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Andrew John Foley

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DYNAMICS OF MICROBIAL COMMUNITIES ASSOCIATED WITH *TPHA ANGUSTIFOLIA* (CATTAIL) LITTER IN A WETLAND ECOSYSTEM: EFFECTS OF LITTER MANIPULATIONS ON MICROBIAL DECAY PROCESSES

by

Andrew John Foley III

Thesis
Submitted to the Department of Biology
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In partial fulfillment of the requirements
for the degree of
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in
Biology

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DYNAMICS OF MICROBIAL COMMUNITIES ASSOCIATED WITH *TYPHA ANGUSTIFOLIA* (CATTAIL) LITTER IN A WETLAND ECOSYSTEM: EFFECTS OF LITTER MANIPULATIONS ON MICROBIAL DECAY PROCESSES

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ABSTRACT

Quantification of microbial processes involved in plant decomposition is important in understanding energy flow and nutrient recycling in wetlands. Prior studies examining emergent plant decomposition have often used litter that had been prematurely harvested (senescent) and/or manipulated (oven-dried). I examined the effects of litter manipulations on microbial decay dynamics associated with litter of the emergent macrophyte Typha angustifolia. Plant litter was harvested after senescence and after a period of standing-dead decay. This collected litter was either air-dried or oven-dried, placed into fine mesh (1 mm) litterbags, and submerged in the wetland. Litterbags were retrieved periodically and analyzed for microbial biomass, litter nutrient concentrations, rates of mass loss, fungal production, and microbial respiration. Significant differences were observed in measured microbial parameters and litter nutrients for all four litter treatments. These results point to contrasting patterns in microbial and nutrient dynamics during emergent macrophyte decay as a result of frequently employed litter manipulations.
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INTRODUCTION

Land-water interfaces (e.g., wetlands) are among the most productive ecosystems on earth and function as important metabolic gateways in freshwater systems (Wetzel 2001). Emergent vascular plants, such as cattail (Typha) and common reed (Phragmites), are a conspicuous feature in freshwater marshes and often account for the bulk of the annual plant biomass produced within these habitats (e.g., Mitsch and Gosselink 1993; Wetzel and Howe 1999; Windham 2001). Only a negligible amount of this plant biomass is directly consumed by herbivores during the growing season (e.g., Dvorak and Imof 1998). Instead, most of the plant biomass produced enters the detrital pool following the senescence and death of the plant shoot, where microbial decomposers (bacteria and fungi) and detritivore consumers play an important role in its eventual breakdown and mineralization (Gessner 2000; Moore and others 2004).

Decomposition of vascular plant matter in aquatic habitats has been intensively studied throughout the last 7 decades (Brinson and others 1981; Polunin 1984; Webster and Benfield 1986; Moran and others 1989; Murkin and others 1989; Szumigalski and Bayley 1996; Thormann and Bayley 1997; Thormann and others 2001; Atkinson and Cairns 2001). Methods used to study plant litter decomposition in aquatic environments were originally adapted from terrestrial ecologists (Falconer and others 1933; Lunt 1933; Bärlocher 1997), where plant litter was collected, dried, and packaged into litterbags of varying mesh sizes in known amounts. Litter bags would then be deployed within the study system and collected periodically for determination of elemental concentrations (e.g., N and P), litter mass loss, and possibly other various biological assays (e.g., fungal and bacterial biomass and
production rates; Moran and Hodson 1989; Boulton and Boon 1991; Thormann and others 2003; Thormann and others 2004a, 2004b).

In freshwater marshes, important details to consider when examining emergent plant decay are both the spatial and temporal conditions under which plant matter naturally decomposes. In the case of emergent vascular plants, collapse of plant material into the water does not take place immediately following senescence and death. Instead, it remains standing for long periods of time, resulting in the accumulation of dead plant litter (Boulton and Boon 1991; Newell 1993; Bärlocher 1997; Gessner 2000). Several studies have established that this standing dead litter is pervasively colonized and degraded by microbial assemblages, particularly fungi (Newell 1993; Bärlocher and Biddiscombe 1996; Kuehn and Suberkropp 1998a; 1998b; Kuehn and others 1999). Hence, considerable initial decomposition and microbial conditioning of emergent plant litter can occur prior to its collapse into the aquatic environment.

Most studies describing the decomposition of emergent plant matter have focused exclusively on litter decay processes occurring at the sediments or under submerged conditions. In many instances, these studies have followed litter mass loss, litter nutrient concentrations (N and P), and microbial dynamics using plant litter that had been prematurely harvested (e.g., living green or recently senesced; e.g., Moran and others 1989) and/or manipulated in some way (e.g., oven-dried material) prior to its placement in the natural environment (see Newell 1993, references therein). However, as Newell (1993) and others (e.g., Bärlocher 1997) pointed out, the initial composition of plant litter (e.g., living green) and/or various manipulations (e.g., oven-drying) of plant litter are likely to introduce experimental artifacts that underestimate important decomposer assemblages present within
decaying litter. For example, Bärlocher (1997) suggested that enclosing pre-dried litter in litterbags placed at the sediment surface short-circuits the natural standing decay stage where substantial prior litter decay would already have occurred. Consequently, such experimental conditions may result in an inaccurate description of the microbial and nutrient cycling dynamics associated with litter during “natural” plant decomposition.

This study investigated the effects of two potentially influential variables, drying technique (i.e., air-dried vs. oven-dried) and age of litter at collection (i.e., senescent vs. standing dead), on the microbial decay and nutrient dynamics associated with submerged decomposing litter of the emergent macrophyte *Typha angustifolia*. Our hypothesis was that litter age (i.e., senescent vs. standing dead) and drying manipulations (e.g., oven-drying vs. air-drying) would significantly alter microbial litter decay processes and litter associated nutrient dynamics (i.e., nitrogen and phosphorus).
MATERIALS AND METHODS

Study Site

This study was conducted in a small lake-littoral emergent wetland located at Independence Lake, Washtenaw County, Michigan, USA (N42°24.21, W083°48.16). This lake has a large fringing littoral wetland that is dominated by a dense, nearly monospecific, stand of *T. angustifolia*. Characteristics of the lake and *Typha* stand examined are summarized in Table 1.

Field Procedures

Sediment surface and water temperatures were continuously monitored (every 30 min.) throughout the study period using 2 Onset Stowaway data loggers (Figure 1). In addition, surface water samples were collected adjacent to the *Typha* stand during 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 (SRP, TP, NO₃, NH₄) (see Table 1). Specific conductance and pH were measured using a YSI Model 33 specific conductivity meter and a Orion Model 420 bench-top pH meter, respectively. Alkalinity and dissolved nutrient concentrations were determined following procedures outlined in Lind (1985).

Two separate collections of *Typha* leaf blades were conducted. The first collection was conducted shortly after plant senescence in September 2000. During that time, approximately 300 grams of standing *Typha* leaf blades were collected, returned to the laboratory, and air dried under ambient laboratory conditions. After drying, this plant material was stored at room temperature until the following spring. The second plant litter
collection was conducted in early spring of 2001, where approximately 300 grams of standing dead plant litter was collected, returned to laboratory, and air-dried under ambient laboratory conditions. This litter represented decaying plant matter that had been naturally standing over the winter period. As a consequence, two age classes of litter were collected (i.e., recently senescent and standing dead). Both of these litter age classes were evenly divided, with one half of each age class receiving an additional oven-drying treatment at 80°C for 48 hours. The resulting plant litter was used to examine the decomposition of four litter treatments: senescent plant litter (S), senescent plant litter that was also oven-dried (SOD), standing dead plant litter (STD), and standing dead plant litter that was also oven-dried (STDOD).

Approximately 3 grams of litter from each of the four litter treatments were separately enclosed into fine mesh (1 mm) litterbags, fastened to 15’ PVC pipe supports, and submerged just above the sediments (early May) in four randomly selected plots established within the Typha stand. Four litter bags from each litter treatment (1 from each plot) were collected on each sample date, placed into separate plastic containers on ice, and returned to the laboratory for determination of plant litter mass loss as % ash-free dry mass remaining (AFDM), microbial (bacteria and fungi) biomass and production, rates of microbial respiration, and litter nutrient contents (C:N:P).

Mass Loss and Nutrient Content

Intact plant material was removed from collected litter bags and gently rinsed of attached sediment and macroinvertebrates. Plant litter was then cut into 2-cm pieces (~ 0.9 g) for determination of all microbial parameters (see below). The remaining litter was frozen
(-20°C) and lyophilized (Labconco, freeze dryer 5) to dryness. Dried plant material was
ground to 40 mesh (~ 400 µm particle size) using a Wiley mill, and subsamples were
combusted overnight at 550°C to determine ash contents. The % AFDM remaining of the
plant material within the litterbags was calculated as the sum of the remaining plant litter,
plus the subsections removed for microbial analyses. Plant litter decay rates (k) were
calculated by using a nonlinear \( M_t = M_o \cdot e^{-kt} \) regression model, where \( M_t \) is the AFDM at
time t (d), and \( M_o \) is the initial AFDM in litter bags.

