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Contributions of fungi to carbon flow and nutrient cycling from standing dead *Typha angustifolia* leaf litter in a temperate freshwater marsh

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Abstract

Standing dead plant litter often constitutes a large fraction of the detritus in many freshwater marshes and lake littoral habitats. Despite this evidence, microbial decay processes in standing litter and its contribution to wetland carbon and nutrient cycling have rarely been quantified. We examined the contribution of fungi to carbon flow and nutrient cycling from *Typha angustifolia* during senescence and standing litter decomposition. Naturally standing *Typha* leaves were collected in August and then periodically over 1 yr. We quantified losses in leaf carbon (C), fungal biomass, and fungal production rates and constructed a partial budget estimating C flow into fungal decomposers. Additionally, we determined leaf litter N and P concentrations to assess the effect of fungi on detrital nutrient dynamics. Significant losses in leaf C occurred during plant senescence and standing litter decay ($\sim$ 55%). Fungal biomass increased during litter decay, reaching a maximum of 106 $\pm$ 7 mg C g^{-1} detrital C. Cumulative fungal production totaled 123 mg C g^{-1} initial detrital C, indicating that 22% of the *Typha* leaf C lost was assimilated into fungal biomass. Fungi also transformed and immobilized nutrients within *Typha* leaves, with fungal N and P accounting for $>$ 50% of the total detrital N and P during later stages of leaf decay. Significant transformation and decomposition of emergent macrophyte litter occurs during the standing dead phase, and a large portion of the plant C and nutrients are channeled into and through fungal decomposers.

Detrital pathways typically dominate the flow of carbon and the recycling of nutrients in terrestrial and aquatic ecosystems (Cebrian 1999; Moore et al. 2004). As a result, heterotrophic microbial decomposers can be viewed as important components of the ecosystem, since many of the fundamental processes related to carbon flow and elemental cycling are intimately coupled with and influenced by their metabolic activities (i.e., biomass production, respiration, nutrient immobilization, or mineralization). However, although widely regarded as important in carbon and nutrient cycling, our quantitative understanding of microbial processes within many ecosystems remains limited (Cebrian 2004), particularly in regard to the role of fungal decomposers.

Freshwater marshes are widely recognized as among the most productive ecosystems on earth (Mitsch and Gosselink 2007). Emergent vascular plants, such as *Typha* (cattails) and *Phragmites* (reed), are a common vegetative feature within many freshwater marshes and often constitute a large fraction of the annual plant biomass produced (Mitsch and Gosselink 2007). Most of this plant biomass enters the detrital pool, where heterotrophic microbes colonize, enzymatically degrade, and assimilate plant litter carbon and nutrients in amounts that bring about the decomposition and mineralization of plant material. The resulting microbial biomass associated with plant litter in turn serves as an important food resource for detritus-feeding consumers (Silliman and Newell 2003), which through their feeding activities help facilitate the further breakdown and recycling of plant litter carbon and nutrients.

Despite the well-known importance of emergent vascular plant detritus in freshwater marshes, the natural ontogeny of plant litter decay within these systems has rarely been investigated (Gessner et al. 2007). Furthermore, litter decay processes within freshwater marshes have rarely been examined in relation to the decay activities (i.e., secondary production, respiration) of the microbial communities that influence nutrient retention or release and the fate of carbon. For example, in most emergent macrophytes, abscission and collapse of plant matter to the sediments or overlying surface waters does not typically occur following plant shoot senescence and death. As a result, initial microbial colonization and decomposition of plant matter (leaf blades or shoots) begins in an aerial standing dead position. Large amounts of standing dead plant litter have been reported in freshwater marshes (Asaeda et al. 2002), where it often represents a significant fraction of the total detrital mass.

Studies dating back more than 100 yr (Saccardo 1898) have established that filamentous fungi pervasively colonize and reproduce on and within standing dead litter of emergent macrophytes in both salt and freshwater marsh habitats (Kohlmeyer and Kohlmeyer 1979; Ellis and Ellis 1997). More recent studies over the last several decades have provided evidence that these fungal communities can accumulate significant quantities of biomass within decaying standing litter (Gessner et al. 2007) and are metabolically adapted to the harsh physical conditions (fluctuations in temperature, water availability) that prevail within the standing litter environment (Kuehn et al. 2004). Collectively, these findings imply that litter-inhabiting fungi are

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Table 1. Selected characteristics of Independence Lake surface waters and the littoral *T. angustifolia* stand, where standing dead leaf decomposition studies were conducted. Values are the means ± 1 SD, with ranges in parentheses. Surface water samples were collected between August 2002 and 2003 ($n = 11$).

Parameter	Value
Lake surface area (km^2)	0.77
Littoral *Typha* stand surface area (km^2)	~0.14
Peak aboveground *Typha* production (g C m^{-2} y^{-1})	
2002	1045±186
2003	993±98
Shoot density (No. m^{-2})	109±42(43–179)
Water depth along transect (cm)	3.3±5.0(0–12)
Conductivity (μS cm^{-1})	315±31(263–373)
pH	7.4±0.5(6.7–8.1)
Total alkalinity (mg $CaCO_3$ L^{-1})	120±19(85–140)
SRP (μg L^{-1})	13±7(6–25)
Total P (μg L^{-1})	21±11(10–46)
NH_4^+-N (μg L^{-1})	38±16(14–71)
NO_3^--N (μg L^{-1})	16±5(8–28)

effective in colonizing, transforming, and assimilating plant litter carbon and nutrients at levels sufficient to support somatic mycelial growth and subsequent development of sexual (ascomata, basidiomata) and asexual reproductive structures (Newell 2001*a*).

Despite abundant evidence of standing litter accumulation in freshwater marsh habitats and extensive fungal colonization, few studies have quantified rates of carbon flow into fungi during standing litter decomposition (Newell et al. 1995; Findlay et al. 2002; Verma et al. 2003) or assessed their potential contribution to plant litter carbon and nutrient transformations (Gessner et al. 2007). As a result, fungal decay processes within standing litter compartments of freshwater marshes have not been recognized as a potential pathway in wetland biogeochemical cycles (Mitsch and Gosselink 2007). This lack of knowledge concerning fungal decay processes in freshwater marshes suggests that our overall understanding of carbon and nutrient cycling in marshes is incomplete.

