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The role of fungi and invertebrates in litter decomposition in mitigated and reference wetlands

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ABSTRACT

Wetland plant litter decomposition influences many wetland processes and is itself driven by a complex web of interacting parameters. Invertebrates and fungi make up one portion of that web by processing organic material; however, their role is poorly understood. To explore invertebrate and fungal influence on plant litter decomposition rate, we measured the decomposition of litter in three mitigated (created wetlands) and three reference wetlands in the Mid-Atlantic Highlands of West Virginia, USA. Litter decomposition rates and most invertebrate metrics were not statistically different between mitigated and reference wetlands; only oligochaetes (worms) and the functional feeding group (FFG) collector/gatherers had numbers that were statistically higher in mitigated wetlands. Invertebrate metrics were able to explain 25% (FFG) to 31% (taxonomic groups) of variance during the first phase of decomposition (<224 days) and 15% (FFG) to 21% (taxonomic groups) during the second phase (≥224 days). Shredders, collector/gatherers, and omnivores were more strongly associated with early phases of decomposition, while oligochaetes and omnivores were most strongly associated with trends in decomposition during the later phase. Fungal biomass, as measured by ergosterol concentration, was similar between mitigated and reference wetlands and was significantly higher in the first phase of litter decomposition than the second phase, but was not statistically correlated with litter decomposition rate. Decomposition influences many aspects of wetland function, making the variables that determine decomposition rates important for assessing and mitigating for lost wetland function.

1. Introduction

Wetlands provide many ecosystem services. When natural wetlands in the United States are filled in or destroyed legally, new wetlands are created or previously existing wetlands are restored or enhanced with the intention of replacing lost net ecological function. In order to accomplish that goal, we need to understand the web of interacting forces that support wetland function. Plant litter decomposition is an important part of the web and influences the physical and chemical properties of wetland soils (Mitsch and Gosselink, 2007), nutrient availability and cycling (Prentki et al., 1978; Facelli and Pickett, 1991), primary productivity (Brinson et al., 1981; Xiong and Nilsson, 1997), and organic matter accumulation (Gambrell and Patrick, 1978; Xiong and Nilsson, 1997). These processes link decomposition to overall wetland services such as invertebrate and wildlife habitat through primary production and detritus availability (Burdett and Watts, 2009; Taylor and Batzer, 2010), to carbon storage through organic matter accumulation (Bridgham et al., 2006), to sediment and mineral retention through primary productivity and organic matter accumulation (Braskerud, 2000; Rooth et al., 2003), and to stream nutrient availability through nutrient cycling (Richardson, 1994; Mitsch and Gosselink, 2007).

Invertebrates contribute to wetland services by playing an important role in litter decomposition (Fazi and Rossi, 2000; Wu et al., 2009). Several studies have implicated invertebrates, particularly invertebrates belonging to the collector/gather and shredder functional feeding groups (FFG) in contributing to plant litter decomposition (Merritt and Lawson, 1979; Brinson et al., 1981; Inkley et al., 2008; Tiegs et al., 2013). Clams (Scatolini and Zedler, 1996), snails (Balcombe et al., 2005a; Meyer and Whiles, 2008), amphipods (Meyer and Whiles, 2008), isopods (Balcombe et al., 2005a), leeches (Meyer and Whiles, 2008), and some hemipterans (Brown et al., 1997) have all been found to have lower abundances in created wetlands, with differences attributed to lower dispersal

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rates. If differences in invertebrate communities exist in mitigated wetlands, they could affect wetland function through slower litter decomposition.

Microbial colonization also contributes to litter decomposition through bacterial (Kuehn et al., 2000; Jackson and Vallaire, 2007) and fungal processes (Gessner and Chauvet, 1994; Findlay et al., 2002). This study focused on fungal biomass, which is easy to quantify by measuring ergosterol (a sterol present in fungal cell membranes and absent from animal and plant cells) in leaf litter (Newell et al., 1988; Kuehn et al., 2000). Fungal colonization and decomposition begins after senescence, but while plant litter is still standing (Facelli and Pickett, 1991; Kuehn et al., 2000; Chimney and Pietro, 2006) and continues after submergence (Bauer et al., 2003; Kuehn et al., 2011; Zhang et al., 2014). Kuehn et al. (2011) found that 22% of leaf Carbon from *Typha angustifolia* was assimilated into fungal biomass. It is largely unknown how microbial communities in mitigated wetlands compare to those in natural communities.

Mesh litter bags have long been used to assess both decomposition rates and the role of macroinvertebrates on decomposition (Witkamp and Olson, 1963; Merritt and Lawson, 1979; Stewart and Davies, 1989; Vasilas et al., 2013). In this study, we used two sizes of mesh for the litter bags to create a continuum of invertebrates by size and study the role of invertebrate biomass on decomposition. We hypothesize that decomposition rates are similar between mitigated and reference wetlands and that both invertebrates and fungi influence decomposition rates. Our primary objective was to compare plant litter decomposition among wetland types (mitigated vs reference wetlands) in the Mid-Atlantic Highlands, USA. Our second objective was to determine if invertebrate biomass and fungal biomass was correlated with decomposition rate or wetland type.

2. Materials and methods

2.1. Study area

Leaf breakdown rates were measured at three mitigated and three reference wetlands located in the Mid-Atlantic Highlands region of West Virginia, USA. The three mitigated wetlands (Leading Creek, Sugar Creek, Hazelton) were constructed by the West Virginia Division of Highways (WVDOH) to compensate for wetland losses associated with the Corridor H and Mon-Fayette Expressway system projects (Table 1). The three reference wetlands (Meadowville, Upper Deckers Creek, and Bruceton Mills) were chosen based on the following factors: their proximity to mitigated sites (to minimize differences in climatic events); their similarity in elevation and wetland classification; and their relative degree of disturbance (minimal disturbance on their edge and no disturbance in the interior). Both mitigated and reference wetlands had some level of disturbance on their edge in the form of roads, grazing, or cultivated land. All wetlands were associated with streams and received water from overbank flooding, with hillslope runoff and groundwater as additional sources. All wetlands also had a mixture of flooded and exposed conditions for the majority of the year, with brief periods of deeper flooding, but mitigated wetlands tended to have a higher percentage of open water and ponded areas than reference sites. Reference sites tended to have more scrub–shrub areas than the mitigated sites, and Leading Creek, Meadowville, and Upper Deckers Creek had portions of scrub–shrub and young forest. Although water depth, temperatures, and pH varied throughout the year, there were no statistical differences between wetland types ($p \geq 0.2$; Gingerich, 2010).