Plant litter C and N concentrations were determined using a CE Instruments EA 1110
CHN analyzer (ThermoQuest Italia, Milan, Italy). Phosphorus concentrations were
determined using a Bran-Lubbe Autoanalyzer II System (Bran-Lubbe methods: 696-82W and
455-762/A) (automated ascorbic acid method) following high temperature CuSO_4/sulphuric
acid digestion (see Su and others 2007).

**Bacterial Biomass**

The enumeration of bacteria associated with litter was determined using
epifluorescence microscopy after staining with SYBR Green (Molecular Probes, Inc.; Noble
and Fuhrman 1998). Collected litter subsections were placed in 20 ml glass scintillation vials
containing 10 ml of 2% (v/v) filtered (0.2 µm) formalin containing sodium pyrophosphate
buffer (0.1% w/v). In addition, replicate litter subsections were also dried (105°C) and ashed
to estimate AFDM of litter subsections within preserved formalin samples. Bacterial cells
attached to decaying plant litter were detached by probe ultrasonication (Cole-Parmer, Inc.,
power 50 W) for 1 min on ice (see Su and others 2007). After ultrasonication, samples were
gently mixed and aliquots (~30-200 µl) placed into a 25 mm glass vacuum filtering apparatus
containing 2 ml of filtered (0.2 µm) sterile distilled water. An additional 2 ml of sterile
distilled water was added to insure a homogenous suspension of bacterial cells prior to
filtration. Samples were then vacuum (≤ 20 kPa) filtered through 25 mm supported Anodisc
filters (25 mm, 0.2 µm, Whatman), stained for 15 minutes with SYBR Green (in darkness),
and mounted on glass slides with 30 µl of antifade solution (see Noble and Fuhrman 1998).
Bacterial cells were enumerated (1000x magnification) in a minimum of 10 fields (≥350
cells) using a Leica DMRB epifluorescence microscope and assigned to one of six categories
according to size and shape. Biovolume estimates (µm³) for each size class were calculated
from length (l) and width (w) measurements using the formula: \( V = \frac{1}{4} w^2 (l-w) \pi + \frac{1}{6} w^3 \pi \). Biovolume estimates were converted to bacterial carbon (fg C) using a formula (\( C = 89.5 \cdot V^{0.59} \)) that accounts for size dependent differences in carbon density of bacterial cells (Simon and Azam 1989).

**Fungal Biomass and Production**

Litter associated fungal biomass was estimated by the extraction and quantification of
ergosterol from plant litter (Gessner and Newell 2002). Frozen samples were lyophilized,
weighed, and ergosterol extracted in alcoholic KOH (0.8% KOH in methanol, extraction
volume 10 ml) for 30 minutes at 80ºC in tightly capped tubes with constant stirring. Solid
phase extraction was used to clean the resultant crude extract (Gessner and Schmitt 1996),
and ergosterol was purified and quantified by high-pressure liquid chromatography (HPLC).
A HPLC (LC-10AT pump and SPD-10A UV-VIS detector, Shimadzu Scientific Inc., Kyoto,
Japan) was used for separation and analysis of ergosterol. The mobile phase was HPLC
grade methanol at a flow rate of 1.5 ml min⁻¹. Ergosterol was detected at 282 nm (retention
time = ~ 7.5 min) and was identified and quantified based on comparison with ergosterol standards (Fluka Chemical Co.). Ergosterol was converted to fungal carbon assuming a conversion factor of 10 μg ergosterol mg⁻¹ fungal C and 50% C in fungal dry mass (Gessner and Newell 2002).

Fungal growth rates were determined from rates of \[^{14}C\] acetate incorporation into ergosterol (Gessner and Newell 2002). Litter subsections from the litterbags were incubated in sterile 20 ml plastic scintillation vials containing 4 ml of filtered (0.45 μm pore size) lake water and 5 mM Na \[^{14}C\] acetate (specific activity = 37 MBq mmol⁻¹) for 5 h at ambient lake water temperature. Incorporation of \[^{14}C\] acetate label was ceased by placing vials on ice and immediately filtering the contents. Filters and litter pieces were washed twice with 4 ml of filtered lake water, placed in scintillation vials, and stored at -20°C until analyzed. Frozen samples were treated similar to regular ergosterol extraction methods (Gessner and Newell 2002). They were lyophilized and weighed, ergosterol was extracted, and samples were analyzed as above. Ergosterol fractions eluting from the HPLC column were collected in 20 ml glass scintillation vials using an automated Advantec SF-3120 fraction collecting system. Collected fractions were mixed with 10 ml of scintillation fluid (ICN EcoLume, MP Biomedicals Inc.), and radioactivity was determined using a Beckman LS 6500 Scintillation Counter that automatically corrected for quenching. Acetate incorporation rates were converted to fungal growth rates assuming 12.6 μg fungal biomass nmol⁻¹ acetate incorporated (see Gessner and Newell 2002).
Microbial Respiration

Microbial respiration rates associated with decomposing *T. angustifolia* litter were estimated from measurements of dissolved oxygen consumption. Subsections of litter material were placed into custom-made glass chambers containing ~ 30 ml of membrane filtered (0.2 µm) lake water. Changes in dissolved oxygen concentration within chambers were then monitored every 30 s for 30 minutes using oxygen electrodes (YSI, model 5100) connected to a data acquisition module and printer. All measurements were conducted in darkness at corresponding lake water temperatures. As a control, filtered lake water was also monitored for oxygen consumption. Respiration rates were calculated as the slope of regression lines from samples minus the slope of the lake water control. Rates of O\(_2\) consumption were converted to rates of CO\(_2\) evolution, assuming a respiratory quotient (RQ) of 1. Afterwards, the litter samples used to determine respiration rates were dried at 105°C to determine dry mass.

Enzyme Activity

Microbial extracellular enzyme activity associated with decaying plant litter was quantified using standard fluorometric assay methods established by Sinsabaugh and others (2002). Subsamples (0.2 g) of lyophilized, ground (40 mesh) plant litter were placed into 125 ml Nalgene bottles containing 125 ml of acetate buffer (50 mM, pH 5). Samples were then homogenized using a Brinkman polytron, and the activities of five extracellular enzymes were assayed. Cellulolytic activity was measured by using assays for β-1,4-glucosidase (EC 3.2.1.21), celllobiohydrolase (EC 3.2.1.91), and β-1,4-xylosidase (EC 3.2.1.37). Corresponding fluorometric (methylumbelliferyl) substrates were 4-MUB-β-D-
glucoside, 4-MUB-β-D-cellobioside, and 4-MUB-β-D-xyloside, respectively. Assays for acid phosphatase (EC 3.1.3.2) and β-N-acetyl-b-glucosaminidase (EC 3.2.1.30), enzymes involved in the hydrolysis of organic phosphorous and nitrogen compounds, were assayed using 4-MUB-phosphate and 4-MUB-N-acetyl-β-glucosaminide, respectively. All assays were conducted at 23°C using procedures previously described by Sinsabaugh and others (1994). Enzyme activities are expressed in units of μmol of substrate converted g⁻¹ detrital C hour⁻¹.

Data Analyses

All statistical analyses were performed using SYSTAT v10.2 (Systat Software Inc., Richmond, CA, USA), with differences considered significant at the p < 0.05 level. Rates of plant litter decomposition (-k) between treatments were compared with analysis of covariance (ANCOVA). All other data were analyzed using a three-way ANOVA (factors: drying treatment, litter age, time). Symbols presented in figures illustrate the mean ± 1 SE (n=4). Error bars that are not visible denote a SE smaller than the plotted symbol. Data were transformed when necessary to meet the assumptions of ANOVA (i.e., normality and heteroscedasticity; Zarr 1999). If data transformations were necessary, most data were transformed using the natural logarithm. Percent phosphorus data were transformed using a quarter root transformation (x¹⁄₄), fungal production data required a reciprocal root (i.e., -1·x⁻¹) transformation, and ash-free dry mass remaining (AFDM) and phosphatase enzyme data both required a square root transformation (i.e., x¹⁄₂).
RESULTS

Study Site

Throughout the 450 days of this study, the mean water level along the transect was 12.4 ± 10.3 cm (mean ± SD) and fluctuated from 0 cm in the summer (July, August, and September) to a maximum of around 27 cm in late spring and early summer (May and June, Table 1). Average water temperature (°C) throughout the 450 days was 14.6 ± 1.85 °C (mean ± SE), with extreme temperatures ranging from 5°C in November to 20°C in June (Figure 1). Alkalinity and pH fluctuated by less than 20% throughout the study (Table 1). The specific conductance (μS cm⁻¹) was naturally high (e.g., 258 μS cm⁻¹ to 350 μS cm⁻¹), due to the limestone geology of the region.