In this study, we examined litter mass loss patterns and fungal growth dynamics associated with naturally standing leaf blades of the emergent macrophyte *Typha angustifolia*. We estimated the seasonal biomass and production rates of fungi during plant senescence and early standing litter decomposition to estimate the contribution of fungal decomposers to carbon flow processes during the standing litter decay phase. We also related the dynamics of fungal decomposers to changes in plant litter N and P to assess the potential importance of fungi to detrital nutrient cycling.

Methods

Study site—This study was conducted in a small lake littoral emergent wetland (~ 0.14 km^2) located at Independence Lake, Washtenaw County, Michigan (42°24.21′N, 083°48.16′W). This lake has a large fringing littoral marsh that is dominated by a dense, nearly monospecific stand of *T. angustifolia*. Characteristics of the lake surface waters and *Typha* stand during the study period are summarized in Table 1.

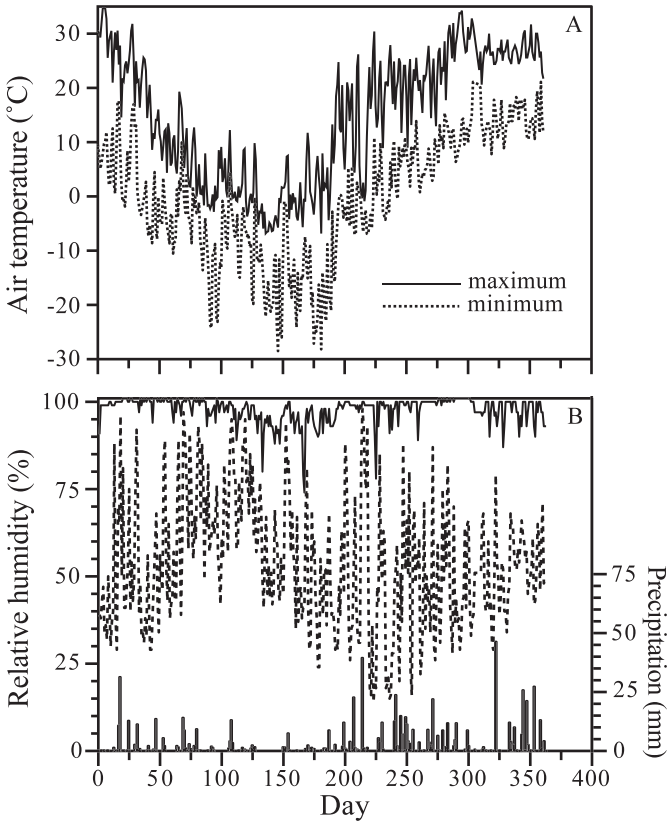

Fig. 1. Changes in (A) maximum (solid line) and minimum (dashed line) air temperature and (B) relative humidity and daily total precipitation (vertical bars) during the annual study period.

Annual aboveground plant production—Annual aboveground net production of *T. angustifolia* was estimated from *Typha* standing crop biomass at peak growth during the end of the growing season in August 2002 and 2003. Living *T. angustifolia* shoots were counted and collected within 0.0625-m^2 subplots in six randomly selected locations along a 100-m transect extending through the *Typha* stand. Collected plant material was returned to the laboratory and dried at 60°C to a constant weight, and subsamples were ground with a Wiley mill (40 mesh screen, ~ 400-μm particle size). Subsamples of ground plant matter were analyzed for carbon and nitrogen concentrations with a carbon nitrogen (CN) analyzer (CE Elantech).

Field procedures—Air temperature and relative humidity (Fig. 1) were continuously monitored every 10 min throughout the study period with the use of two Onset Hobo H8 Pro series data loggers placed midway (~ 1.5 m height) within *Typha* plant canopy. Daily precipitation data (Fig. 1) were recorded and provided by the Ann Arbor municipal airport, located ~ 19 km south of the marsh study site. Surface water samples were collected on each

sampling date, placed on ice in a cooler, and returned to the laboratory for analysis of pH, specific conductance, alkalinity, and concentrations of dissolved nutrients (soluble reactive phosphorus [SRP], total phosphorus [TP], NO_3^-, and NH_4^+). Specific conductance and pH were measured with a Yellow Springs International (YSI) Model 33 specific conductivity meter and a Orion Model 420 bench-top pH meter, respectively. Alkalinity and dissolved nutrient concentrations were determined according to procedures outlined in Lind (1985). Chemical characteristics of collected lake surface waters are summarized in Table 1.

Aboveground standing leaf blades of *T. angustifolia* (2002 cohort) were collected monthly from August to December 2002 and March to August 2003 within six randomly selected subplots (0.0625 m^2) along the 100-m transect extending through the *Typha* stand (as above). During each sampling period, one leaf blade was arbitrarily collected from two separate *Typha* shoots within each subplot (upper leaf portion, mean distal length of collected leaf blades 114 ± 28 cm) to estimate losses in leaf carbon. Two additional leaf blades within each subplot were also arbitrarily collected to determine litter-associated fungal biomass and rates of fungal production. Collected samples were immediately returned to the laboratory and processed.

Leaf mass loss and nutrient concentrations—Carbon loss of standing *T. angustifolia* leaf blades was estimated by losses in leaf area–specific mass (Gessner 2001). Collected leaf blades were scanned on a flatbed scanner, and leaf surface areas were determined by the software program Canvas (version 3.5.5) for Macintosh. After scanning, leaf blades were frozen (−20°C), lyophilized to dryness, and weighed. Dried leaf litter was then ground with a Wiley mill, and subsamples were used to determine litter carbon, nitrogen, phosphorus, and glucosamine concentrations. Initial area-specific leaf carbon mass was estimated by dividing the leaf carbon mass at the initial sampling date (August, fully living green leaves) by the corresponding leaf area. Percent leaf carbon mass remaining during subsequent sampling dates was calculated by determining changes in area-specific carbon mass relative to the mean initial leaf area–specific carbon mass. Leaf area–specific mass loss rates (k) were calculated with the use of an exponential model ($N_t = N_0 e^{-kt}$), where N_t is the carbon mass remaining at time t (days), N_0 is the initial carbon mass, and e is the base of the natural logarithm. Plant litter carbon and nitrogen concentrations were determined with a CN analyzer (as above). Phosphorus concentrations were determined by spectrophotometric procedures (molybdate–ascorbic acid method) after combustion (500°C) and hot HCl extraction of ground litter samples.

