Effects of Detritivores on Detritus Breakdown in Wetlands: Do Detritivores Matter?



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I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

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Pledge

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TABLE OF CONTENTS

Abstract……...3

Introduction……3

Methods……...……7

Study Site……7
Invertebrate Sampling and Processing……....7
Experimental Design and Setup……..7
Statistical Analysis……..8

Results……8

Table 1..……9
Figure 1……..9
Table 2……...10

Discussion…… 10

Detritus Decomposition……10
Detritus Type…….11
Detritivore effect……11
Conclusion……..13

Acknowledgements……..13

References Cited…..…….14

ABSTRACT

Detritus decomposition is an important contributor to the productivity of wetlands; however, there is controversy over the role of animal detritivores in this process. Previous studies have demonstrated that detritivores (caddisflies, stoneflies, etc.) accelerate the breakdown of detritus with leaves in streams, but few studies have quantified the relative roles of animal detritivores and microbial decomposers in standing water habitats. The goal of this study was to determine the *in situ* role of animal detritivores, including caddisflies and other "cryptic" detritivores, on the breakdown of sedge (*Carex*) and red maple (*Acer* rubrum) leaves in a small, isolated wetland. This experiment assessed the relationship between detritivores and detritus decomposition rates by comparing detritus breakdown in litter packs that included or excluded detritivores. I hypothesized that (1) the presence of detritivores would result in a greater detrital decomposition rate when compared to the trays excluding detritivores, and (2) the relative decomposition rates of detritus composed of red maple leaf would be greater than sedge detritus. I found that red maple leaves decayed faster in comparison to sedge, but that after the first four months of experimentation, that detritivores had no effect on the decomposition of either types of detritus.

INTRODUCTION

Detritus, dead organic material, and the accompanying microbial decomposers are an important source of primary energy and nutrients at the base of food webs in most types of wetlands (Inkley et al., 2008; Oertli, 1993). Autochtonous detritus is derived from aquatic vascular plants within the ecosystem, whereas allochthonous detritus originates from riparian trees and herbaceous plants along the edges of water bodies (Webster and Benfield, 1986).

Wetlands are highly variable in their productivity and diversity because they support both aquatic and terrestrial biota (Gopal et al., 2001). Because of this, many wetlands differ from each other, making it difficult to make generalizations. Despite these difficulties, wetlands are important to study because they contribute to ecosystem services such as carbon cycling, nutrient cycling, and decomposition (Batzer and Sharitz, 2014). It is estimated that estuaries and swamps/floodplains hold the highest global annual value of ecosystem services per hectare (Constanza et al., 1997).

In deciduous woodland swamps, autumn leaf fall is the main pathway for the recycling of nutrients and transfer of energy to animals in the food web (Batzer and Sharitz, 2014). Although detritus lacks a substantial amount of nutrients, microbial and fungal colonization of the detritus provides the nutrition for animal detritivores (Inkley et al., 2008).

Much of what we know about the breakdown of detritus is based on the large amount of research on detritus breakdown in streams. In the stream paradigm for detritus processing, microbial decomposers, especially fungi and bacteria, convert recalcitrant carbon (cellulose, hemicellulose, lignin) into microbial tissue that is readily digested by animal detritivores termed shredders (Webster & Benfield, 1986; Wallace et al., 1997; Webster & Meyer, 1997). These shredders create fine particulate organic matter (FPOM), which is reutilized in benthic food chains and is key in the recycling of nutrients (Anderson and Sedell, 1979; Klemmer et al., 2012).

While the role of animal detritivores in lotic ecosystems has been well researched, the role that shredders play in detritus decomposition in shallow lentic habitats is poorly documented (Inkley et al., 2008). Breakdown begins with a process called conditioning, in which soluble compounds leach out of detritus during the first few days of decomposition and promote

microbial colonization within a bio-film. Recalcitrant structural carbon, such as cellulose and lignin, are then converted into simple carbon compounds by microbial enzymes (cellulase and lignase) (Batzer et al., 2014). However, the degree to which detritivores then accelerate continued detritus decay by shredding the detritus-microbial substrates has mainly been studied under laboratory microcosm or mesocosm conditions (Inkley et al., 2008; Klemmer et al., 2012). Surprisingly, there is only one study conducted in a wetland in which detritus decay rates with and without detritivores has been studied (Wissinger et al. ms.). In that study, detritus decay in leaf packs accessible to detritivores (mainly limnephilid caddisflies) occurred about twice as fast as in leaf packs that excluded detritivores. The paucity of data from other types of wetlands combined with the apparent rareness of wetland taxa described as shredders has led Batzer et al. (2014) to conclude that microbial decomposers are responsible for detritus breakdown in most wetlands, rather than animal detritivores. Thus the question remains, "Do detritivores really matter in wetlands?"