2.2. Decomposition (litterbag) procedures

We collected (September–October 2007) three litter species (common rush [*Juncus effusus* L.], brookside alder [*Alnus serrulata* (Ait.) Willd.], and reed canary grass [*Phalaris arundinacea* L.]) based on common dominant species at mitigated and reference sites in West Virginia (Balcombe et al., 2005b; Veselka IV, 2008) and used the litter bag method to compute litter decomposition rates (Benfield, 1996). Not all wetlands studied had the same dominant species or ratio of dominant species; however, litter mixes can have non-additive decomposition rates compared to single species (Gartner and Cardon, 2004). In an attempt to more closely mimic the natural systems in our study wetlands and the most common species across wetlands (Balcombe et al., 2005b; Veselka IV, 2008), 20 g of litter was created from a mix of 3:2:1 reed canary grass (10 g), common rush (6.6 g), and brookside alder (3.3 g).

To minimize variability, reed canary grass and common rush leaves and stems were clipped and collected as they senesced but while still standing (Marsh et al., 2000; Bedford, 2005). We collected brookside alder leaves with a STIHL model SH 85 D Shredder Vacuum/Blower (STIHL Incorporated, Virginia Beach, VI) reversed to suck leaves into the tube. Brookside alder leaves that were not

Table 1
List of three mitigated and three reference wetland study sites in West Virginia, including site name, year created, county and closest town, size (ha), wetland classifications, and differences in mean air temp, water temp, water depth, hydroperiod, and pH. Environmental measurements were taken every two weeks in each wetland from December 2007 to December 2009. Standard error (S.E.) is presented in parentheses under each mean. Analysis of correlations between environmental factors and decomposition rates can be found in Gingerich et al. (2014).

Site name (year created)	County and closest town	Size (ha)	Wetland classifications* at Site	Air Temp. (°C)	Water Temp. (°C)	Water depth (cm)	Hydroperiod†	pH
Mitigated								
Leading Creek (1995)	Montrose, Randolph Co.	17	AB, EP, **SS**‡	9.56 (0.62)	7.16 (0.40)	8.05 (1.30)	0.49 (0.05)	6.30 (0.07)
Sugar Creek (1995)	Meadowville, Barbour Co.	11	**EP**, SS	10.92 (0.58)	7.88 (0.41)	6.34 (1.08)	0.43 (0.05)	6.09 (0.06)
Hazelton (2006)	Hazelton, Preston Co.	2.7	UB, AB, **EP**	6.39 (0.85)	7.70 (0.90)	2.88 (0.69)	0.20 (0.04)	6.93 (0.08)
Reference								
Meadowville	Meadowville, Barbour Co.	11.7	EP, **SS**	10.51 (0.63)	7.83 (0.50)	2.23 (0.51)	0.28 (0.05)	6.37 (0.09)
Upper Deckers Creek	Masontown, Preston Co.	2.1	AB, **SS**, F	10.10 (0.68)	5.37 (0.46)	8.16 (1.32)	0.35 (0.05)	6.21 (0.02)
Bruceton Mills	Bruceton Mills, Preston Co.	1.4	**EP**, SS	8.07 (0.66)	7.06 (0.64)	3.25 (0.41)	0.50 (0.05)	6.55 (0.05)

* Palustrine: unconsolidated bottom = UB, aquatic bed = AB, emergent persistent = EP, scrub–shrub = SS, forested = F (Cowardin et al., 1979).
† Measured as proportion of days inundated.
‡ Bold text indicates dominant classifications.

intact and any material other than alder leaves were discarded. To minimize differences in litter quality, each species was collected from only one area in a single wetland (Baker et al., 2001; Fennessy et al., 2008). We air-dried all litter for a minimum of 1 week before weighing and bagging it.

We constructed 20 × 20 cm litter bags from 1.27 mm (fine) and 2.8 mm (coarse) vinyl-coated fiberglass window mesh (Benfield, 1996). The fine mesh size was chosen to exclude as many invertebrates as possible while trying to keep from creating a microenvironment that was different from the external wetland environment. The coarse mesh was chosen to allow larger invertebrates access to the litter, while remaining fine enough to keep unprocessed litter fragments from slipping through. Litter bags were constructed with one folded side and three heat-sealed sides, and reinforced with stainless steel staples at 5-cm intervals (Hough and Cole, 2009). Each bag was uniquely marked with a plastic tag (Vargo et al., 1998; Hough and Cole, 2009).

Nine transects were established, using stratified sampling (Taylor and Middleton, 2004), to represent aerial proportions of environmental conditions. Ten wooden stakes were installed at 7.5 m intervals along each transect and one fine and one coarse-mesh bag were attached to each stake with 0.5 m lengths of nylon fishing line (Battle and Golladay, 2001; Anderson and Smith, 2002). Litter bags were placed flat on bare ground or on top of any existing litter to mimic natural litter deposition. If the stake was located in standing water, the litter bag was submerged before being allowed to float or sink, with the intention of minimizing the hydrophobic effect of the mesh. The fine mesh had a greater hydrophobic effect than the coarse mesh and dunking the bags helped remove the effect and the potential bias it created.

In December 2007, ninety of each type of litter bag (180 total) were placed in each wetland for a total of 1080 litter bags. Extra litter bags (1.5× the collected number) were placed in wetlands to compensate for anticipated litter bag losses from environmental disturbance (e.g., currents during flooding) and destruction from wildlife. Six replicates of each litter type were retrieved the same day the bags were placed in the field to calculate the loss of mass due to handling (Benfield, 1996). Four replicates were then retrieved at 1, 3, 5, 7, 11, 17, 24, 32, 42, 52, 65, 78, 91, and 104 weeks. We sampled the four replicates by collecting all litter bags from four randomly chosen stakes in each wetland. A total of 686 litter bags were collected.

Litter bags were transported to the lab on ice, cleared of external material, and opened. Litter was rinsed from the interior of the bag into a 500 μm sieve and sediment was rinsed off. Invertebrates were picked from the litter of all bags and preserved in 80% ethanol for later identification. We oven-dried (65 °C) leaf litter for 7–9 days until a constant mass could be recorded and then ground the litter to a powder in a 2-mm mesh Thomas Wiley Mill (Thomas Scientific, Swedesboro, NJ). Three subsamples of the ground litter were then incinerated to calculate ash-free dry mass (AFDM), which was used for statistical analysis.