Litter Mass Loss and Nutrient Dynamics

Statistically significant differences were observed in litter mass loss patterns between the two litter age treatments, senescent and standing dead, (p < 0.001) with senescent litter losing 47% of initial mass, and standing dead litter losing 53% of initial mass after 450 days (Figure 2). Significant differences were also observed between air-dried litter and oven-dried litter (p = 0.001), even though air-dried and oven-dried litter had similar mass loss after 450 days (i.e., ~ 50% mass loss). Decay rate coefficients (-k) were not significantly different between the four litter treatments (p > 0.05, ANCOVA; Table 2). As expected, time (i.e., days) had a statistically significant effect on litter mass loss patterns (p < 0.001, F = 346.379). There was a significant interaction between age of litter and time (days) that is apparent after 100 days (Figure 2, p < 0.001), indicating different patterns of decay through time.
Total litter nitrogen content (mg N litterbag\(^{-1}\)) showed no difference between drying treatments (p > 0.05); however, significant differences were observed between litter age treatments (p < 0.001, F = 522.507). Senescent litter total N decreased from 41 to 37 mg during the study period, whereas total N increased from 19 to 22 mg for standing dead litter (Table 3). As expected, time (i.e., days) had a significant effect (p < 0.001, F = 8.308) on total N contents for all litter treatments. In addition, there was a statistically significant interaction (p < 0.05) between time and age of litter (i.e., senescent and standing dead).

Significant differences were also observed in total litter phosphorus content (mg P litterbag\(^{-1}\)) through time (p < 0.001, F = 43.094). Significant differences were observed for total litter phosphorus content (P) between litter age (p < 0.001, F = 183.211) where total P for standing dead litter increased from 1.1 to 1.8 mg and decreased from 2.3 to 2.2 mg for senescent litter over the 450-day study period (Table 3). The effect of drying treatment (i.e., oven-dried and air-dried litter) showed statistically significant differences (p < 0.05), where total P associated with air-dried litter increased from 1.8 to 2.0 mg compared to an increase from 1.5 to 2.1 mg for oven-dried litter over the 450-day study period (Table 3). A significant interaction was observed for total P between time and litter age (p < 0.05).

Litter N concentration (% N by weight) differences were significant between litter age treatments (p < 0.001, F = 372.37). Litter N concentration associated with senescent litter increased from 1.3 to 2.2% during the study period, whereas N concentration in standing dead litter increased from 0.7 to 1.6%. The effect of drying was also significant (p < 0.05), where N concentrations associated with air-dried litter increased from 1.0% to 1.9% compared with N concentrations associated with oven-dried litter, which increased from 0.9% to 1.9% (Table 3). Time had a significant effect on N concentrations (p < 0.001, F =
which was expected. There was a significant (p < 0.05) interaction between litter age and time for N concentration.

Significant differences were also observed in litter P concentrations (% P by weight) over time (p < 0.001, F = 123.18). In addition, litter P concentrations were also significantly effected by litter age (p < 0.001, F = 94.97), where senescent litter P concentrations increased from 0.08 to 0.13%, yet standing dead litter P concentrations increased from 0.04 to 0.13% (Table 3). Drying treatment was statistically significant between oven-dried and air-dried litter (p < 0.001, F = 20.41), where air-dried litter increased from 0.06 to 0.13% in P concentration, and from 0.05 to 0.13% in oven-dried litter (Table 3). There were significant interactions between time and litter age (p < 0.001) and time and drying treatment (p < 0.05) for P concentrations.

Carbon to nitrogen (C:N), carbon to phosphorus (C:P), and nitrogen to phosphorus (N:P) ratios were all significantly different through time (p < 0.001, Figure 3 and 4). C:N ratios were significantly different between age treatments (p < 0.001, F = 348.82), with senescent litter decreasing from 44 ± 2 to 25 ± 1 (mean ± SE) and standing dead litter from 80 ± 8 to 33 ± 3. Statistically significant differences (p < 0.05) were also observed in C:N between air-dried and oven-dried litter, where air-dried litter decreased from 63 ± 12 to 29 ± 4, and oven-dried litter decreased from 62 ± 10 to 29 ± 2. An interaction was observed between time and litter age (p < 0.001).

C:P ratios were significantly different between litter age treatments (p < 0.001, Figure 3). Senescent litter decreased from 1740 ± 145 to 973 ± 59, and standing dead litter decreased from 3341 ± 432 to 907 ± 95. C:P ratios were significantly different between drying treatments (p < 0.001), with air-dried litter decreasing from 2308 ± 435 to 947 ± 83,
and oven-dried litter decreasing from 2773 ± 594 to 933 ± 78. Significant interactions were observed between time and litter age (p < 0.001) and time and drying treatment (p < 0.05).

N:P ratios were significantly different between litter age treatments (p < 0.001, F = 24.56), with senescent litter decreasing from 40 ± 3 to 38 ± 1 over the 450-day study (Figure 4). Additionally, an interaction for N:P was observed between time and litter age (p < 0.05). Drying treatment had a statistically significant effect on N:P (p < 0.001), illustrated by a decrease in air-dried litter from 37 ± 3 to 33 ± 3, where oven-dried litter decreased from 45 ± 6 to 32 ± 3. There was also a significant interaction of N:P between time and drying treatment (p < 0.05), and a statistically significant interaction was observed between litter age and drying treatment (p < 0.05).

**Microbial Biomass and Production**

A significant increase (p < 0.001, F = 116.57) in bacterial biomass (mg C g\(^{-1}\) AFDM) was observed for all four litter treatments (i.e., senescent, senescent oven-dried, standing dead, and standing dead oven-dried) throughout the study period (Figure 5). On average, bacterial biomass accounted for < 1% of total microbial biomass (bacterial + fungal) among all four litter treatments throughout the entire 450-day study. There were only four sample dates where bacterial biomass accounted for > 1% of total microbial biomass (e.g., day 450 for senescent and oven-dried senescent litters, 1.026% and 1.025%, respectively, and oven-dried standing dead litter on days 44 and 450, 1.195% and 1.098%, respectively). However, there was no statistical significance of drying treatment on bacterial biomass (p > 0.05). Significant differences were observed between the two litter age treatments for bacterial
biomass (p < 0.05). Senescent litter showed approximately a 180-fold increase during the 450-day experiment, whereas standing dead litter had approximately a 270-fold increase.

Significant differences were observed in fungal biomass (mg C g\(^{-1}\) AFDM) among all four litter treatments through time (p < 0.001, F = 25.65). On average, fungal biomass accounted for > 99% of total microbial biomass throughout the 450-day study and never declined to < 98% of total microbial biomass. In air-dried litter (i.e., senescent and standing dead litter) on day 0, fungal biomass accounted for 100% total microbial biomass. Litter age had a significant effect on fungal biomass (p < 0.001, F = 31.215), with senescent litter fungal biomass increasing by ~ 2% (e.g., 18.735 mg C g\(^{-1}\) AFDM at day 0 to 19.089 mg C g\(^{-1}\) AFDM at day 450), whereas fungal biomass associated with standing dead litter increased by ~ 60% (e.g., 13.304 mg C g\(^{-1}\) AFDM at day 0 to 21.391 mg C g\(^{-1}\) AFDM at day 450; Figure 6). The largest differences in fungal biomass were observed during the first 114 days of the study (Figure 6), where standing dead litter contained 69% less fungal biomass than senescent litter (i.e., 19.58 mg C g\(^{-1}\) AFDM and 28.09 mg C g\(^{-1}\) AFDM, respectively).

Significant differences were also observed in fungal biomass between litter drying treatments (p < 0.001, F = 442.36). Air-dried litter showed a decrease in fungal biomass from 24.87 mg C g\(^{-1}\) AFDM to 21.97 mg C g\(^{-1}\) AFDM, whereas oven-dried litter increased in fungal biomass from 7.16 mg C g\(^{-1}\) AFDM to 18.5 mg C g\(^{-1}\) AFDM throughout the study. Although fungal biomass associated with oven-dried litter was consistently less than that associated with air-dried litter throughout the study period, it is most apparent during the first 114 days of the study (Figure 6). Average fungal biomass associated with air-dried senescent litter was 28.09 mg C g\(^{-1}\) AFDM, and senescent oven-dried litter average, for the first 114 days, was 10.12 mg C g\(^{-1}\) AFDM (Figure 6). Standing dead litter (i.e., air-dried) and standing dead
oven-dried litter also showed the largest differences during the first 114 days of the study (e.g., 19.58 mg C g\(^{-1}\) AFDM versus 6.89 mg C g\(^{-1}\) AFDM, respectively). There were significant interactions between time (i.e., days) and litter age (p = 0.007) and time and drying treatment (p < 0.001). Additionally, a significant interaction was observed between time, litter age, and drying treatment (p < 0.05).