Living fungal biomass and production—Living biomass and instantaneous growth rates of fungi associated with standing dead *T. angustifolia* leaf litter were estimated from concentrations of ergosterol and rates of [1-^{14}C]acetate incorporation into ergosterol, respectively (Gessner 2005; Suberkropp and Gessner 2005). Two leaf sections (~ 2-cm-long subsamples, middle portion of each leaf blade) were cut from collected leaf blades. Leaf samples were placed into sterile 20-mL glass scintillation vials containing 3.95 mL of filtered (0.7-μm, Whatman GF/F) marsh water and allowed to hydrate for at least 2 h at corresponding field temperatures. Afterward, a 50-μL aliquot of a Na[1-^{14}C] acetate (MP Biomedicals) solution was added to each sample, resulting in a final concentration of 5 mmol L^{-1} Na[1-^{14}C]acetate (specific activity = 48.5 MBq $mmol^{-1}$). Samples were incubated for 4–5 h at corresponding field temperatures. One additional vial (kill control) containing two leaf sections received formalin (2%, v/v, final concentration) before the addition of [1-^{14}C]acetate label. After incubation, incorporation of [1-^{14}C]acetate label was stopped by placing sample 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 into clean scintillation vials, and stored frozen at −20°C until analyzed.

Frozen litter samples were lyophilized to dryness, weighed, ergosterol-extracted, and partially purified by solid-phase extraction. Ergosterol in samples was separated and quantified by a Shimadzu high-pressure liquid chromatography (HPLC) system using a LichroSpher 100 RP-18 column (0.46 × 25 cm, mobile phase HPLC-grade methanol, flow rate of 1.5 mL min^{-1}). Ergosterol was detected at 282 nm and identified and quantified on the basis of comparison with ergosterol standards (Fluka Chemical). Ergosterol fractions eluting from the HPLC 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), and radioactivity assayed by using a Beckman LS6500 Scintillation Counter corrected for quenching and radioactivity observed in control samples. Ergosterol concentrations and radioactivity within ergosterol fractions were determined as the average of two HPLC sample injections per plot.

Glucosamine concentrations—Glucosamine concentrations within leaf litter samples were also determined as another potential biochemical indicator molecule of total (living + dead) fungal mass. Samples were extracted and digested using a modified procedure originally described by Ekblad and Näsholm (1996). Briefly, subsamples of lyophilized, ground plant litter (~ 20–30 mg) were extracted in 0.2 mol L^{-1} NaOH (10 mL total volume) for 6 h at room temperature (~ 20°C) in sterile 50-mL polypropylene centrifuge tubes with plug-seal caps. After 6 h, samples were vortexed (~ 10 s) and centrifuged (10 min at 2000 revolutions per min [rpm]), the supernatants were removed carefully by aspiration. An additional 10 mL of fresh 0.2 mol L^{-1} NaOH was added, and samples were vortexed (~ 10 s). Samples were then placed in a dry-bath heating block at 100°C for 17 h. After 17 h, samples were removed, cooled to room temperature, vortexed (~ 10 s), and centrifuged (10 min at 2000 rpm), and the supernatants were aspirated (as above). Samples were then washed four times each with 20 mL of distilled water following the same procedure (i.e., vortexed, centrifuged, and supernatants aspirated). The resulting chitinosan (i.e., deacetylated

polyglucosamine) in NaOH-washed pellets was then hydrolyzed to glucosamine residues in 8 mol L^{-1} HCL (10 mL total volume) for 6 h at 110°C in a dry-bath heating block.

Glucosamine residues in hydrolyzed samples were then converted to 9-fluorenylmethylchloroformate (FMOC-Cl) derivatives and analyzed by HPLC. Sample acid hydrolysate (20 μL) was placed into a 1.5-mL screw-capped microcentrifuge tube and neutralized to ~ pH 7 with the addition of ~19 μL of 8 mol L^{-1} NaOH. Borate buffer (211 μL of 0.5 mol L^{-1}, pH 7.0) was added to microcentrifuge tubes and briefly vortexed, and glucosamine residues were derivatized for 30 min at 20°C by the addition of 250 μL of 9-fluorenylmethylchloroformate (15 mmol L^{-1} in acetonitrile). After derivatization, the FMOC–glucosamine samples were transferred to a 1.5-mL screw-capped HPLC autosample vial, mixed with an additional 500 μL of borate buffer (0.5 mol L^{-1}, pH=7.0), and stored at 4°C until analyzed.

Separation and analysis of FMOC–glucosamine derivatives was performed by HPLC on a LichroSpher 100 RP-18 column. The mobile phase solutions were 5 mmol L^{-1} ammonium formate (pH 4.5) and HPLC-grade acetonitrile at a total flow rate of 1.0 mL min^{-1}. A binary gradient was performed from 30% acetonitrile : 70% ammonium formate to 100% acetonitrile over 13 min. The column was then regenerated with 100% mobile phase acetonitrile for 4 min before returning to initial running conditions (total run time 21 min). FMOC–glucosamine was detected with a Shimadzu (RF-10AXL) fluorescence detector (excitation 260 nm, emission 330 nm) and was identified and quantified on the basis of comparison with FMOC-derivatized glucosamine standards (Sigma Chemical).

Fungal growth and production estimates: conversion factors—For determination of litter-associated living fungal biomass, ergosterol concentrations were converted to fungal C assuming a conversion factor of 5 μg ergosterol (mg fungal dry mass)$^{-1}$, and 43% C in fungal dry mass (Findlay et al. 2002; Gessner and Newell 2002). Rates of acetate incorporation into ergosterol were multiplied by 12.6 μg fungal biomass (nmol acetate)$^{-1}$ incorporated to obtain an hourly fungal growth rate (μ, % h^{-1}) (Gessner and Newell 2002). Because [1-^{14}C]acetate incorporation assays involved submergence of leaf litter samples, estimates of daily growth rates (μ, % d^{-1}) of fungi inhabiting standing litter were determined with relative humidity data, which reveal total daily time periods (hours) in which fungi are metabolically active (Kuehn et al. 2004). Fungi 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. Daily growth rates (μ, % d^{-1}) were calculated by multiplying the hourly fungal growth rate (μ, % h^{-1}) by the time period (h d^{-1}) in which standing leaf litter was exposed to ~ 100% relative humidity. Daily rates of fungal production (mg C [g detrital C]$^{-1}$ d^{-1}) were subsequently calculated by multiplying the adjusted daily growth rate (μ) by the litter-associated fungal biomass (B).