2.3. Invertebrates

We identified invertebrates to family, classified to FFG, and tallied individuals by using previously described methods and resources (Bland and Jaques, 1978; Dindal, 1990; Peckarsky et al., 1990; Stehr, 1991; Chu and Cutkomp, 1992; Merritt and Cummins, 1996; Ubick et al., 2005; Wolfenbarger et al., 2008). Some individuals proved problematic to identify to family, therefore leeches (Hirundinea), worms (Oligochaeta), and mites (Acarni) were identified to subclass, and slugs (Stylommatophora) were identified to order, but all were included in analysis with families. Taxonomic groups that could not be identified to specific feeding guilds (scrapers, filterers, predators, collector/gatherers, shredders) were identified to the general groups of herbivores or omnivores. Because terrestrial invertebrates have greater diversity and less available information on their FFG, they were often identified as herbivores, omnivores, or predators and made up a larger portion of those groups than aquatic species. Total dry mass of oligochaetes was 2.5× greater than the next taxonomic group; therefore, they were separated out into their own group for FFG analysis. Total biomass and detritivore metrics were calculated both with and without the inclusion of oligochaetes. Richness was expressed as the number of taxonomic groups/litter bag. Invertebrate biomass (mg/litter bag) was obtained by oven-drying samples at 55 °C for ≥48 h to a constant mass (0.0001 g) and using an analytic scale (Balcombe et al., 2005a).

2.4. Ergosterol

Fungal biomass was estimated for fine mesh litter bags by the extraction and quantification of ergosterol from ground litter (Newell et al., 1988; Kuehn et al., 2000) using a modification of the cold ethanol procedure described in Richardson and Logendra (1997). We mixed 0.2 g of ground litter and 1 mL of absolute ethanol in 2-mL, screw-cap microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA) in a FastPrep FP120 (Q-biogene, Irvine, CA) with agitation at 6.0 m s^{-1} for 30 s. Ergosterol was then extracted for 30 min by rotating, end-over-end at 15 rpm, on a Glas-Col (Terre Haute, IN) mini-rotator. Samples were centrifuged for 10 min at 10,000 rpm in a VSB-14 microcentrifuge (Shelton Scientific, Shelton, CT) before the supernatant was removed and filtered through a 0.22-μm nylon filter microcentrifuge tube (Costar, Corning, NY) by centrifugation for 2 min at 10,000 rpm.

Ergosterol was analyzed by high-performance liquid chromatography (HPLC) on a 150 mm × 4.6 mm Phenomenex Prodigy 5-μm ODS3 reverse phase C18 column (Phenomenex, Torrance, CA). HPLC conditions were described previously (Panaccione and Coyle, 2005) and consisted of a model 600 pump controller with an in-line degasser, a model 717plus autosampler, and a model 2487 absorbance detector (all from Waters Corp., Milford, MA). Samples were eluted isocratically with 100% methanol at a flow rate of 1.0 mL min^{-1}, and peaks were monitored at 280 nm. Ergosterol eluted at ~9.0 min and was quantified by the external standard method using a pure compound (UV absorption in MeOH, λ_{max} = 282 with shoulders at 269 and 293) obtained from a commercial source (MP Biomedicals, Solon, OH). The presence of ergosterol was confirmed by comparison of HPLC retention times and UV absorption of the unknown peak and pure standard. Ergosterol is expressed as μg ergosterol mg^{-1} dry weight litter.

2.5. Data analysis

We used an exponential decay rate to model leaf litter decomposition and calculate decomposition rate:

$$\frac{y_t}{y_o} = e^{-kt}$$

where k is the instantaneous decomposition rate constant ($year^{-1}$), y_t is the AFDM at time t (years), and y_o is the initial AFDM (Olson, 1963; Brock et al., 1985).

Normality was checked using the Shapiro–Wilk test (shapiro.test {stats}) in Program R (version 2.10.1) and parameters were transformed to more closely approximate normality. All count data were log transformed, decomposition rate of litter was inverse square root ([1/−(sqrt x)] + 1) transformed, and ergosterol was sqrt transformed. Correlations between invertebrate metrics were checked visually using a scatterplot matrix (pairs {graphics}) and with the Pearson's correlation (cor {stats}) in Program R. Diversity and richness were highly correlated (r > 0.75), therefore

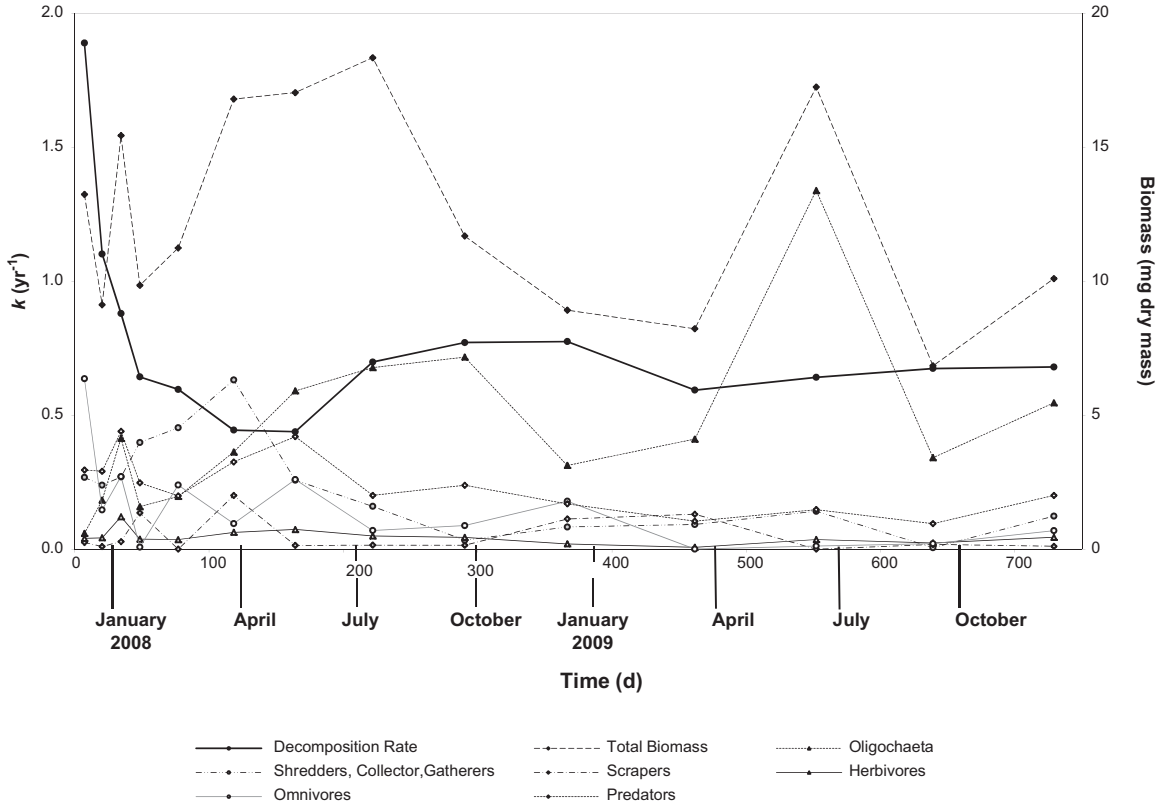

Fig. 1. Litter decomposition rate constant k (year^{-1}) and invertebrate functional feeding group biomass (mg dry mass litter) from litter bags collected from three mitigated and three reference wetlands in the Mid-Atlantic Highlands region, December 2007 to December 2009.