As expected, differences in fungal production rates (mg g\(^{-1}\) AFDM d\(^{-1}\)) between the four litter types were also statistically significant through time (p < 0.001, F = 39.582, Table 4). Litter age was not significant (p > 0.05), with senescent litter and standing dead litter both showing similar overall decreases throughout the study period (i.e., 0.640 ± 0.16 mg g\(^{-1}\) AFDM d\(^{-1}\) to 0.516 ± 0.11 mg g\(^{-1}\) AFDM d\(^{-1}\) and 0.515 ± 0.22 mg g\(^{-1}\) AFDM d\(^{-1}\) to 0.467 ± 0.07 mg g\(^{-1}\) AFDM d\(^{-1}\), respectively). However, significant differences were observed for fungal production rates between drying treatments (p < 0.05). Rates of fungal production associated with air-dried litter decreased from 0.767 ± 0.19 mg g\(^{-1}\) AFDM d\(^{-1}\) to 0.525 ± 0.10 mg g\(^{-1}\) AFDM d\(^{-1}\) over the 450 day study, whereas rates associated with oven-dried litter increased from 0.388 ± 0.15 mg g\(^{-1}\) AFDM d\(^{-1}\) to 0.458 ± 0.07 mg g\(^{-1}\) AFDM d\(^{-1}\) (Table 4).

Microbial Respiration

Statistically significant differences were observed in microbial respiration (µg C g\(^{-1}\) AFDM h\(^{-1}\)) through time (p < 0.001). The largest differences were observed during the first 9 days of the study period (Figure 7), where microbial respiration associated with air-dried litter was more than 10 times greater than that of oven-dried litter (77 ± 9 µg C g\(^{-1}\) AFDM h\(^{-1}\) vs. 7 ± 2 µg C g\(^{-1}\) AFDM h\(^{-1}\), respectively). Significant differences in rates of microbial respiration were observed with litter age (p = 0.046, Figure 6), with senescent litter
increasing from 40 ± 23 µg C g⁻¹ AFDM h⁻¹ at day 0 to 112 ± 17 µg C g⁻¹ AFDM h⁻¹ at day 450), and standing dead litter increasing from 38 ± 15 µg C g⁻¹ AFDM h⁻¹ at day 0 to 123 ± 21 µg C g⁻¹ AFDM h⁻¹ at day 450). Significant differences were also observed between drying treatment (p < 0.001, F = 19.278), with respiration on air-dried litter increasing by from 68.628 µg C g⁻¹ AFDM h⁻¹ at day 0 to 121.968 at day 450. Corresponding rates of respiration associated with oven-dried litter increased 10-fold during the study period (i.e., 10.682 µg C g⁻¹ AFDM h⁻¹ at day 0 to 113.876 µg C g⁻¹ AFDM h⁻¹ at day 450). Additionally, there was a significant interaction between time and drying treatment (p < 0.001).

**Enzyme Activity**

β-1,4-glucosidase activity (µmol mg⁻¹ C h⁻¹) associated with all four litter treatments was statistically significant through time (p < 0.001), with the largest decrease in activity occurring in the first 65 days for all four litter treatments (e.g., ~ 80%, Figure 8). The effect of litter age was statistically significant (p < 0.001), with β-1,4-glucosidase activity associated with senescent litter decreasing from 0.835 ± 0.283 to 0.147 ± 0.009 µmol mg⁻¹ C h⁻¹ during the entire 450-day study, whereas standing dead litter decreased in activity from 0.566 ± 0.007 to 0.141 ± 0.003 µmol mg⁻¹ C h⁻¹ (Figure 8). The largest decrease in β-1,4-glucosidase activity occurred during the first 65 days of the study, where senescent litter decreased by 0.83 ± 0.28 µmol mg⁻¹ C h⁻¹ at day 0 to 0.13 ± 0.03 µmol mg⁻¹ C h⁻¹ at day 65 (i.e., 84% decline) compared to the remaining 385 days of the study, where β-1,4-glucosidase activity increased 0.129 ± 0.034 to 0.147 ± 0.009 µmol mg⁻¹ C h⁻¹ (i.e., 13% increase). Similarly, standing dead litter showed the largest decrease (i.e., 78% decrease) in β-1,4-glucosidase activity during the first 65 days 0.566 ± 0.007 to 0.121 ± 0.006 µmol mg⁻¹ C h⁻¹.
with a 16% increase in activity during the remaining 385 days of the study (i.e., 121 ± 0.006 up to 141 ± 0.003). Statistically significant differences were observed in drying treatment, with β-1,4-glucosidase activity associated with oven-dried litter decreasing in activity from 0.907 ± 0.234 to 0.152 ± 0.006 µmol mg⁻¹ C h⁻¹ during the 450-day study compared to air-dried litter, which decreased in activity from 0.495 ± 0.043 to 0.135 ± 0.001 µmol mg⁻¹ C h⁻¹. Again, the largest decrease in β-1,4-glucosidase activity associated with air-dried and oven-dried litter occurred during the first 65 days of the study, where activities associated with air-dried litter decreased by 80% (0.495 ± 0.043 µmol mg⁻¹ C h⁻¹ on day 0 to 0.097 ± 0.011 µmol mg⁻¹ C h⁻¹ on day 65), compared to a 39% increase in activity during the remaining 385 days of the study. Oven-dried litter also had the largest decrease in the first 65 days, decreasing from 0.907 ± 0.003 µmol mg⁻¹ C h⁻¹ at day 0 to 0.154 ± 0.004 µmol mg⁻¹ C h⁻¹ at day 65 (i.e., 83% decrease) with only a 1% decrease occurring during the remaining 385 days. Additionally, significant interactions occurred between time and litter age (p < 0.05) and time and drying treatment (p < 0.001).

Differences in β-1,4-xylosidase (µmol mg⁻¹ C h⁻¹) activity associated with all four litter treatments were statistically significant through time (p < 0.001, F = 156.831), with the largest activity occurring in the first 86 days of the 450-day experiment (Figure 9). Litter age was statistically significant (p < 0.001), with β-1,4-xylosidase activity associated with senescent litter declining from 0.057 ± 0.018 to 0.008 ± 0.001 µmol mg⁻¹ C h⁻¹ (i.e., 86% decrease) by the end of the study period, and activity associated with standing dead litter declining by 92% (i.e., from 0.091 ± 0.003 to 0.007 ± 0.001 µmol mg⁻¹ C h⁻¹; Figure 9). During the first 86 days of the experiment, β-1,4-xylosidase activity had the largest decrease, where standing dead decreased from 0.091 ± 0.003 to 0.014 ± 0.001 µmol mg⁻¹ C h⁻¹, and
senescent litter decreased from 0.057 ± 0.018 to 0.008 ± 0.001 µmol mg\(^{-1}\) C h\(^{-1}\). Drying treatment showed statistically significant differences (p < 0.001, F = 136.222), where β-1,4-xylosidase activity associated with oven-dried litter decreased from 0.089 ± 0.004 to 0.008 ± 0.001 µmol mg\(^{-1}\) C h\(^{-1}\), and air-dried litter decreased from 0.059 ± 0.019 to 0.007 ± 0.001 µmol mg\(^{-1}\) C h\(^{-1}\). The largest decreases in β-1,4-xylosidase activity occurred during the first 86 days, with air-dried litter declining from 0.059 ± 0.019 to 0.009 ± 0.002 µmol mg\(^{-1}\) C h\(^{-1}\) and oven-dried litter decreasing from 0.089± 0.004 to 0.128 ± 0.002 µmol mg\(^{-1}\) C h\(^{-1}\).

From day 86 until the end of the study period, β-1,4-xylosidase activity associated with oven-dried litter continued to decrease from 0.128 ± 0.002 to 0.008 ± 0.004 µmol mg\(^{-1}\) C h\(^{-1}\), whereas activity associated with air-dried litter decreased 0.009 ± 0.002 to 0.007± 0.001 µmol mg\(^{-1}\) C h\(^{-1}\). Significant interactions occurred between time and litter age (p < 0.001); time and drying treatment (p <0.001); litter age and drying treatment (p < 0.001); and time, litter age, and drying treatment (p < 0.001).

