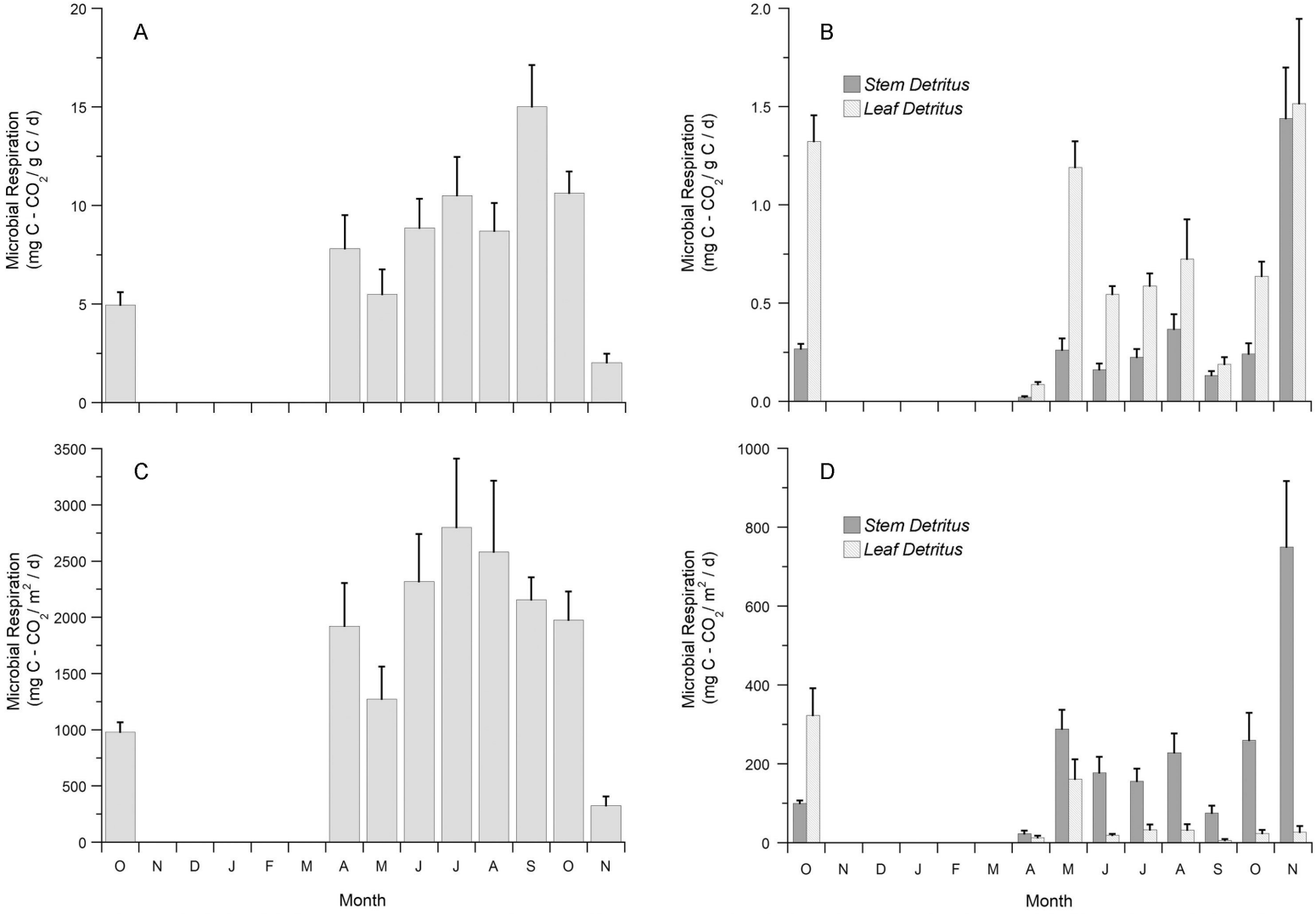

Figure 9: Microbial community respiration as CO_2 per gram detrital carbon per day in benthic litter layer (A) and aerial detritus (B). Microbial community respiration as CO_2 per square meter of *Typha* stand per day in benthic litter layer (C) and aerial detritus (D). Values indicate means ± 1 SE, n = 6.

	Benthic Litter	SD Stems	SD Leaves
Mean Annual Biomass			
Bacteria (gC m^{-2})	0.364	0.037	0.004
Fungi (gC m^{-2})	15.4	13.0	7.4
Annual Production			
Bacteria (gC m^{-2} yr^{-1})	244.4	6.5	0.7
Fungi (gC m^{-2} yr^{-1})	113.3	34.7	8.0
Annual Microbial Respiration (gC m^{-2} yr^{-1})	551.00	45.40	11.50
Production:Biomass			
Bacteria (P/B)	672.3	174.3	178.4
Fungi (P/B)	7.3	2.7	1.1
Turnover Time			
Bacteria (d)	0.54	2.09	2.05
Fungi (d)	49.69	136.41	337.30
Annual Microbial Growth Efficiency (%)	39.37	47.60	42.93

Table 1: Annual microbial production and respiration in benthic and standing-dead (SD) decomposition phases within the *Typha* stand during the study period.