richness was used because it was better able to predict litter decomposition (lower Akaike Information Criteria value; Burnham and Anderson, 2002) than diversity when tested in a single parameter regression model. Analysis of variance (ANOVA) tested the influence of mesh size (fine, coarse), wetland type (mitigated, reference), collection date, and biomass of invertebrate metrics (collected from litter bags) on decomposition rate using a linear mixed effects (lme {nlme}) model in Program R. Wetland was treated as a random effect and stakes were experimental units. Regression tree analysis was performed using mvpart {mvpart} in Program R to identify quantitative differences in decomposition rates based on the biomass of taxonomic groups, FFG, and all invertebrate metrics (De'ath and Fabricius, 2000). Regression trees were pruned, based on percent of variance explained, to prevent over-fitting the data.

3. Results

3.1. Decomposition

Decomposition of litter was not statistically different between litter bag mesh sizes and wetland types. Proportion of mass remaining for fine mesh ($\bar{x} = 28.3$, S.E. = 1.8) and coarse mesh ($\bar{x} = 26.1$, S.E. = 1.7) bags were similar ($F_{1,41} = 1.05$, $p = 0.312$). Litter decomposition rate constants were rapid initially, likely due to rapid mass loss from leaching (Fig. 1). They then continued to slow until 119 to 168 days, after which decomposition rates rose slightly and leveled off to an average rate of 0.69 year^{-1} for the rest of the study period, with only slight fluctuations that were likely due to seasonal effects.

The ANOVA indicated a significant interaction between wetland type and mesh size for decomposition rate constant (Table 2); therefore, average decomposition rate constants of meshes were tested within each wetland type. For mitigated wetlands, mean k for fine mesh ($\bar{x} = 0.69$, S.E. = 0.04) and coarse mesh ($\bar{x} = 0.78$, S.E. = 0.04) bags were not significantly different ($F_{1,315} = 3.60$, $p = 0.059$). For reference wetlands, values of mean k for fine mesh ($\bar{x} = 0.87$, S.E. = 0.06) and coarse mesh ($\bar{x} = 0.77$, S.E. = 0.04) bags were again not significant ($F_{1,320} = 1.38$, $p = 0.241$). The significant interaction therefore was a product of fine mesh bags having a higher decomposition rate in reference wetlands, but a lower mean rate in mitigated wetlands. Collection date also had a significant effect on decomposition rate, indicating that rates changed over time. Because decomposition rate was similar among mesh sizes and wetland types, all litter bags were combined for invertebrate analysis.

3.2. Invertebrates

We picked 7973 individuals from the 642 collected litter bags and identified them to 125 taxonomic groups (120 families, one order, and four subclasses). Oligochaetes (worms), formicids (ants),

Table 2
Analysis of variance results for plant litter decomposition, expressed as average decomposition rate constant k (year^{-1}), in six wetlands (three mitigated, three reference) in West Virginia, December 2007 to December 2009. Wetland type (mitigated, reference), mesh size (fine, coarse), date ($n = 14$), and their interactions were all tested. Date and the interaction between type and mesh were significant ($p < 0.05$).

Effect	Num DF	Den DF	F value	p Value
Type	1	583	0.17	0.680
Mesh	1	583	0.22	0.637
Date	13	583	14.54	**<0.001**
Type × mesh	1	583	4.75	**0.030**
Type × date	13	583	0.9	0.557
Mesh × date	13	583	0.32	0.990
Type × mesh × date	13	583	0.81	0.650

Table 3
Overall means per bag, standard errors (S.E.), and maximums for five invertebrate metrics, seven functional feeding groups (FFG), and the top 20 taxonomic groups by mass (mg dry mass). Minimums were 0 for all metrics.

Invertebrate metric	Family	Mean (mg)	S.E. (mg)	Max (mg)
Abundance		12.3	2.68	1011
Richness		2.3	0.09	13
Diversity		0.53	0.024	2.3
Biomass (with oligochaetes)		14.92	1.524	482.9
Biomass (without oligochaetes)		13.16	1.200	353.5
Detritivores (with oligochaetes)		1.94	0.954	471.3
Detritivores (without oligochaetes)		0.18	0.036	11.6
Predators and parasites		10.78	1.148	353.5
Shredders		0.14	0.042	14.5
Collector/gatherers		0.11	0.022	7.6
Scrapers		0.02	0.019	12.1
Filterer/collectors		0.00	0.000	0.3
Herbivores		1.97	0.289	91.3
Omnivores		0.01	0.005	2.7
Oligochaeta (subclass)		4.51	0.738	235.7
Hymenoptera	Formicidae	1.76	0.954	471.0
Stylommatophora		1.36	0.269	90.6
Isopoda	Asellidae	0.85	0.239	113.5
Veneroida	Sphaeriidae	0.77	0.333	181.8
Diptera	Chironomidae (l)†	0.46	0.224	97.9
Diptera	Tipulidae (l)	0.46	0.100	29.1
Araneae	Pisauridae	0.46	0.095	29.7
Ephemeroptera	Leptophlebiidae (l)	0.44	0.208	114.6
Coleoptera	Hydrophilidae (l)	0.42	0.058	12.4
Basommatophora	Physidae	0.37	0.216	119.4
Decapoda	Cambaridae	0.31	0.306	199.1
Megaloptera	Corydalidae (l)	0.27	0.114	56.8
Basommatophora	Planorbidae	0.24	0.094	35.5
Coleoptera	Carabidae (a)	0.24	0.044	12.2
Basommatophora	Lymnaeidae	0.16	0.068	39.9
Isopoda	Armadillidiidae	0.13	0.047	20.2
Chordeumatida	Conotylidae	0.11	0.037	20.2
Hirudinea (subclass)		0.11	0.032	11.6
Coleoptera	Dystiscidae (l)	0.10	0.039	18.9