Cellulobiohydrolase activity (µmol mg\(^{-1}\) C h\(^{-1}\)) associated with all four litter treatments was statistically significant through time (p < 0.001), where the largest decrease in activity occurred during the first 65 days of the experiment (i.e., ~ 82%, Figure 10). Statistically significant differences (p < 0.001) were observed between litter age treatments (e.g., senescent and standing dead litter) where cellulobiohydrolase activity associated with standing dead litter decreased from 0.218 ± 0.005 to 0.057 ± 0.002 µmol mg\(^{-1}\) C h\(^{-1}\), whereas senescent litter decreased in activity from 0.316 ± 0.087 to 0.059 ± 0.004 µmol mg\(^{-1}\) C h\(^{-1}\) over the entire 450 day experiment. Again, the majority of cellulobiohydrolase activity decreased during the first 65 days of the experiment, where senescent litter decreased from 0.316 ± 0.087 to 0.041 ± 0.01 µmol mg\(^{-1}\) C h\(^{-1}\), and standing dead litter decreased from 0.218
± 0.005 to 0.051 ± 0.009 µmol mg⁻¹ C h⁻¹. In contrast, both senescent and standing dead litter experienced overall increases in cellobiohydrolase activity during the remaining 385 days (e.g., 0.041 ± 0.01 to 0.059 ± 0.004 µmol mg⁻¹ C h⁻¹ and 0.051 ± 0.009 to 0.057 ± 0.002, respectively). Statistically significant differences were observed in cellobiohydrolase activity between drying treatments (p < 0.001), with air-dried litter declining from 0.209 ± 0.011 to 0.054 µmol mg⁻¹ C h⁻¹ and oven-dried litter from 0.325 ± 0.080 ± 0.063 ± 0.002 µmol mg⁻¹ C h⁻¹ throughout the 450-day study, with the largest decrease in cellobiohydrolase activity occurring during the first 65 days. Significant interactions were observed between time and litter age (p < 0.05) and between time and drying treatment (p < 0.001).

Acid phosphatase activity (µmol mg⁻¹ C h⁻¹) associated with all four litter treatments changed significantly through time (p < 0.001). The largest decline in activity occurred during the first 65 days for all four litter treatments (Figure 11). Oven-dried litters showed a slight increase in activity between day 9 and day 30 before continuing to decline, while air-dried litters showed steady declines during the first 65 days of the study (Figure 11). No significant differences were observed between litter age treatments (p > 0.05), while statistically significant differences were observed between drying treatments. Throughout the 450-day study, acid phosphatase activity associated with air-dried litter declined from 0.141 ± 0.026 to 0.045 ± 0.001 µmol mg⁻¹ C h⁻¹, compared to oven-dried litter, where acid phosphatase activity declined from 0.317 ± 0.004 to 0.054 ± 0.009 µmol mg⁻¹ C h⁻¹. The largest decrease in acid phosphatase activity occurred during the first 65 days in both air-dried litter (i.e., from 0.141 ± 0.026 to 0.026 ± 0.001 µmol mg⁻¹ C h⁻¹) and oven-dried litter (i.e., from 0.317 ± 0.004 to 0.086 ± 0.004 µmol mg⁻¹ C h⁻¹). Statistically significant
interactions were observed between time and litter age (p < 0.05) and time and drying treatment (p < 0.05).

β-N-acetyl-b-glucosaminidase activity (µmol mg⁻¹ C h⁻¹) changed significantly through time (p < 0.001, F = 43.478), with the largest decrease in activity (i.e., ~ 82%) occurring during the initial 86 days of the experiment (Figure 12). The effect of litter age was also statistically significant (p < 0.001), with β-N-acetyl-b-glucosaminidase activity associated with senescent litter decreasing from 0.199 ± 0.060 to 0.080 ± 0.008 µmol mg⁻¹ C h⁻¹ throughout the 450 day study, where activity associated with standing dead litter decreased from 0.138 ± 0.002 to 0.075 ± 0.006 µmol mg⁻¹ C h⁻¹. Similar activity patterns were observed between litter age treatments during the initial 86 days, where senescent litter decreased from 0.199 ± 0.060 to 0.028 ± 0.003 µmol mg⁻¹ C h⁻¹ and standing dead by 0.138 ± 0.002 to 0.041 ± 0.006 µmol mg⁻¹ C h⁻¹. However during the remaining 364 days, an increase in activity was observed for both litter age treatments, with senescent litter increasing from 0.028 ± 0.003 to 0.080 ± 0.008 µmol mg⁻¹ C h⁻¹, and activity associated with standing dead litter increasing from 0.041 ± 0.001 to 0.75 ± 0.006 µmol mg⁻¹ C h⁻¹.

Statistically significant differences (p < 0.001, F = 56.960) were also observed between drying treatments throughout the 450-day experiment, with air-dried litter decreasing in activity from 0.128 ± 0.010 to 0.068 ± 0.001 and oven-dried litter decreasing from 0.209 ± 0.053 µmol mg⁻¹ C h⁻¹ (Figure 12). The bulk of the decrease in activity occurred during the first 86 days where air-dried activity decreased from 0.128 ± 0.010 to 0.032 ± 0.006 µmol mg⁻¹ C h⁻¹ and oven-dried from 0.209 ± 0.053 to 0.037 ± 0.004 µmol mg⁻¹ C h⁻¹; however, during the remaining 364 days of the experiment, an increase in activity was observed in both air-dried litter and oven-dried litter (e.g., 0.032 ± 0.006 to 0.068 ± 0.001 µmol mg⁻¹ C h⁻¹ and
0.037 ± 0.004 to 0.087 ± 0.003 µmol mg\(^{-1}\) C h\(^{-1}\), respectively). Statistically significant interactions were also observed between time and litter age (p < 0.001) and time and drying treatment (p < 0.001).
DISCUSSION

I examined the effects of the commonly-employed methods of collecting litter prematurely (e.g., senescent) and litter manipulations (e.g., oven-drying vs. air-drying) on microbial litter decay processes associated with decaying litter of the emergent macrophyte *Typha angustifolia*. The underlying hypothesis of this research was that litter age (i.e., senescent vs. standing dead) and drying manipulations (e.g., oven-drying vs. air-drying) would significantly alter microbial litter decay processes and litter-associated nutrient dynamics (i.e., nitrogen and phosphorus).

*Litter Mass Loss*

Litter mass loss patterns were significantly different between litter age treatments, with standing dead litter having greater levels of mass loss than corresponding senescent litter. Litter mass loss patterns were also significantly different between drying treatments, with air-dried litter having lost more mass during the first 5 months of the study than oven-dried litter. Although comparisons in mass loss patterns can sometimes prove difficult (e.g., different hydrologic and seasonal periods, and litter quality; Bedford 2005), *Typha* mass loss patterns observed in this study were similar to recent studies examining submerged *Typha* decomposition (e.g., Vargo and others 2000; Su and others 2007). For example, Su and others (2007) examined the effects of sedimentation on submerged/sediment leaf litter decay and microbial dynamics associated with *Typha angustifolia* and observed between 55% dry weight remaining after ~117 days. Also, Vargo and others (1998), examined the *Typha* decay and observed ~60% dry weight remaining after ~117 days.
Although significant differences were observed in overall mass loss patterns, the measured decay rates (k) for all four litter treatments were not significantly different (Table 2), and were slightly slower than previously published rates for various *Typha* species (Hill 1985; Nelson and others 1990; van der Valk and others 1991; Neely 1994; Bärlocher and Biddiscombe 1996; Chimney and Pietro 2006). For example, both Neely (1994) and Bärlocher and Biddiscombe (1996) reported decay rates of -0.0072 d\(^{-1}\) for submerged/sediment associated *Typha latifolia* litter, and earlier studies by van der Valk (1991) observed even higher decay rates (-0.0131 d\(^{-1}\)). Nelson and others (1990) examined litter decay differences between two types of *Typha* litter (living and senescent). They observed decay rates of -0.011 d\(^{-1}\) for senescent litter, but much higher (> 2 times) decay rates were observed for green litter (i.e., -0.0241 d\(^{-1}\)), suggesting that the higher levels of labile carbon and nutrients in living green plant matter resulted in the faster decay rate observed. Other *Typha* decay studies observed similar decay rates to this study (Vargo and others 1998; Gonçalves and others 2004; Su and others 2007). For example, Vargo and others (1998) observed decay rates of ~ -0.0018 d\(^{-1}\) using standing dead litter, while Gonçalves and others (2004) observed decay rates of -0.0017 d\(^{-1}\) using senescent litter. Based on the variety of observed decay rates in the literature, it may be difficult to directly compare mass loss patterns and decay rates since study sites and *Typha* litter are likely to differ markedly in environmental condition (e.g., temperatures and hydrologic periods, see Su and others 2007) and the intrinsic quality of the plant litter (see Bedford 2005), respectively.
Robinson and Gessner (2000) observed that nutrient additions (i.e., N and P amendment) significantly increased deciduous leaf litter decay rates in Alpine stream. Likewise, Nelson and others (1990) compared rates of decomposition and nutrient release between *Typha* litter toppled prematurely by muskrats (i.e., green) and *Typha* that naturally senesced. They found that there were 21 and 36 times more N and P, respectively, in toppled litter (i.e., green) than in senescent litter and that decomposition rates for green litter were higher (e.g., green litter -0.0241 d\(^{-1}\) vs. -0.011 d\(^{-1}\) for senescent litter). Davis (1991) also found nutrient enrichment to increase decay rates of *Typha* litter. In the present study, recently senescent litter was more nutrient-rich (i.e., lower C:N, C:P) than corresponding standing dead litter throughout the study period (see below). As a consequence, I expected that the increased presence of litter-associated nutrients (N and P) would result in a faster rate of decay for senescent *Typha* litter. However, the similarity in decay rates between senescent and standing-dead litter observed in this study did not support this hypothesis. It is possible that sediment/surface water nutrient conditions at Independence Lake were sufficient enough to support microbial nutrient demand, despite clear differences in nutrient concentrations (N and P) between litter types.