Cumulative fungal production was calculated by estimating daily fungal production rates over the study period (Suberkropp et al. 2010). This raw data set was subsequently used to estimate cumulative fungal production using Monte Carlo Simulation (*see* below). To obtain estimated daily fungal production values for days between sampling dates, the following criteria were assumed and calculated: (1) average hourly fungal growth rates (μ, % h^{-1}) and litter fungal biomass (mg C [g detrital C]$^{-1}$) for half of the days between the sampling events were assumed to be equal to the growth rate and fungal biomass on one sampling date, and growth rates and fungal biomass for the other half of the days within the sampling interval was assumed to be equal to the corresponding values observed on the next sampling date. (2) Daily growth rates (μ, % d^{-1}) for days between sampling intervals were calculated (as above) by multiplying the hourly fungal growth rate (μ, % h^{-1}) by the specific daily time period (h d^{-1}) in which relative humidity was ~ 100%. Daily growth rates between sampling intervals were also temperature adjusted to account for daily changes in temperatures (Kuehn et al. 2004). (3) Fungal production for each day (as above) was calculated by multiplying the adjusted daily growth rate (μ) by the litter-associated fungal biomass (B). Rates of fungal production during the winter sampling interval (January–February) were not determined and assumed to be zero.

Data analyses—Statistical analyses were performed with SYSTAT software, with differences considered significant at the $p < 0.05$ level. To reduce heteroscedasticity, data were either $\log(X+1)$ or reciprocal root transformed before analysis. Appropriate transformations were selected by inspection of residuals for homoscedasticity and normality. Data were analyzed separately with one-way ANOVA with time as the factor. We used Monte Carlo Simulation Analysis (Microsoft Excel, PopTools add-ins) to estimate cumulative fungal production. The raw data set of estimated daily fungal production over the study period was resampled with replacement, and the data were recombined to produce 10,000 sets, from which the mean ± 1 SD and 95% CI were calculated. Approximated variance of transformed variables (P : B ratio, fungal yield) was performed using the delta method (Salkind 2007).

Results

Leaf blade mass loss and nutrient dynamics—Significant carbon loss of *T. angustifolia* leaf blades occurred while in the natural standing position (ANOVA, $F_{10,55} = 23.15$, $p < 0.001$), with 55% mass loss observed over the annual study period (Fig. 2). Initial leaf area–specific carbon mass of living (fully green) leaf blades of *T. angustifolia* collected at the end of the growing season in August 2002 (peak biomass) averaged 9.14 ± 0.49 mg C cm^{-2} (mean ± SE). Shortly after this initial collection, plant senescence began with leaf senescence (yellowing) progressing from the leaf tip to the base. Within 47 d, average leaf area–specific mass of *Typha* leaf blades declined to 7.34 ± 1.05 mg C cm^{-2}, indicating that a large portion of the leaf mass was likely lost as a result of leaching, plant translocation of shoot photosynthate to belowground rhizomes, or both (*see* also nutrients below). Following senescence, carbon loss of *T.*

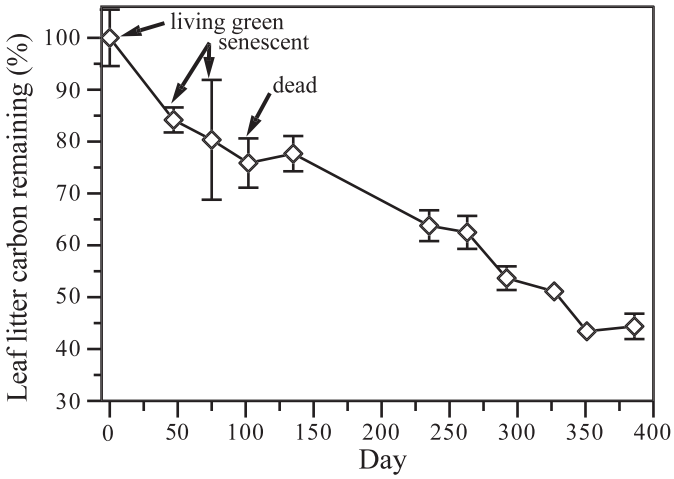

Fig. 2. Percent area-specific leaf C remaining of standing *T. angustifolia* leaves. Symbols indicate the mean ± 1 SE (n = 6).

angustifolia leaf blades continued (Fig. 2), with the average leaf area–specific mass decreasing significantly to 4.05 ± 0.22 mg C cm^{-2} by the end of the study period ($p < 0.001$, Tukey). The decay coefficient (k d^{-1}) with a nonlinear regression model was −0.00206 ± 0.00015 asymptotic standard error ASE (r^2 = 0.74, estimated N_0 = 94.0 ± 3.5% ASE) for postsenescence area-specific leaf mass loss from day 74 onward. Note that overall mass loss pattern and decay rate observed can be considered a conservative estimate because it is based only on losses in leaf area–specific mass. Additional losses in leaf mass because of fragmentation were not taken into account.

Significant decreases in litter N and P concentrations (%) were also observed during plant senescence (ANOVA, $F_{10,55}$ = 20.47 and 43.28, respectively, $p < 0.001$), resulting in corresponding increases in litter C : N and C : P ratios (Fig. 3A,B). Initial N and P concentrations of living (green) leaf blades averaged 1.77% ± 0.08% and 0.17% ± 0.01%, and decreased to 0.52% ± 0.08% and 0.04% ± 0.005% during leaf senescence and early standing decay (by day 74, November), respectively. After this initial decrease, N concentrations increased significantly ($p < 0.001$, Tukey) during litter decomposition to 1.31% ± 0.05% by the end of the study, resulting in a decrease in litter C : N ratios (Fig. 3A). Corresponding P concentrations increased only slightly during litter decomposition (to 0.06% ± 0.002%), resulting in a modest decrease in litter C : P ratios (Fig. 3B). However, this decrease was not statistically significant ($p >$ 0.05, Tukey). Litter N : P ratios increased significantly (ANOVA, $F_{10,55}$ = 19.15, $p < 0.001$) during the study period, illustrating the disproportionate retention or accumulation of N vs. P in *Typha* standing leaf litter (Fig. 3C).