† Indicates adult (a) or larvae (l).

and stylommatophores (slugs) accounted for 78.7% of the total biomass (9696 mg dry mass) of invertebrates collected (Table 3). Formicids, chironomids (midge larvae), oligochaetes, and asellids (aquatic pill bugs) accounted for 67.5% of total individuals picked from litter bags. Invertebrates were significantly higher by mass in coarse mesh litter bags than in fine mesh bags for nearly all metrics (Table 4). Only mean diversity was higher in fine mesh bags, and only shredder, scraper and oligochaete biomasses were similar between coarse and fine mesh bags. Predators were the most abundant FFG, accounting for 72.2% of the total dry mass. Herbivores were the second most abundant feeders in litter bags, accounting for 13.2% of total dry mass. When oligochaetes were included within the grouping of detritivores, they comprised 13.0% by mass; but when oligochaetes were removed, only 1.2% of the total dry mass was detritivores. Collector/gatherers were 12.5% of the total dry mass when oligochaetes were grouped with them and 0.75% when oligochaetes were excluded. Only 2.7% of individuals (0.93% by mass) could not be placed in any FFG.

Most invertebrate metrics were similar between mitigated and reference wetlands (Table 4). A total of 4099 individuals (72.9%

Table 4
Comparisons of means per bag and standard errors (S.E.) using analysis of variance (ANOVA) for five invertebrate metrics, seven functional feeding groups, and oligochaetes, expressed as dry mass (mg), among litter bag mesh sizes and wetland types.

	Fine mesh		Coarse mesh				Mitigated wetlands		Reference wetlands			
Invertebrate metric	Mean	S.E.	Mean	S.E.	($F_{1,635}$)	*p* Value	Mean	S.E.	Mean	S.E.	($F_{1,635}$)	*p* Value
Abundance	5.60	1.3	19.0	5.2	18.86	***<0.001***	12.7	4.1	11.8	3.5	0.66	0.427
Richness	1.6	0.10	2.9	0.15	55.97	***<0.001***	1.9	0.12	2.6	0.14	2.26	0.133
Diversity	0.68	0.04	0.38	0.03	47.91	***<0.001***	0.41	0.03	0.64	0.04	3.33	0.068
Total mass (without Oligochaeta)	2.95	0.52	17.91	2.55	55.42	***<0.001***	10.88	1.96	9.94	1.80	0.06	0.803
Total mass (with Oligochaeta)	6.30	0.95	23.58	2.83	39.8	***<0.001***	17.83	2.39	12.06	1.90	6.87	***0.009***
Detritivores (without Oligochaeta)	1.85	0.50	7.48	1.36	25.53	***<0.001***	5.09	1.30	4.22	0.69	0.14	0.708
Detritivores (with Oligochaeta)	5.20	0.93	13.15	1.82	16.84	***<0.001***	12.03	1.84	6.34	0.93	9.75	***0.002***
Predators	0.85	0.10	4.02	0.42	52.95	***<0.001***	1.92	0.28	2.94	0.35	1.00	0.320
Shredders	0.01	0.01	0.06	0.02	3.74	0.055	0.05	0.02	0.03	0.01	1.14	0.292
Collector/gatherers	4.41	0.90	9.16	1.58	5.51	***0.020***	10.45	1.67	3.17	0.70	7.17	***0.008***
Scrapers	0.39	0.20	1.31	0.55	0.75	0.386	0.97	0.53	0.73	0.24	0.01	0.944
Filterers	0.12	0.08	1.43	0.66	3.95	***0.047***	0.73	0.35	0.81	0.57	0.01	0.905
Herbivores	0.16	0.04	0.76	0.16	12.76	***<0.001***	0.46	0.11	0.45	0.13	0.01	0.933
Omnivores	0.14	0.04	6.14	1.97	25.64	***<0.001***	3.09	1.29	3.17	1.49	0.03	0.863
Oligochaeta	3.35	0.80	5.67	1.24	2.75	0.101	6.94	1.35	2.12	0.59	11.16	***0.001***

by mass) were collected from mitigated wetland bags and 3874 individuals (27.1% by mass) were collected from reference wetland bags. Differences in mass between wetland types were mostly due to oligochaetes, with total biomass including oligochaetes, detritivore biomass including oligochaetes, and oligochaetes all being significantly higher in mitigated wetlands. Collector/gatherers also were significantly higher in mitigated wetlands. Reference wetlands had higher mean richness, diversity, predator biomass, filterer biomass, and omnivore biomass, but none were significantly different.

Biomass for most FFG peaked prior to 224 days, then decreased and leveled off for the remainder of the study (Fig. 1); only oligochaete biomass peaked later, at 546 days. Because of this shift in invertebrate composition, decomposition was split into two phases (early: <224 days; late: ≥224 days) and regression tree analysis was run separately on each phase. When only taxonomic groups were analyzed, regression tree analysis revealed that in the first phases of decomposition, trends in limnephilid (shredder caddisfly) biomass were most strongly associated with high decomposition rates, but when limnephilid biomass was <0.15 mg then decomposition was lower and slug biomass was associated with decomposition (Fig. 2a). Higher larval dytiscid (predatory beetle) biomass also was associated with higher decomposition rates. In the second phase of decomposition (Fig. 2b), adult hydrophilid (collector/gatherer beetle) biomass was most strongly associated with higher decomposition rates, followed by oligochaete biomass.

When FFG were analyzed with regression tree analysis, we found that high collector/gatherer biomass along with high shredder biomass led to the largest decomposition rates during the first phase (Fig. 3a). When collector/gatherer biomass was low, omnivore biomass was the greatest determinant of decomposition rates followed by herbivore biomass. In the second phase of decomposition (Fig. 3b), higher rates were associated primarily with oligochaete biomass, followed by omnivore biomass.

When all invertebrate metrics were analyzed together, taxonomic groups were the most strongly associated metric with decomposition rate and the regression tree yielded the same results as taxonomic groups only (Figs. 2a and 4a). However, the regression tree for the second phase of decomposition was a mix of invertebrate metrics, FFG, and taxa (Fig. 4b). Adult hydrophilid biomass was associated with the largest decomposition rates, but when it was <1.43 mg, oligochaetes biomass was associated with higher decomposition rates. Higher taxonomic richness and total biomass also were associated with the fastest decomposition rates.