Total litterbag N content (mg per litterbag) was not significantly different between drying treatments (air-dried litter vs. oven-dried litter). However, total N content was significantly different between litter age treatments (senescent vs. standing dead). Temporal patterns in N content also varied between senescent and standing dead litter. Both senescent and standing dead litter experienced net N mineralization during the initial month of this study, which may be attributed to initial leaching processes after submergence of litter bags.
This initial leaching phase is often observed when plant litter enters the aquatic milieu (see Boulton and Boon 1991; Bärlocher 1997; Gessner 1999). Overall, senescent litter experienced a net mineralization of N, whereas standing dead litter experienced net immobilization of N. Moran and Hodson (1989) observed similar results using senescent *Typha* litter, where N concentration increased and C: N ratios decreased (see below) during the study period. This observation supports the hypothesis that senescent litter would not have undergone initial microbial conditioning and decomposition during the standing dead phase and thus would contain substantially more nutrients than standing dead litter. This difference between litter types during decomposition (i.e., senescent vs. standing-dead) illustrates how varying nutrient dynamics (i.e., mineralization vs. immobilization) patterns can occur as a result of the initial litter substrate (methodology) used at the time of litter bag construction (i.e., type/age of litter utilized).

Temporal patterns in total litter P content (mg litterbag$^{-1}$) were also significantly different between litter age treatments, with standing dead litter P content increasing more than 60 times that of senescent litter P during the study period. Similar to the trends observed in N content, patterns of P content showed a net mineralization during the initial month of the study, which was likely due to initial leaching processes. The observed large increase in fungal biomass associated with standing dead litter (see following section) indicates that there was net P immobilization occurring with the standing dead litter over the 450-day study period. The differences observed between litter age for P content support our hypothesis that standing dead litter, which has been conditioned, is not as rich in nutrients as senescent litter. Therefore the increase in P content is likely due to the litter-associated increase observed in microbial growth as they immobilize P from the environment.
Total litter P content was also significantly different between drying treatments. Both litter treatments (i.e., air-dried and oven-dried litter) increased in total P content, but air-dried litter P content increased by 3 times less than oven-dried litter. Thus, immobilization of P was greater in oven-dried litter. The difference in fungal biomass likely explains why there was more P immobilization occurring with oven-dried litter than air-dried litter. Fungal biomass associated with oven-dried litter increased 13 times more than that associated with air-dried litter, and, thus, more P could be immobilized within fungi on the oven-dried litter than air-dried litter.

Litter N concentrations (% N by weight) in all age and drying treatments increased throughout the 450-day study, and differed significantly between litter age treatments and drying treatments. Drying treatments also showed significant differences in P concentrations. These data indicate that N and P immobilization was occurring more readily in oven-dried litter. Since air-dried litter likely maintained its natural microbial fauna, as it was not manipulated through oven-drying, it seems logical that oven-dried litter can be colonized more readily, and therefore N and P would be immobilized more quickly.

C:N, C:P, and N:P ratios all differed significantly between litter age treatments and drying treatments. All litter began the study with the same amount of mass, yet the high N content of senescent litter resulted in a low C:N ratio relative to standing dead litter C:N ratios. Similar results were observed for C:P ratios. The data support our hypothesis that senescent litter, having avoided the standing dead phase, would be nutrient-rich when compared to standing dead litter.

Significant differences were also observed in C:N and C:P ratios between air-dried and oven-dried litter. The differences between air-dried and oven-dried litters are probably
more a matter of differential nutrient immobilization (via associated microbial biomass). For all litter treatments, it is interesting to note that C:N and C:P ratios both displayed net increases during the first 2 weeks of this study. These increases are probably due to leaching of molecules rich in N and P (Polunin 1984; Boulton and Boon 1991; Bärlocher 1997; Gessner and others 1999; Gessner 2000). After the first 2 weeks of this study, C:N and C:P ratios declined steadily for the next 4.5 months until finally leveling off. This trend was inversely related to total microbial biomass, specifically fungal biomass, similar to patterns observed by Su and others (2007).

N:P ratios for all litter treatments decreased during the 450-day study period. N:P ratios decreased more in standing dead litter than in senescent litter. This difference in N:P ratios between litter age treatments is likely due to the increased net immobilization of P in standing dead litter compared to senescent litter during the study period. This supports our hypothesis that standing dead litter, which has been conditioned, would not be as rich in nutrients as senescent litter. N:P ratios were also significantly different between drying treatments. N:P ratios associated with oven-dried litter decreased more than air-dried litter N:P ratios. It is likely this is due to the greater net immobilization of P observed in oven-dried litter in comparison to air-dried litter. This supports our hypothesis that oven-drying litter would eliminate existing microbial fauna and therefore cause increased microbial activities after submergence.

Trends for litter nutrient dynamics (N and P) are similar to those observed in other vascular plant decomposition studies (Nelson and others 1990; Vargo and others 1998; Su and others 2007). For example, Su and others (2007) examined the effects of sedimentation on submerged/sediment leaf litter decay and microbial dynamics associated with *Typha* litter
and found that P concentrations (% P) increased throughout the study period. Su and others (2007) also found that during the initial 100-150 days of the study, P was being immobilized and that N was immobilized throughout the decay study. Additionally, Vargo and others (1998) examined the impact of sedimentation on *Typha* decay and observed an initial decrease of N concentration followed by a gradual increase in N concentration during the study period, yet, in contrast, observed a decrease in P concentrations during the study period. However, litter nutrient dynamics (N and P) observed in other studies contrasted with this study (Moran and Hodson 1989; Thormann and Bayley 1997). For example, Thormann and Bayley (1997) examined the decomposition of *Typha* in a lacustrine marsh and a floating sedge fen and observed C:N ratios ranging from 9-15 throughout the study period, which is lower than what was observed in this study.

*Microbial Biomass and Production*

In the present study, drying manipulations of *Typha* litter (i.e., oven-dried vs. air-dried) did not have a significant effect on the development and biomass accumulation of bacterial decomposers. In contrast, significant differences in bacterial biomass patterns were observed between senescent and standing-dead *Typha* litter, where senescent litter had more bacterial biomass than standing dead litter. Litter that has collapsed to the sediment-water interface will leach dissolved organic matter (DOM), which ultimately provides surface-associated decomposers (i.e., bacteria) essential carbon and nutrients for growth. Differences observed in bacterial biomass between litter age treatments may be a result of nutrient differences observed between senescent and standing dead litter (see above). Hence, studies using prematurely collected litter (senescent) versus more natural litter (standing dead) may
observe unnatural increases and biomass dynamics in litter-associated bacterial decomposers. In all treatments, bacterial decomposers were a minor component of the microbial community.

Our results are similar to bacterial dynamics observed in other decomposition studies employing standing dead, air-dried litter (Komínková and others 2000; Kuehn and others 2000; Findlay and others 2002; Su and others 2007). For example, Su and others (2007) observed initially low bacterial biomass after submergence, with biomass increasing steadily during the study period, but never accounting for more than 6% of total microbial biomass. Komínková and others (2000), investigating microbial dynamics associated with *Phragmites australis*, observed similar patterns in bacterial biomass (increase in biomass through time) after the litter was submerged. However, they never observed bacterial biomass accounting for > 10% of total microbial biomass. This is in direct contrast with reports by other authors, using senescent and manipulated (i.e., ground) vascular plant litter, which found that bacteria were the major mediators of vascular plant decomposition under submerged or sediment conditions (Benner and others 1986; Moran and Hodson 1989).