Fungal biomass, production, and nutrient immobilization—Significant increases in ergosterol concentrations were observed in standing *Typha* leaf blades during decomposition (ANOVA, $F_{10,55}$ = 49.94, $p < 0.001$). Initial ergosterol concentrations in leaves (day 0, August) were

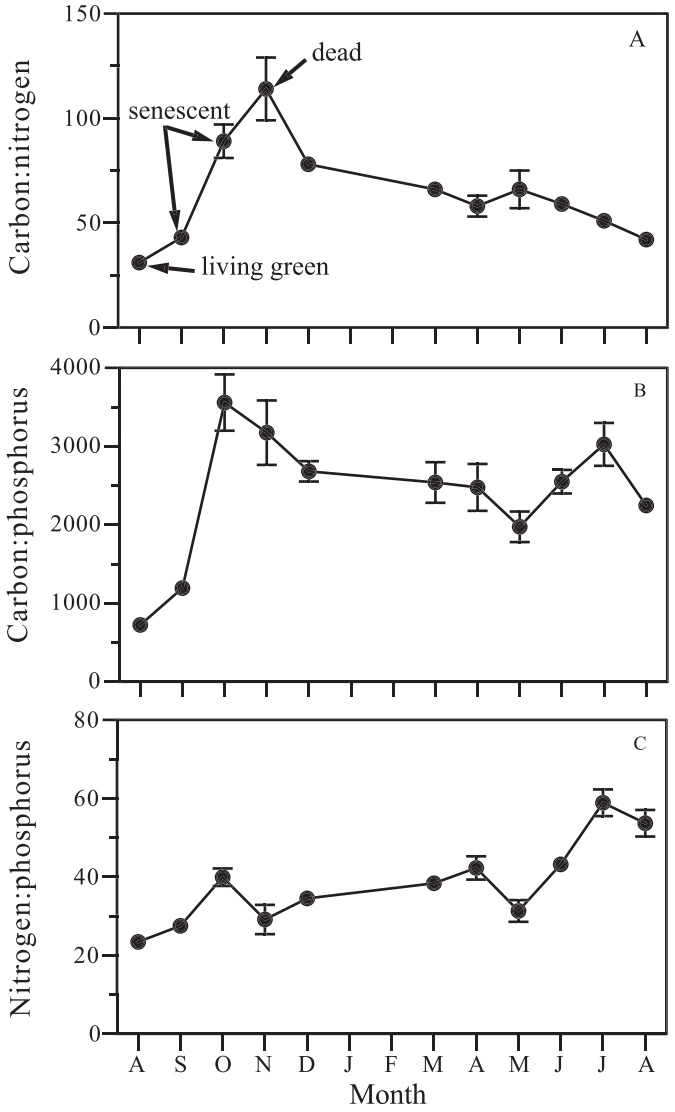

Fig. 3. Changes in (A) carbon : nitrogen, (B) carbon : phosphorus, and (C) nitrogen : phosphorus ratios in standing *T. angustifolia* leaves. Symbols indicate the mean ± 1 SE (n = 6).

low, averaging 0.07 ± 0.02 mg (g detrital C)$^{-1}$ (Fig. 4). Ergosterol concentrations increased markedly during senescence and early litter decay, reaching 0.68 ± 0.05 mg (g detrital C)$^{-1}$ by December (day 107). Concentrations then remained relatively stable until the last sampling period, when they increased significantly to 1.23 ± 0.08 mg (g detrital C)$^{-1}$ ($p < 0.001$, Tukey). Estimates of living fungal biomass within standing leaf litter ranged between 6 ± 2 and 106 ± 7 mg C (g detrital C)$^{-1}$ (Fig. 4).

Changes in glucosamine concentrations associated with *Typha* leaves followed a similar pattern as ergosterol concentrations (r = +0.84, $p < 0.001$, Pearson) (Fig. 4), with glucosamine increasing significantly during litter decomposition (ANOVA, $F_{10,55}$ = 32.00, $p < 0.001$). Initial glucosamine concentrations in leaf blades averaged 0.68 ± 0.16 mg (g detrital C)$^{-1}$ and increased to 5.4 ± 0.6 mg (g detrital C)$^{-1}$ by the end of the study period. The ratio of

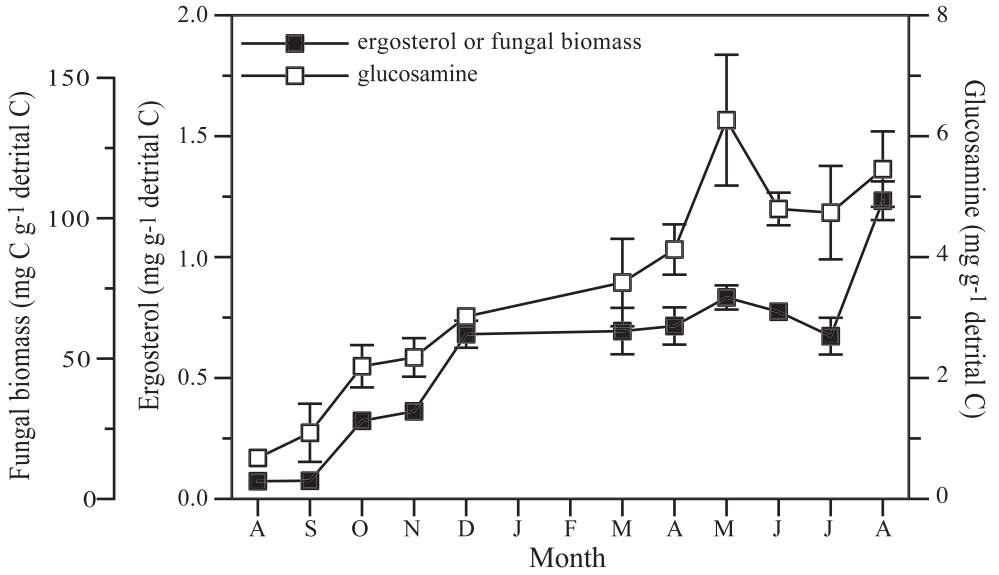

Fig. 4. Dynamics of ergosterol and glucosamine concentrations in standing *T. angustifolia* leaves. Estimated fungal biomass is also indicated. Symbols indicate the mean ± 1 SE ($n = 6$).

ergosterol to glucosamine ranged between 0.10 and 0.23 throughout the study period, with lower ratios being observed in the early fall, spring, and summer periods and higher ratios being observed during the winter. Increases in litter ergosterol and glucosamine concentrations were consistent with observed patterns of leaf mass loss. Area-specific leaf carbon mass, as a measure of leaf litter decomposition, was negatively correlated with both ergosterol ($r = -0.74$) and glucosamine ($r = -0.72$, $p < 0.001$, Pearson) concentrations.