The purpose of this study was to compare the rates of decomposition of red maple leaves (*Acer rubrum*) and sedge (*Carex*) in a small, isolated wetland. It was important to look at a variety of detrital sources and environments in order to generalize the role of detritivores in ecosystems. Many factors influence the decomposition rate of detritus, including leaf type and quality (specifically nitrogen and lignin levels) (Ostrofsky, 1997; Rubbo and Kiesecker, 2004; Webster and Benfield, 1986). Thus, I wanted to compare how the presence and absence of detritivores affected the breakdown of sedge (the dominant herbaceous plants at my study site) and red maple leaves (the dominant allochthonous detrital inputs in the study site).

Another goal of this study was to determine the role of cryptic detritivores, in detritus breakdown. While it is well known that limnephilid caddisflies are detritus shredders, there is

some evidence that other types of benthic grazers and omnivores might contribute to detritus breakdown rates as well (Stoler & Relyea, 2016; Mehring & Maret, 2011; Brady & Turner, 2010). Some species of snails, tadpoles, and amphipods increase the rate of detrital breakdown in streams, but the mechanism in which they accomplish this is unknown; hence their classification as cryptic detritivores (Brady & Turner, 2010, Iwai & Kagaya, 2007, Iwai et al., 2009, Stoler et al., 2016). Thus, while other studies typically focused on only one detritivore (e.g., caddisflies in Inkley et al. 2008, Klemmer et al. 2012), this study is the first to view the combined effects of all benthic invertebrates in a natural wetland habitat.

I designed a standard leaf pack experiment with coarse and fine meshed screens to compare the decomposition rates of sedge (*Carex*) and red maple leaves (*Acer rubrum)* with and without animal detritivores, respectively, in a small temporary marsh. I hypothesized that the detritus exposed to both detritivores and microbes would break down at a faster rate than those only exposed to microbes, because detritivores are thought to act as shredders or grazers, accelerating detritus breakdown (Anderson and Sedell, 1979; Klemmer et al., 2012). Alternate hypotheses included, (1) detritivores would have no effect on detritus breakdown, or (2) detritivores would have a negative effect on detritus breakdown. Furthermore, I hypothesized that detritus composed of red maple leaves would break down at a faster rate than detritus composed of sedge. Alternate hypotheses included, (1) the decomposition rates of sedge and red maple leaves would not differ, and (2) red maple leaves would decompose at a slower rate than sedge detritus.

METHODS

Study Site

This study was conducted in a small isolated wetland located on Pratt Road, Guys Mill, Pennsylvania, USA, (41.693799, -80.018697), from November 2016 to March 2017 (17 weeks). Trays were deployed in November 2016 near the center of the marsh and marked with stakes so that they could be located after the pond froze during winter.

Invertebrate Sampling and Processing

Qualitative samples of invertebrates were collected at and around the location of the detritus trays. Using a standard aquatic D-frame net, invertebrates were collected, preserved in 70% ethanol and identified. A dissecting microscope was used to aid in identification. Invertebrates found in each detritus cage sampled were also collected, identified, and stored in 70% ethanol.

Experimental Design and Setup

A total of 120 detritus trays were created using 17.78 cm x 17.78 cm x 5.84 cm plastic Tupperware containers. Half of the trays contained 5 grams of dried sedge (*Carex)* each and half of the trays contained 5 grams of dried red maple (*Acer rubrum)* leaves each. All of the containers had 10 glass marbles at their bottom. Plastic Mesh was added to the top of each container with hot glue and rubber bands and kept the detritus in the containers. Half of the containers were topped with coarse plastic mesh (20 mm) that allowed macroinvertebrate entry and microbial colonization while the other half were topped with fine plastic mesh (1 mm) that only allowed microbial colonization.

The detritus trays were equally dispersed around a central location within the wetland. In order to mimic litterfall, the trays were deployed the first week of November, at varying depths. Initially, some of the trays sunk to the bottom of the pond, while others initially floated 1-4 inches from the surface or in between the top and bottom; all trays eventually sunk to the bottom. Two coarse and two fine trays of each litter type were removed from the site once every two weeks (8 trays removed every sampling). The final trays were removed in March and these data were used to define the rate of decomposition of sedge and red maple leaves.

Statistical Analysis

JMP version 12.0.1 was used to analyze data from this study. The analysis showed the differences in mass remaining over time between the four treatments. Analysis of Covariance (ANCOVA) was used to compare the detritus mass remaining and time passed as well as the differences between sedge and red maple and the difference between the presence and absence of detritivores. The interactions of each variable were also assessed using a full factorial. Scatter plots were used to pictorially represent the data.

RESULTS

Detritus type ($P<0.00001$) had the greatest effect on decomposition rate of detritus (Table 1). The red maple detritus lost more mass over time than the sedge detritus. Time ($P=0.00002$) had an effect as well; as more days passed, less detrital mass remained. There was also an interacttive effect of detritus type and days passed ($P=0.00345$) on mass remaining.

Table 1. Summary table of the relationships/interactions of each variable. These values show that detritus type and days past have the strongest significance and an interaction between the two was found.