In contrast to bacterial biomass, fungal biomass accounted for > 98-99% of total microbial biomass (bacterial + fungal) for all litter treatments throughout the entire 450-day study period. Fungal biomass associated with air-dried litter was, on average, 4 times greater than that of oven-dried litter, indicating that oven-drying litter prior to its use in decomposition studies can result in the elimination of resident fungal populations and hence litter-associated fungal biomass. Fungal biomass was also significantly different between litter age treatments. Fungal biomass associated with senescent litter was, on average, 1.2 times greater than that found on standing dead litter, further indicating that the higher
nutrient concentrations and availability in senescent litter may have supported greater fungal growth. The disproportionate amount of fungal biomass to bacterial biomass has been observed in similar decay studies using standing dead, air-dried litter (Komínková and others 2000; Kuehn and others 2000; Francoeur and others 2006; Su and others 2007). For example, Su and others (2007) examined the effects of sedimentation on microbial dynamics associated with decaying *Typha* litter and found fungal biomass was consistently > 94% of total microbial biomass. Also, Francoeur and others (2006) observed fungal biomass to account for ≥ 93% of total heterotrophic microbial biomass on decaying *Typha* litter. These observations in fungal biomass conflict with other decomposition studies that used oven-dried senescent vascular plant litter (Benner and others 1986; Moran and Hodson 1989; Thormann and others 2004a). For example, Thormann and others (2004a), using senescent litter, examined the effect of temperature and microbial species number on the decomposition of vascular plant litter. These studies revealed that bacterial decomposers were the dominant mediators in plant litter decomposition. Studies that found bacteria to dominate decomposition used prematurely harvested (senescent) litter that was oven-dried prior to being used in the study.

Similar to previous findings (e.g., Newell and others 1995; Komniková and others 2000; Kuehn and others 2000; Francoeur and others 2006; Su and others 2007), this study also found that fungi play a major role in the decomposition of emergent macrophyte plant litter in submerged environments. Based on these results, it seems that in order to accurately describe microbial (bacteria and fungi) patterns and processes (e.g., nutrient retention and release) associated with naturally decaying emergent macrophytes, studies should employ
standing dead material, and allow it to air dry so as not to significantly interrupt the natural
decay sequence.

Rates of fungal production were not significantly different between senescent and
standing dead litter age groups. However, drying treatment had a significant effect on fungal
production, as oven-dried litter experienced larger initial increases in production than that in
air-dried litter. The significant differences observed between air-dried and oven-dried litter
are likely related to the differences observed between drying treatment for fungal biomass.
Fungal biomass associated with oven-dried litter was initially ~2.5 times lower than what
was observed with air-dried litter, and oven-dried litter increased by 158% compared to < 1%
increase in fungal biomass associated with air-dried litter (i.e., throughout the 450-day
study). These data support our hypothesis that prior manipulations (i.e., oven-drying) can
create artifacts in data that can lead to inaccurate results about microbial dynamics and
subsequent nutrient dynamics.

Fungal production rates measured in this study were within range of those reported by
other authors using standing dead litter. For example, Kuehn and others (2000) examining
microbial parameters associated with *Juncus* litter observed fungal production rates ranging
from 0.073 mg C g\(^{-1}\) AFDM d\(^{-1}\) to 2.836 mg C g\(^{-1}\) AFDM d\(^{-1}\). However, other studies
observed much lower production rates. For example, Su and others (2007) examined
microbial dynamics associated with decaying *Typha* litter and found production rates ranging
from ~ 0.025 mg C g\(^{-1}\) AFDM d\(^{-1}\) to 0.20 mg C g\(^{-1}\) AFDM d\(^{-1}\), and Komniková and others
(2000) observed production rates from only 0.003 mg C g\(^{-1}\) AFDM d\(^{-1}\) to 0.051 mg C g\(^{-1}\)
AFDM d\(^{-1}\). It is possible that the differences observed in fungal production rates could be
related to different hydrologic patterns.
**Microbial Respiration**

In the present study, rates of litter-associated microbial respiration were significantly different between senescent and standing dead litter. Similar to patterns observed with levels of microbial biomass, litter-associated respiration rates were higher on senescent versus standing-dead litter for up to 6 weeks into the study period. Thereafter, respiration rates associated with senescent litter and standing dead litter were similar. Significant differences in microbial respiration rates were also observed between air-dried and oven-dried litter, with rates associated with air-dried litter ~ 10 times greater than those associated with oven-dried litter during the first week of the study. Even though air-dried litter continued to have greater respiration rates throughout the study period, oven-dried litter showed the greatest increase in microbial respiration (i.e., a 10-fold increase) after submergence and subsequent microbial colonization. The differences observed between air-dried litter and oven-dried litter can be partly explained by the differences in fungal biomass between air-dried and oven-dried litter. Initially, fungal biomass was greater in air-dried litter than in oven-dried litter but increased substantially more in oven-dried litter than in air-dried litter during the study period (e.g., 158% increase vs. <1% increase, respectively).

Rates of litter-associated microbial respiration are generally considered a direct measurement of litter decomposition (carbon mineralization), since the litter organic carbon is being assimilated and mineralized by inhabitant microbial decomposers. The increased respiration rates observed in senescent litter can be partly explained by the quality of the litter that was being assimilated by inhabitant microbial decomposers. Senescent litter had not been conditioned by microbial colonizers during the standing dead phase and therefore had more nutrients associated with it than standing dead litter and could support a larger
biomass of microbial communities than standing dead litter. The differences in microbial respiration observed between litter age treatments can be accounted for by the differences in fungal biomass associated with the two litter age treatments, where senescent litter contained more fungal biomass than standing dead litter.

Litter-associated microbial respiration rates for this study support our hypothesis that the use of senescent litter and/or the manipulation of litter (i.e., oven-drying) can lead to artifacts in the understanding of microbial dynamics and subsequent nutrient cycling. Consistent with this study, Komniková and others (2000) reported similar litter-associated microbial respiration rates ranging from 255 ± 40 to 58 ± 18 µg C g\(^{-1}\) detrital C h\(^{-1}\) on Phragmites australis leaf litter. However, they are much lower than rates reported by Su and others (2007), who examined microbial dynamics associated with decomposing Typha litter (e.g., reported mean values of 170 ± 7 and 191 ± 49 µg C g\(^{-1}\) detrital C h\(^{-1}\)), which may be due to different hydrologic regimes between the observed studies.

**Enzyme Activity**

All carbon-acquiring extracellular enzymes (i.e., β-1,4-glucosidase, β-1,4-xylosidase, and Cellobiohydrolase) showed significant differences between litter age treatments and drying treatments. During the first 2.5 months of this study, senescent litter had greater enzyme activity than standing dead litter for all carbon-acquiring enzymes, which is consistent with the higher levels of fungal biomass (~70%) observed in senescent versus standing-dead litter. The differences observed between oven-dried and air-dried litter can also be explained by the increase in fungal biomass (~40%) during the initial 3.5 months of the study, whereas air-dried litter increased only slightly (i.e., ~1%). Considering fungal
biomass was >98-99% of total microbial biomass, it is likely that the pattern observed for carbon-acquiring enzymes among litter treatments is due to inhabitant fungal decomposers. The observed patterns for all carbon-acquiring enzymes support our hypotheses that litter age or prior manipulations (i.e., oven-drying) will affect microbial activity and can create artifacts that lead to inaccurate conclusions regarding microbial dynamics and subsequent nutrient dynamics (i.e., initial death of inhabitant microbial communities). The trends observed for these three carbon-acquiring enzymes are concordant with other studies that used standing dead litter. For example, Su and others (2007) also observed extracellular enzyme patterns being similar to fungal biomass patterns. Also, Francoeur and others (2006) concluded that fungi were responsible for most of the extracellular enzyme production due to the fact that they found fungal biomass to be > 93% of total microbial biomass.

Acid phosphatase, an enzyme responsible for acquiring P, was the unique exception to the general pattern observed in extracellular enzyme activity during this study. Acid phosphatase activity did not differ between litter age treatments. It is possible that ambient P levels associated with Independence Lake lessened any potential differences in phosphatase production. In contrast to acid phosphatase, the activity of the N-acquiring enzyme β-N-acetyl-b-glucosaminidase was significantly affected by litter age. It is likely the observed difference in litter age treatments for the N-acquiring enzyme is due to the initial imbalance of N concentrations associated with both litter ages. β-N-acetyl-b-glucosaminidase activity associated with senescent litter decreased more than activity associated with standing dead litter, probably because standing dead litter had a lower N concentration than senescent litter.
In contrast to litter age, drying treatment (oven-drying vs. air-drying) had a significant effect on phosphatase activity and β-N-acetyl-b-glucosaminidase activity. Oven-dried litter increased in fungal biomass by 40% (i.e., first 3.5 months) and had a 10-fold increase in microbial respiration during the study period. Air-dried litter showed a small increase in fungal biomass (i.e., ~1%) and a small increase in microbial respiration (i.e., 2-fold increase). Considering fungal biomass was >98-99% of total microbial biomass, the increase in β-N-acetyl-b-glucosaminidase activity and phosphatase activity is likely due to fungal activities. Phosphatase and β-N-acetyl-b-glucosaminidase activity observed in this study is similar to what was reported by Su and others (2007), who observed P-acquiring enzyme activities ranging from $0.330 \pm 0.250 \, \mu\text{mol mg}^{-1} \, \text{C h}^{-1}$ to $0.086 \pm 0.081 \, \mu\text{mol mg}^{-1} \, \text{C h}^{-1}$ and N-acquiring enzymes of ~ $0.200 \, \mu\text{mol mg}^{-1} \, \text{C h}^{-1}$. Francoeur and others (2007) also reported consistent N- and P-acquiring enzyme activities as those observed in this study. The significant difference observed in the N-acquiring enzyme between litter age treatments supports our hypothesis that the premature collection of litter (i.e., senescent) may also lead to potential artifacts in data and inaccurate conclusions regarding nutrient dynamics associated with litter decomposition.