Significant increases in instantaneous growth rates (μ) of fungal decomposers associated with *Typha* leaf blades were observed during the study period (ANOVA, $F_{10,55} = 31.87$, $p < 0.001$), with peak daily growth rates occurring after leaf senescence in late fall (November, 2.86% ± 0.47% d^{-1}) and again during the summer season (June, 4.96% ± 0.04%; Fig. 5A). Corresponding rates of fungal secondary production associated with *Typha* leaf blades followed a similar pattern as growth rates (Fig. 5B), with rates ranging from 0.18 ± 0.04 to 3.34 ± 0.47 mg C (g detrital C)$^{-1}$ d^{-1} during the study period. When integrated over the annual study period, estimated cumulative fungal production totaled 123 mg C (g initial detrital C)$^{-1}$, indicating that 22% of the *Typha* leaf carbon lost was transformed and assimilated into fungal biomass (i.e., fungal yield) (Table 2).

The dynamics of nitrogen and phosphorus concentrations within postsenescent decaying *Typha* leaf blades were also positively correlated with both ergosterol ($r = +0.65$ for N and $r = +0.41$ for P) and glucosamine ($r = +0.61$ for N and $r = +0.48$, for P, $p < 0.001$, Pearson) concentrations. Estimated detrital nitrogen and phosphorus present as fungal biomass increased during leaf litter decay, with the contribution of fungal immobilized N and P reaching a

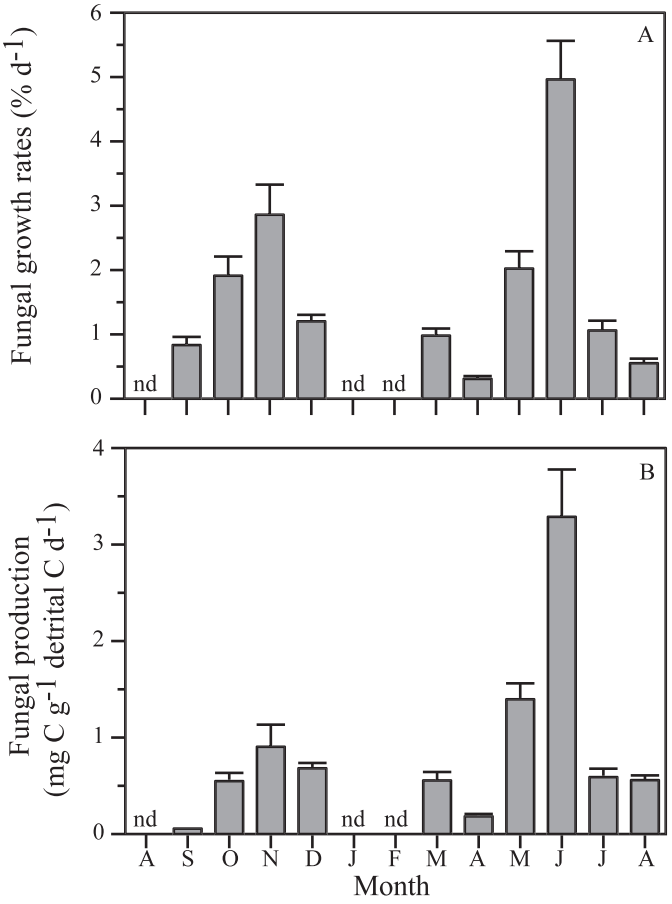

Fig. 5. Dynamics of (A) fungal growth rates and (B) production rates associated with standing *T. angustifolia* leaves. nd, no samples were collected. Symbols indicate the mean ± 1 SE ($n = 6$).

Table 2. Total leaf mass loss, cumulative fungal production, mean biomass, P : B ratio, turnover time, and estimated contribution of fungal decomposers associated with standing dead leaf litter of *T. angustifolia*. Fungal yield coefficient (%) = cumulative fungal production/total leaf mass loss × 100. The percentage of leaf C assimilated by fungi was calculated by dividing cumulative fungal production by the previously reported fungal growth efficiency 32% ± 3.6% (Gulis and Suberkropp 2003). The contribution of fungal assimilation to overall carbon loss from standing dead leaf litter was determined by dividing fungal assimilation by the total leaf C mass loss. Values are the means ± 1 SD. Values for cumulative fungal production represents the means ±1 SD and the 95% confidence interval in parentheses (calculated using Monte Carlo Simulation Analysis).

Parameter	Value
Total leaf mass loss (mg C [g initial leaf C]$^{-1}$)	556±60
Cumulative fungal production (mg C [g initial leaf C]$^{-1}$)	123±10(104–143)
Mean fungal biomass (mg C [g initial leaf C]$^{-1}$)	33±11
P : B ratio*	3.7±1.3
Turnover time (d)*	99±34
Fungal yield coefficient (%)*	22±3
Fungal assimilation of initial leaf C (%)*	38±5
Fungal contribution to overall leaf C loss (%)*	69±5

* Error estimates determined using the delta method (Salkind 2007).

maximum of 60% and 83% of the total detrital N and P, respectively (Fig. 6); assuming 5 μg ergosterol (mg fungal dry mass)$^{-1}$ and 6.5% N and 0.4% P in fungal mass (Beever and Burns 1980; Findlay et al. 2002).

Discussion

Significant changes in *T. angustifolia* leaf carbon mass and nutrients (N and P) were observed during the study period, supporting previous findings from both freshwater and salt marsh ecosystems that appreciable transformation and decomposition of emergent plant matter occurs while it is still attached to the parent plant in a natural standing position (Newell and Porter 2000; Gessner et al. 2007). In *T. angustifolia*, changes in leaf mass and nutrient concentrations occurred during the transition of plant matter from living through senescence and into early stages of litter decomposition. Rapid losses in *Typha* leaf carbon (20%), nitrogen (61%), and phosphorus (77%) were observed during the first 7 weeks, when plant leaves began to senesce and die. During this time, colonization of *Typha* leaves by fungi was low, suggesting that these initial losses were likely mediated via plant translocation to belowground rhizomes (Asaeda et al. 2008; Tylová et al. 2008) or by physical leaching of plant matter during rain or dew-forming events. These initial losses were followed by a more gradual loss in leaf litter carbon, which was accompanied by a concomitant increase in biomass accumulation (ergosterol and glucosamine) of litter-associated fungal decomposers.