3.3. Fungi

Fungi colonized the litter quickly, peaking at 35 days and again with a smaller peak at 77 days (Fig. 5). The first phase of decomposition had a mean ergosterol concentration of 0.083 μg mg^{-1} dry litter (S.E. = 0.004), while the second phase of decomposition had a mean ergosterol concentration of 0.052 μg mg^{-1} dry litter (S.E. = 0.004), which was significantly lower ($F_{1, 312} = 33.62$, $p < 0.001$). Overall mean ergosterol concentration was 0.067 μg mg^{-1} dry litter (S.E. = 0.003), but was not significantly ($F_{1, 234} = 1.17$, $p = 0.280$) related to overall decomposition rate. When first and second phase ergosterol and decomposition were tested separately, ergosterol did not significantly predict decomposition for either phase (first: $F_{1, 151} = 0.46$, $p = 0.499$; second: $F_{1, 154} = 0.154$, $p = 0.695$). Concentrations of ergosterol in leaf litter were also similar ($F_{1, 4} = 0.017$, $p = 0.902$) between mitigated ($\bar{x} = 0.065$, S.E. = 0.004) and reference ($\bar{x} = 0.067$, S.E. = 0.004) wetlands.

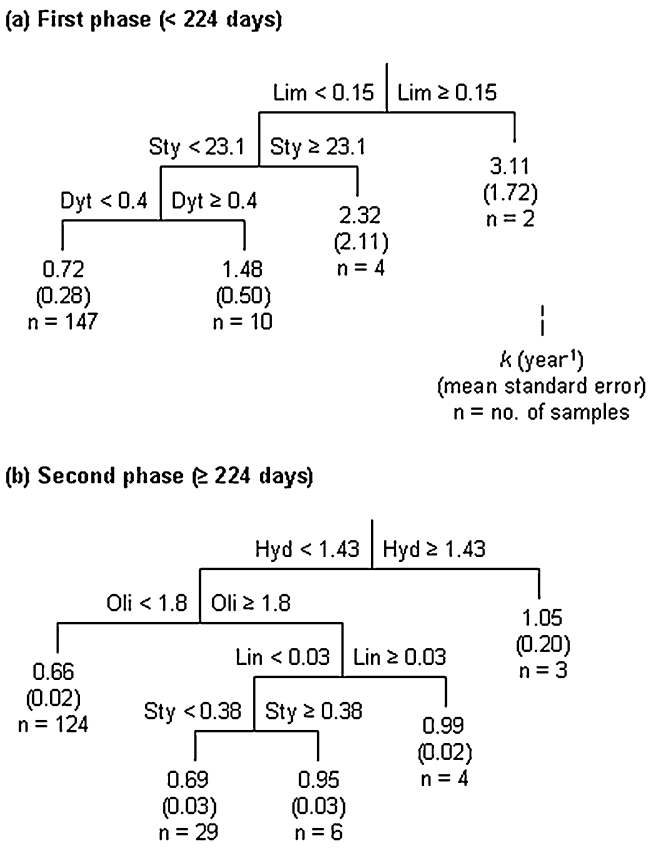

Fig. 2. Regression tree analysis to identify invertebrate taxa, by biomass (mg), associated with trends in the first and second phases of decomposition. Decomposition was measured over two years in three mitigated and three reference wetlands in the Mid-Atlantic Highlands region, USA, December 2007 to December 2009. Divisions in the first phase regression tree explains 24.9% of variance in decomposition rates and the second phase regression tree explains 21.4% of variance.

4. Discussion

4.1. Invertebrates

Our results show that invertebrates played an important role in plant litter decomposition, which is influenced by a wide range of factors that include the chemical composition of the litter (Poi de Neiff et al., 2006; Crawford et al., 2007) and site conditions, such as hydrology (Atkinson and Cairns, 2001; Anderson and Smith, 2002; Poi de Neiff et al., 2006; Gingerich et al., 2014), water chemistry (Conner and Day, 1991; Verhoeven and Arts, 1992; Gingerich et al., 2014), and temperature (Middleton et al., 1992; Álvarez and Bécares, 2006; Gingerich et al., 2014). In particular, related to our study wetlands, hydrology, water pH, soil temperature, and air temperature influenced decomposition rates in single species litterbags of brookside alder, reed canary grass, and broadleaf cattail (Gingerich et al., 2014) litter. Our research found that 24.9 to 30.9% of variance in decomposition rate during the first phase and 14.9 to 21.4% of the variance in the second phase of litter decomposition was correlated with invertebrate metrics, suggesting that invertebrates contribute significantly to litter

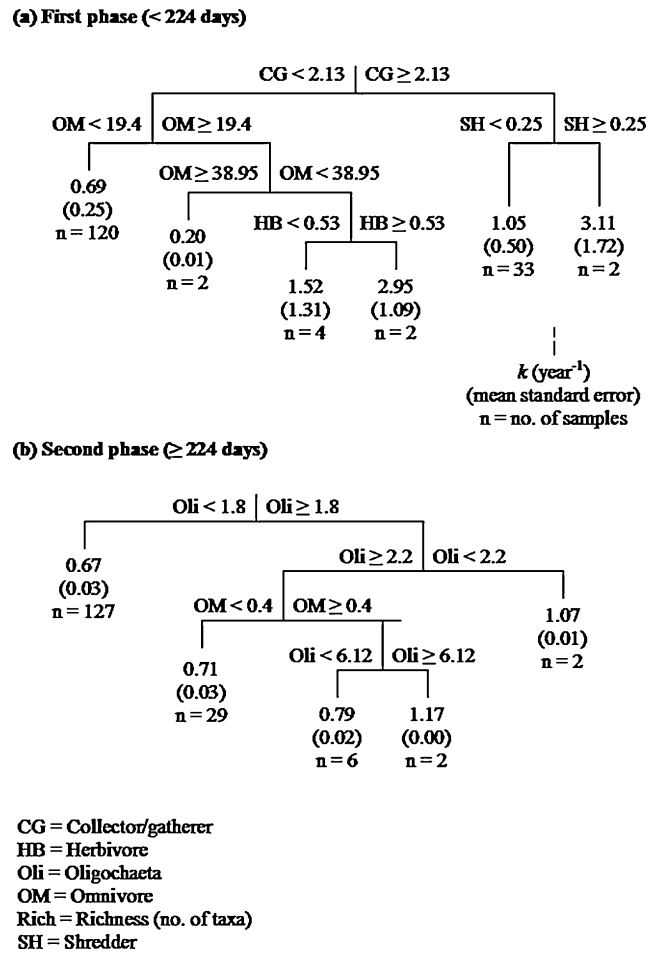

Fig. 3. Regression tree analysis to identify invertebrate functional feeding groups (FFG), by biomass (mg), associated with trends in the first and second phases of decomposition. Decomposition was measured over two years in three mitigated and three reference wetlands in the Mid-Atlantic Highlands region, USA, December 2007 to December 2009. Divisions in the first phase regression tree explain 30.8% of variance in decomposition rates and the second phase regression tree explains 14.9% of variance.