In conclusion, one of the most fundamental components of wetland food webs is detritus. These ecosystems are detritus-based systems, and the availability of detritus and associated microbial decomposers has profound effects on all wetland trophic levels and ecosystem functioning (Moore and others 2004). Some authors have discussed the problem with pre-drying (i.e., oven-drying) plant litter, hypothesizing that it could affect weight loss and can lead to inaccurate conclusions regarding litter mass loss and nutrient dynamics (Gopal and others 1982, Boulton and Boon, 1991; Bärlocher 1997). Others have expanded
upon this hypothesis by arguing that oven-drying or any manipulation to the litter (i.e., pre-mature collection of litter or senescent litter use) could create artifacts leading to incorrect conclusions about wetland ecosystem dynamics and function by altering microbial community activities (Boulton and Boon, 1991; Newell 1993; Gessner and others 1999; Bärlocher 1997; Gessner 2000).

This study is the first to illustrate the effects of two influential methods, pre-drying (i.e., oven-drying) litter and the pre-mature collection of litter (i.e., the use of senescent litter), on microbial decay and nutrient dynamics associated with submerged decomposing litter of the emergent macrophyte *Typha angustifolia*. Our data indicate that either of these methods can potentially introduce litter artifacts, leading to inaccurate conclusions regarding microbial and nutrient cycling dynamics associated with plant litter during decomposition. In particular, our data indicate that the use of senescent litter increases N and P concentrations and that oven-drying litter reduces microbial activity. This study also illustrated that fungi play a very important role in the decomposition of plant litter. In summary, our results support the review assertion of Boulton and Boon (1991), that the results obtained in plant litter decomposition studies are largely a result of the underlying methods employed.
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Table 1. Selected characteristics of Independence Lake surface waters and of the littoral *Typha angustifolia* stand where studies were conducted. Values are the means ± 1 SD. Surface water from the lake was sampled adjacent to the *Typha* stand on each sampling date between August 2001 and 2002 (N = 12).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake surface area (ha)</td>
<td>77.7</td>
</tr>
<tr>
<td>Size of <em>Typha</em> stand examined (m)</td>
<td>~ 25x100</td>
</tr>
<tr>
<td>Water depth along transect (cm)</td>
<td>12.4 ± 10.3</td>
</tr>
<tr>
<td>Water Temperature (°C)</td>
<td>14.6 ± 10.3</td>
</tr>
<tr>
<td>Above ground production (gC/m²/y) 2001-2002</td>
<td>1095 ± 199</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>317 ± 18.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.1 ± 0.08</td>
</tr>
<tr>
<td>Total Alkalinity (mg CaCO₃/l)</td>
<td>150 ± 7.89</td>
</tr>
<tr>
<td>TP (µg/l)</td>
<td>18 ± 11</td>
</tr>
<tr>
<td>NH₄⁺-N (µg/l)</td>
<td>110 ± 209</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/l)</td>
<td>12 ± 11</td>
</tr>
</tbody>
</table>
Table 2. Mass loss rates associated with *Typha* litter from Independence Lake Wetland. k: litter decay rate; M₀: initial ash-free dry mass in litter bags; ASE: asymptotic standard error.

<table>
<thead>
<tr>
<th>Litter Type</th>
<th>k ± ASE (d⁻¹)</th>
<th>m₀ ± ASE(%)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senescent (S)</td>
<td>-0.0014 ± 0.000099</td>
<td>99.4 ± 0.5</td>
<td>0.8056</td>
</tr>
<tr>
<td>Senescent Oven-Dried (SOD)</td>
<td>-0.0012 ± 0.000106</td>
<td>100.0 ± 0.6</td>
<td>0.7440</td>
</tr>
<tr>
<td>Standing Dead (STD)</td>
<td>-0.0016 ± 0.000113</td>
<td>99.5 ± 0.3</td>
<td>0.8167</td>
</tr>
<tr>
<td>Standing Dead Oven-Dried (STDOD)</td>
<td>-0.0018 ± 0.000118</td>
<td>99.8 ± 0.7</td>
<td>0.8334</td>
</tr>
</tbody>
</table>
Table 3. Beginning and ending nitrogen (N) and phosphorus (P) concentrations associated with the four *Typha* litter types used during this decay experiment. Values indicate mean ± SE.

<table>
<thead>
<tr>
<th>Litter Type</th>
<th>Day</th>
<th>N concentration (%)</th>
<th>P concentration (%)</th>
<th>Total N content (mg)</th>
<th>Total P content (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Senescent</strong></td>
<td>0</td>
<td>1.342 (±0.047)</td>
<td>0.080 (±0.007)</td>
<td>41.19 (±1.14)</td>
<td>2.47 (±0.31)</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>2.190 (±0.118)</td>
<td>0.125 (±0.008)</td>
<td>37.88 (±2.98)</td>
<td>2.17 (±0.22)</td>
</tr>
<tr>
<td><strong>Senescent Oven Dried</strong></td>
<td>0</td>
<td>1.189 (±0.037)</td>
<td>0.065 (±0.004)</td>
<td>38.41 (±2.08)</td>
<td>2.10 (±0.18)</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>2.118 (±0.092)</td>
<td>0.123 (±0.006)</td>
<td>40.54 (±1.97)</td>
<td>2.36 (±0.19)</td>
</tr>
<tr>
<td><strong>Standing Dead</strong></td>
<td>0</td>
<td>0.659 (±0.041)</td>
<td>0.041 (±0.004)</td>
<td>19.29 (±1.26)</td>
<td>1.20 (±0.12)</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>1.596 (±0.149)</td>
<td>0.130 (±0.014)</td>
<td>22.79 (±2.08)</td>
<td>1.86 (±0.21)</td>
</tr>
<tr>
<td><strong>Standing Dead Oven Dried</strong></td>
<td>0</td>
<td>0.748 (±0.079)</td>
<td>0.036 (±0.005)</td>
<td>22.73 (±2.47)</td>
<td>1.08 (±0.16)</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>1.636 (±0.128)</td>
<td>0.137 (±0.016)</td>
<td>22.04 (±2.18)</td>
<td>1.81 (±0.16)</td>
</tr>
</tbody>
</table>
Table 4. Fungal production associated with different litter types throughout the 450-day study. Statistically significant differences were observed through time (p < 0.001) and drying method (p = 0.007). Values indicate the mean ± SE.

<table>
<thead>
<tr>
<th>Litter Type</th>
<th>Fungal Production (mg g(^{-1}) AFDM d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Senescent</td>
<td>0.787 ±0.026</td>
</tr>
<tr>
<td>Senescent Oven Dried</td>
<td>0.493 ±0.206</td>
</tr>
<tr>
<td>Standing Dead</td>
<td>0.748 ±0.285</td>
</tr>
<tr>
<td>Standing Dead Oven Dried</td>
<td>0.283 ±0.020</td>
</tr>
</tbody>
</table>
Figure 1. Sediment surface and water temperature (°C) data collected at the sediment-water boundary. Data were collected over the entire 450-day study period using 2 data loggers. The gap in temperature data from day 144 to 198 was due to temperature logger failure.
Figure 2. *T. angustifolia* leaf litter percent ash-free dry mass (AFDM) remaining in mesh litter bags through time. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 3. Carbon:Nitrogen (C:N) and Carbon: Phosphorus (C:P) ratios associated with decaying *T. angustifolia* litter, respectively. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 4. Nitrogen:Phosphorus ratios (N:P) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 5. Bacterial biomass (mg C g\(^{-1}\) AFDM) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 6. Fungal biomass (mg C g\(^{-1}\) AFDM) associated with decaying \textit{T. angustifolia} litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 7. Rates of microbial respiration (µg C g⁻¹ AFDM h⁻¹) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 8. $\beta$-1,4-glucosidase activity ($\mu$mol mg$^{-1}$ C h$^{-1}$) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 9. β-1,4-xylosidase activity (µmol mg⁻¹ C h⁻¹) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 10. Cellobiohydrolase activity (μmol mg\(^{-1}\) C h\(^{-1}\)) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 11. Acid Phosphatase activity (µmol mg⁻¹ C h⁻¹) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.
Figure 12. β-N-acetyl-b-glucosaminidase activity (µmol mg⁻¹ C h⁻¹) associated with decaying *T. angustifolia* litter. Senescent, Senescent Oven-dried, Standing Dead, and Standing Dead Oven-dried litter, respectively. Symbols indicate the mean ± 1SE (n = 4). Lack of error bars indicates SEs smaller than plotted symbol.