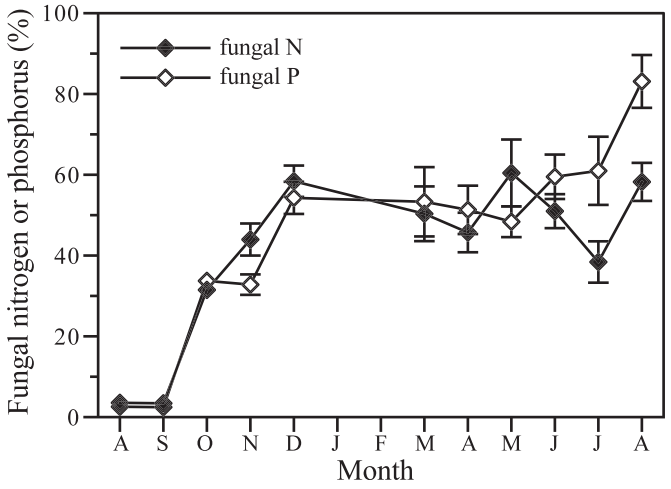

Fig. 6. Estimated amount of total detrital nitrogen and phosphorus sequestered in standing leaf–associated fungal biomass. Symbols indicate the mean ± 1 SE (n = 6).

Similar findings have been reported by Newell et al. (1995), Kuehn et al. (1999), and Gessner (2001) for decaying naturally standing leaf litter of the freshwater emergent macrophytes *Carex walteriana*, *Erianthus giganteus, and Phragmites australis*, respectively. For example, Gessner (2001) observed appreciable mass (~ 30%) and nutrient losses during senescence and early litter decomposition in tagged, naturally standing *P. australis* leaf blades. Patterns of mass loss, nutrient loss, and fungal colonization were consistent with the sequential senescence, death, and microbial decay of *P. australis* leaves, which started with leaf blades attached at the base of shoots and continued upward along the shoot axis with time as leaves began to senesce, die, and decompose (Gessner 2001). Taken together, results of these former studies and the present investigation demonstrate that emergent plant decomposition is not a simple process, as critical transformations of plant carbon and nutrients occur throughout the progression of plant matter through senescence and into standing litter decomposition, before its collapse to marsh sediments or overlying surface waters.

The presence of notable carbon loss and nutrient changes in standing emergent plant matter illustrates that our functional understanding of *natural* plant litter decomposition within these wetland ecosystems is incomplete. To date, much of our knowledge of plant decay processes in freshwater marshes comes from traditional litter bag studies that were conducted at or within the marsh sediments or overlying surface waters (Polunin 1984; Rothman and Bouchard 2007; Fennessy et al. 2008). Furthermore, many of these studies examined decay patterns using plant material that had been prematurely harvested (living green or recently senesced) manipulated (e.g., oven-dried), or both—a widespread practice that continues even today (Rothman and Bouchard 2007; Fennessy et al. 2008). These methodological approaches eliminate, either partially or completely, the initial standing decay phase and often lead to serious artifacts (e.g., altered

litter nutrient quality, elimination of active microbial community) that do not reflect the natural decay (carbon and nutrient) processes within emergent macrophyte stands. If plant and microbial transformations are important in the geratology and early decay stages of emergent macrophytes, as the present and former studies demonstrate, then future investigators of emergent plant decay should reflect carefully (Bärlocher 1997) and not employ methods that fundamentally alter or interrupt the physical state of plant litter or its natural sequence of decay.

In ecosystems, decomposition of plant matter involves a variety of processes that result in the production of consumer biomass (microbial and invertebrate or vertebrate detritivore), release of CO_2 and nutrients (N and P) through metabolic activities (mineralization), and release of other substances, such as dissolved organic matter and fine particulate organic matter (Gessner et al. 1999). Currently, much of our quantitative information on microbial processes in freshwater marshes comes from investigations of microbial communities associated with plant matter at or within the wetland sediments (Gutknecht et al. 2006; Rejmánková and Sirová 2007; Wright et al. 2009). This emphasis likely reflects an ongoing paradigm in salt and freshwater marsh ecology that carbon and nutrient cycling pathways are largely mediated via aerobic and anaerobic sediment microbial (bacterial) communities (Mitsch and Gosselink 2007; Reddy and Delaune 2008; Kayranli et al. 2010). As a result, the role of fungal decomposers in wetland biogeochemical processes has not been fully explored or explicitly included in wetland ecosystem carbon and nutrient flow models (Mitsch and Gosselink 2007; Reddy and Delaune 2008; but *see* Newell and Porter 2000). This view contrasts sharply with studies in freshwater stream systems, where stream-dwelling aquatic hyphomycetes have been widely accepted as an ecologically important group of microorganisms that are central to litter decomposition and food resources for inhabitant detrital (macroinvertebrate) consumers (Findlay 2010).

In the present study, fungi associated with *Typha* leaves accumulated substantial levels of biomass during standing litter decay, with peak estimates of living fungal mass approaching $\sim$ 11% of the total leaf detrital mass. These findings agree with reports from both salt and freshwater marshes indicating that fungal decomposers pervasively colonize (self-extending hyphal ''tubular reactors''; Newell 1996) both standing and submerged litter of emergent vascular plants, often accounting for $>$ 99% of the total litter-associated microbial biomass (Newell and Porter 2000; Gessner et al. 2007). Gessner (2001) observed a gradual increase in fungal biomass (ergosterol) associated with standing dead leaf litter of *P. australis*, with peak estimates of fungal mass accounting for $>$ 4% of the total detrital mass. Furthermore, as observed in the present study, Gessner (2001) also reported that fungal biomass concentrations (ergosterol) were negatively correlated ($r = -0.76$), with patterns of *P. australis* leaf mass loss, implying that fungi are most likely important participants in *P. australis* leaf decomposition.