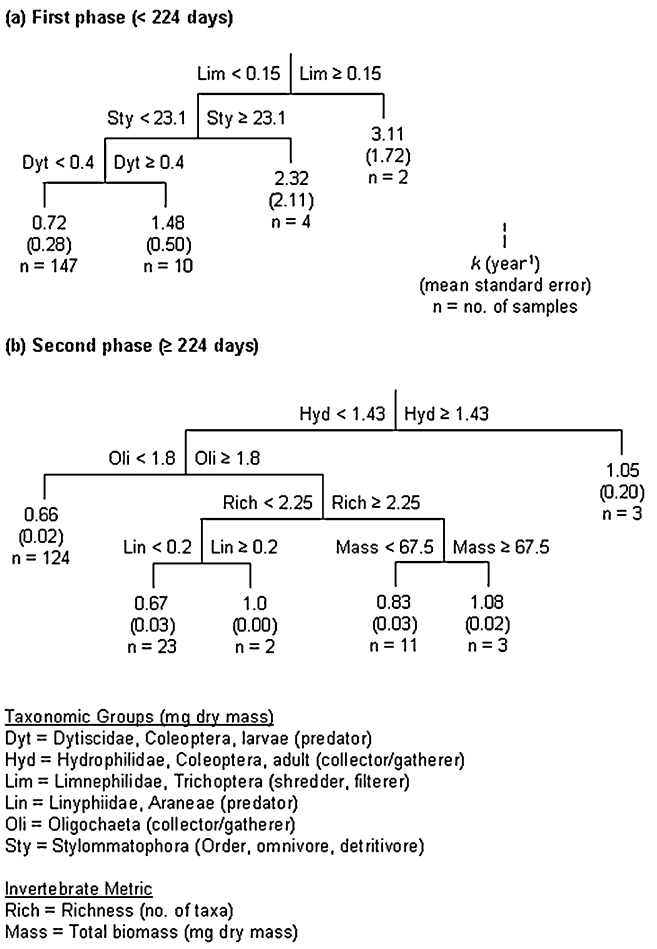

Fig. 4. Regression tree analysis to identify invertebrate metrics associated with trends in the first and second phases of decomposition. Decomposition was measured over two years in three mitigated and three reference wetlands in the Mid-Atlantic Highlands region, USA, December 2007 to December 2009. Divisions in the first phase regression tree explain 24.9% of variance in decomposition rates and the second phase regression tree explains 20.7% of variance.

processing in wetlands, despite there being many potential factors influencing litter decomposition rate.

Although it is not surprising that invertebrate biomass was significantly different between coarse and fine mesh litter bags for nearly all metrics analyzed; with the coarse mesh allowing larger invertebrates access to the litter bags and resulting in greater invertebrate biomasses, it was surprising that decomposition rates were similar in spite of these differences. If invertebrate biomass directly affected decomposition rate, we would predict that the significant difference of invertebrate biomasses would lead to a significant difference in decomposition rates. There are a few possibilities why this was not the case. The most likely reason is that shredders performed the largest amount of litter processing (Zilli et al., 2008; Galizzi et al., 2012). Shredder biomass was similar among mesh sizes and wetland types, and no differences in decomposition were found. The other FFG may not have directly processed litter enough to influence decomposition rate, despite significantly different biomasses. Second, there may be diminishing processing capacity as invertebrate biomass increases. If other FFG contributed to litter processing, increased numbers may have led to a decrease in litter processing capacity per individual due to competition for litter and space. Third, slight differences in light transmittance, temperature, and water entry may contribute to differences in decomposition rates, even though this technique of varying mesh sizes has been endorsed as a means of evaluating the role of invertebrates in decomposition studies (Bokhorst and Wardle, 2013).

It is also possible that increased predators influenced decomposition rates through top-down control of decomposers. This is supported by the fact that the predator FFG had the largest total biomass and that predator families were included in the clipped regression trees. Dytiscid larvae, an aquatic family of beetles, were included in the clipped regression trees and suggest that predation in aquatic systems may be influencing litter decomposition rate. The terrestrial spider family Linyphiidae was also included in the clipped regression trees and suggest that predation may be influencing litter decomposition rate in terrestrial systems as well.

Regression tree analysis revealed trends in invertebrates associated with decomposition and supported the importance of shredders (Figs. 2a and 4a), but suggested they only play a primary role in the first phase of decomposition. In the second phase of decomposition, oligochaete and omnivore biomass appear to have the strongest association with decomposition rate. In the first phase of decomposition, soft leaf tissue and high fungal colonization may have attracted many invertebrates to the decomposing litter. Collector/gatherers, shredders, and omnivore numbers peaked, and high prey numbers attracted predators. As the litter decomposition transitioned from the early phases into the late phase, most invertebrate numbers declined and leveled off, except oligochaetes, whose