Observations of substantial fungal biomass accrual in decaying standing litter implies that inhabitant fungal decomposers must be effective in enzymatically acquiring and assimilating sufficient quantities of plant litter C and nutrients (N and P) to support pervasive mycelial growth. In the present study, rates of fungal secondary production (based on ^{14}C incorporation) associated with *Typha* leaf litter ranged between 10 and 314 μg fungal C (g detrital C)$^{-1}$ h^{-1}, indicating that under optimal environmental conditions (i.e., periods of water availability), fungi colonizing standing dead *Typha* leaves are metabolically active and assimilate plant litter carbon at levels conducive to considerable production of fungal biomass. Furthermore, on the basis of estimates of N and P in fungal biomass, fungi associated with *Typha* leaf litter also appeared to be effective in acquiring and immobilizing a sizable fraction of the detrital N and P, suggesting that elemental (N and P) stoichiometry within decaying *Typha* litter is strongly influenced by the growth and biomass accumulation of inhabitant fungal decomposers.

Similar production rates have been reported for fungal communities inhabiting both standing and collapsed plant litter in freshwater and salt marsh habitats (Newell and Porter 2000; Newell 2001*b*; Gessner et al. 2007). Newell (2001*b*) reported remarkably similar production rates ranging between 60 and 309 μg fungal C (g detrital C)$^{-1}$ h^{-1} for fungi inhabiting standing leaves of the salt marsh grass *Spartina alterniflora* over a 3-yr period (assuming 43% and 50% C in fungal mass and organic matter, respectively). Additionally, fungal decomposers associated with standing *S. alterniflora* and *P. australis* litter could also effectively immobilize and retain nearly all of the available N and P in decomposing plant matter (Newell and Porter 2000; van Ryckegem et al. 2006).

Quantification of elemental fluxes (e.g., C, N, and P) to various litter decay products (e.g., CO_2, microbial, or consumer biomass) has been proposed as a means to enhance our overall understanding of plant litter decomposition in ecosystems (Gessner et al. 1999). Such data help clarify the functional role of microbial assemblages (bacteria and fungi) and consumers (e.g., invertebrates) in organic matter processing (Hieber and Gessner 2002) and could provide unique insights into the potential effects of eutrophication on detrital carbon and nutrient flow pathways in ecosystems (Baldy et al. 2007; Suberkropp et al. 2010). Data collected in the present study allows the construction of a partial budget to assess the contribution of fungal decomposers to overall leaf carbon loss in *T. angustifolia*. When fungal production estimates were integrated over a daily and then annual study period (i.e., accounting for daily time periods of high water availability, $\sim$ 100% relative humidity), fungal production associated with standing *Typha* leaves totaled 123 mg C (g initial leaf C)$^{-1}$, indicating that 22% of the observed *Typha* leaf C lost during the study period was transformed and assimilated into fungal biomass (fungal yield; Table 2). Furthermore, estimates of cumulative fungal production during the study period were significantly related to cumulative losses in *Typha* leaf carbon (Fig. 7), providing additional evidence that fungi played an important role in the transformation of plant carbon throughout the postsenescence period of standing litter decomposition. If fungal growth efficiency is

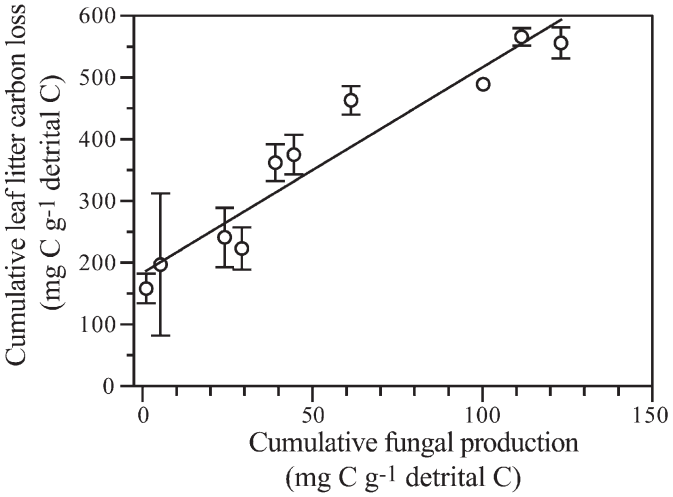

Fig. 7. Relationship between cumulative fungal production and cumulative leaf litter carbon loss in *T. angustifolia*. Linear regression model ($F_{1,8} = 90.55$, $p < 0.001$) : Leaf C loss = 3.33 (fungal production) + 183.27, adjusted $r^2 = 0.91$.

assumed to be ~ 32% (Gulis and Suberkropp 2003), then total fungal assimilation (biomass production + respiration) could account for 69% of the total observed *Typha* leaf carbon lost (Table 2), with the remaining 31% of the C lost through a combination of plant translocation (senescence), leaching, and other nonfungal detrital processes (e.g., bacterial).

Estimates of fungal carbon assimilation from *T. angustifolia* concur with the growing number of studies in other decompositional systems that have estimated the contribution of fungal decomposers to overall carbon loss from decaying plant litter (Newell et al. 1996; Pascoal and Cássio 2004; Baldy et al. 2007). For example, Newell et al. (1996) reported biomass yields ranging between 56% and 87% for fungal decomposers inhabiting standing leaf litter of *S. alterniflora,* with the highest biomass yields observed in litter containing higher nitrogen availability. Likewise, Gessner (2001) estimated that fungal assimilation could account for ~ 50% of the observed mass loss in standing dead *P. australis* leaves.

Similar findings have also been reported for fungi associated with emergent plant litter under sediment or submerged conditions, suggesting that fungal decomposers are also important mediators in C and nutrient flow after collapse of plant litter to the sediments or overlying surface waters (Kuehn et al. 2000; Mancinelli et al. 2009; but *see* Buesing and Gessner 2006). Kuehn et al. (2000) reported that fungal assimilation accounted for 68% of the observed litter mass loss of *Juncus effusus* litter during submerged decomposition. In a more recent study, Mancinelli et al. (2009) observed that fungal respiration alone accounted for ~ 21% of the detrital C lost during submerged decomposition of *P. australis* leaves.

Microorganisms are key players in virtually all biogeochemical cycles, and assessing their composition and quantitative role in ecosystem processes is an important issue in contemporary ecology (Prosser et al. 2007). Although rarely used in freshwater marshes, application of the fungal secondary production method is providing key quantitative data concerning the role and contribution of fungi in marsh carbon cycling. In salt and freshwater marshes, where fungi have been studied under *natural* conditions, they have been found to play an important role in the cycling of carbon and nutrients (N and P). Hopefully, as future researchers explore and apply methods for investigating fungal participation during litter decomposition, their quantitative role in wetlands will become better understood, recognized, and incorporated into ecosystem models that depict important carbon and nutrient flows and processes.

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