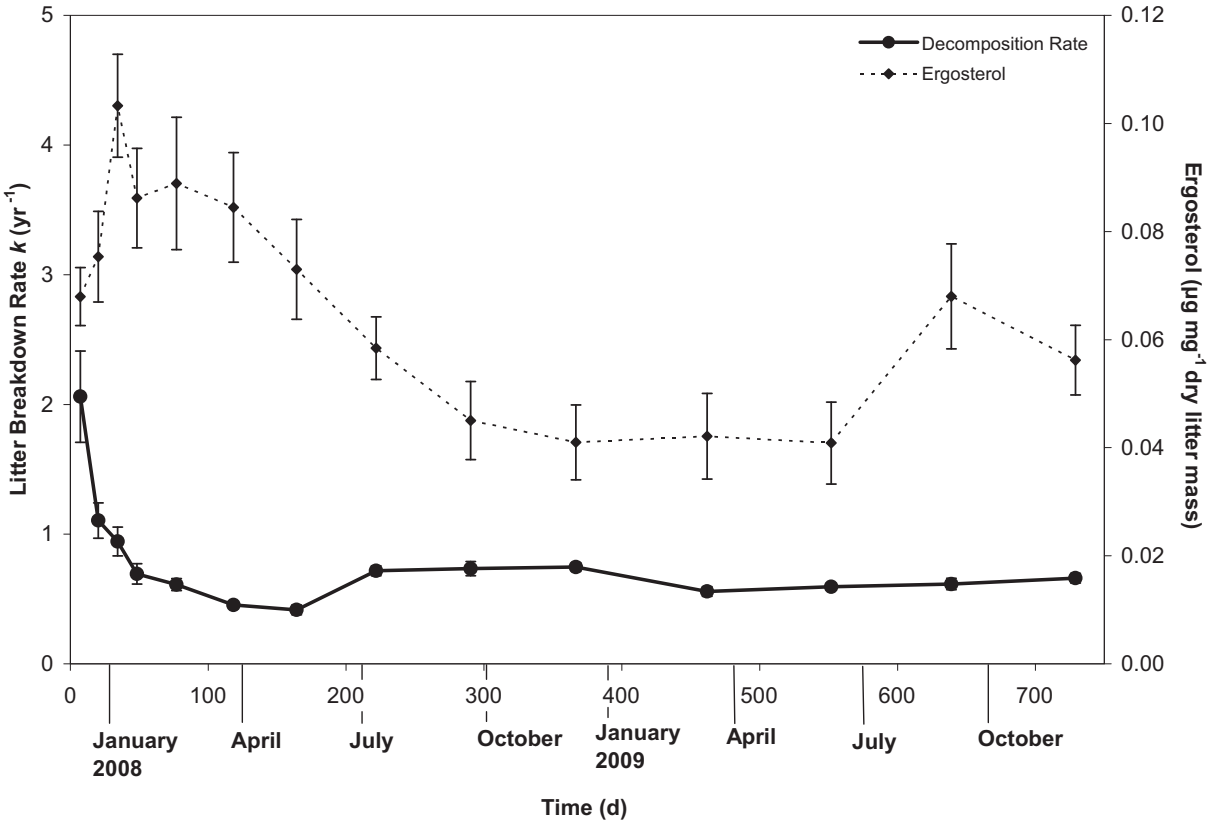

Fig. 5. Graph of litter decomposition rate constant k (year^{-1}) and fungal biomass (μg ergosterol mg^{-1} dry mass litter) from litter bags collected from three mitigated and three reference wetlands in West Virginia, USA, December 2007 to December 2009.

numbers increased. This may be because oligochaetes were able to process the remaining tougher tissues of the litter, the microbial community changed leading to changes in consumer community composition (Giordano et al., 2014; Rivera-Usme et al., 2015), or because over time the litter bags were better incorporated into the top soil horizon, allowing oligochaetes better access to the material.

When all metrics were analyzed collectively, taxonomic groups were more strongly associated with trends in decomposing litter than FFG, abundance, richness, or diversity. This suggests that within FFG, certain taxa were more strongly associated with decomposition, and possibly contributed more to decomposition rate, than the group as a whole. As invertebrate numbers declined in late phase decomposition, taxa richness and total biomass became more important but were still preceded in the regression tree by individual taxa. Our results indicate that the alternating wetting and drying periods may be the driver promoting distinct aquatic and terrestrial invertebrate response to decomposition rate.

Though this study demonstrates the role of invertebrates on decomposition, it needs to be noted that the sampled species composition may not represent the actual species composition influencing plant litter decomposition. Dobson (1991) found that invertebrate communities sampled from litter bags did not adequately reflect natural community composition. In this study, all litter bags were collected during the middle of the day, which may have misrepresented invertebrates with diel migrations, such as oligochaetes, asellids, and chironomids (Erman, 1973; Ola et al., 2001), all of which were among the most abundant species found in this study. Second, as noted earlier, predator abundances likely influence decomposition through top-down control of decomposers. Predator numbers were extremely high in litter bags, and predator taxa were included in regression trees indicating trends strongly associated with plant litter decomposition rate.

4.2. Fungi

Our study indicated increased biomass of fungi in decomposing litter between 0 and 100 days, followed by a decline in fungal biomass that stabilized between days 300 and 400. Despite the relatively higher fungal biomass during the time of most rapid litter decomposition, fungal biomass was not significantly associated with decomposition rate. A top-down effect of invertebrates on fungal communities may have been a confounding variable that could potential obscure a correlation between fungal biomass and decomposition rate (Webster and Benfield, 1986). As fungal biomass was consumed by invertebrates along with leaf litter, fungal biomass would be increasingly altered as litter decomposition rates increased.

During early phases of decomposition, microbes condition the plant litter, releasing nutrients and facilitating decomposition. Once most nutrients have been processed and soft material has been broken down, the role of microbes diminishes (Godshalk and Wetzel, 1978; Brinson et al., 1981). This is supported by the decline and leveling off of ergosterol levels around 300 days.

This is one of only a few studies comparing fungi in created and reference wetlands, and the only study that has compared fungi in created and reference wetlands using litter decomposition as a basis. Confer and Niering (1992) compared colonization by mycorrhizal fungi in roots in created and natural wetlands and found higher colonization rates in roots from created wetlands, attributing the difference to higher nutrient availability. The fact that ergosterol levels were similar in litter from mitigated and reference wetlands indicated the presence of fungal communities of

similar biomass in mitigated and natural wetlands. Invertebrates may have been consuming fungi and creating a top-down effect, but invertebrate biomass for all metrics, except oligochaetes, were similar between wetland types, which may result in similar consumptions rates among wetland types and therefore allow fungal biomass to remain similar.

5. Conclusion

Overall, litter decomposition rates were similar among mitigated and reference wetlands and across varying invertebrate communities. Invertebrates were more abundant in coarse mesh bags and were comparable or more abundant in mitigated wetlands. Oligochaetes and collector/gatherer numbers were higher in mitigated wetlands. Shredders, collector/gatherers, and omnivores were associated with trends in litter decomposition during the first phase, but oligochaetes and omnivores were most strongly associated with decomposition trends in the second phase of decomposition. Based on ergosterol levels, fungi colonized the leaf litter quickly, peaking at 35 days, then declined and leveled off by 300 days. Ergosterol levels were significantly higher in early phases of decomposition than the later phase and were similar among wetlands types. Ergosterol levels were not significantly correlated with litter decomposition rates.

Invertebrate metrics were able to explain 24.9% to 30.9% of variance in decomposition during the earlier phase and 14.9% to 21.4% of the variance in the later phase of litter decomposition. These numbers represent substantial portions of a dynamic process that involves many interacting forces and phases, of which invertebrates and fungi comprise only a portion. Though we found low measurable influence of fungi on decomposition, it is likely that their contribution was more significant than our results reflect due to the complexity of separating out the contributions from microbial communities, invertebrates, and abiotic factors. Further studies are needed to more fully identify the associations between biological variables and litter decomposition.

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