Source	LogWorth	PValue
Detritus Type	6.322	0.00000
Days Passed	4.649	0.00002
Detritus Type*Days Passed	2.462	0.00345
Detritivores?	0.652	0.22278
Detritivores?*Days Passed	0.183	0.65631
Detritivores?*Detritus Type	0.110	0.77670
Detritivores?*Detritus Type*Days Passed	0.104	0.78619

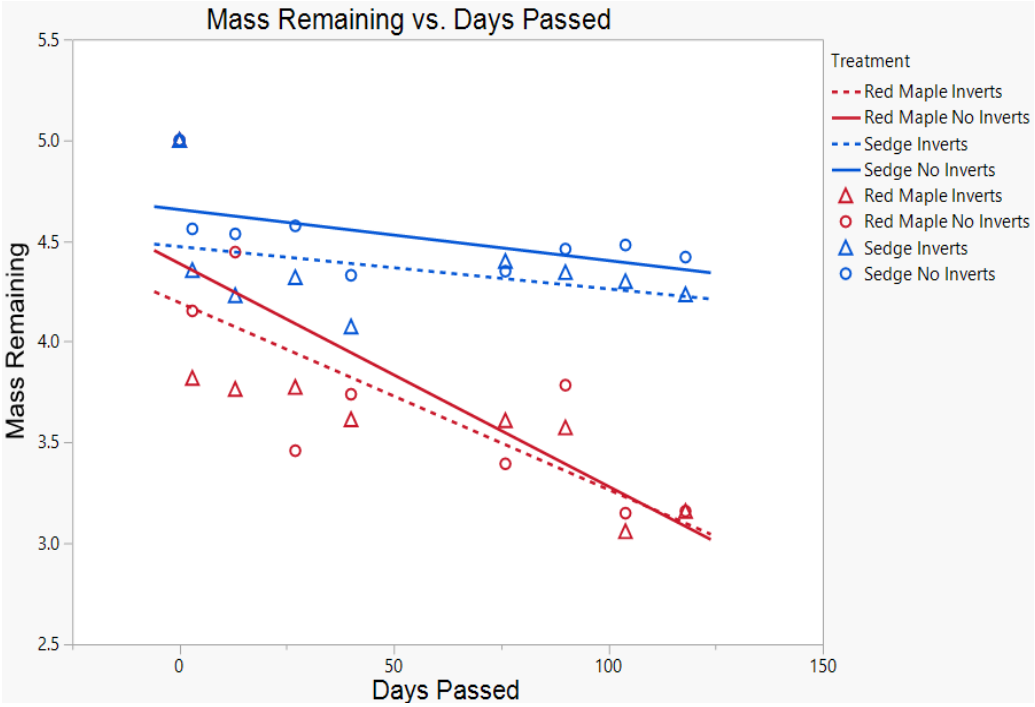

Figure 1. Mass remaining of detritus over time. Treatments included Red Maple detritus with and without invertebrates and sedge detritus with and without invertebrates. There was a significant difference between treatments (*P*=0.00001), red maple showing greater decrease in mass over time than sedge. *Note. Y-axis starts at 2.5g.

This time x detritus interaction represents the divergence in time between the mass remaining for maple leaves vs. that for the sedge detritus (Figure 1). Presences vs. absence of detritivores had no effect on the decomposition rate of either detritus type by the March sample date (*P*=0.22278, Figure 1). Invertebrate identification (Table 2) lists all of the organisms found

at the study site. The coarse treatments allowed access to all listed invertebrates, whereas the fine treatments only showed the presence of the water flea and the seed shrimp.

Table 2. Summary table of invertebrates found at study site.

	Invertebrate	
1)	*Limnephilus indivisus*	Cased Caddisfly
2)	*Ptilostomus ocellifera*	Cased Caddisfly
3)	*Hydrophilidae Coleoptera*	Beetle larva
4)	*Hydroporina Dytiscidae*	Beetle
5)	*Gastropoda*	Snail
7)	*Culicidae*	Mosquitoe Larvae
8)	*Sphaeriidae Pisidium*	Clams
9)	*Stratiomyidae odontomyia*	Fly larvae
10)	*Cladocera daphniidae*	Water flea
11)	Ostracodas	Seed Shrimp
12)	Hirudinea	Leech
13)	Tubificid oligochaeta	Aquatic Worm

DISCUSSION

Detritus Decomposition

Both autochthonous and allochthonous detritus decomposition serve as a primary food base for organisms in wetlands, which contribute to ecosystem services such as carbon cycling, nutrient cycling, and decomposition (Batzer et al., 2014). While there have been many studies of detritivore effects on detritus decomposition in running water ecosystems, few have been conducted in shallow lentic habitats (Batzer et al., 2014). Furthermore, no *in situ* studies have been conducted in a wetland. This study uniquely examines the holistic effects of the environment and all of the organisms within the study site. The goal of this study was to compare detritus decomposition rates between red maple leaves (allochthonous detritus) and sedge (autochthonous detritus), with and without the effects of detritivores.

Detritus Type

In my study, red maple leaves decayed faster than sedge detritus both with and without detritivores. This result is consistent with previous studies in which red maple leaves (k = 0.0042 day^{-1}) had a faster decay rate than sedge detritus (k = 0.0028 day^{-1}) (Webster and Benfield, 1986, Grout et. al, 1997, Melillo et. al., 1982). One possible explanation is that the red maple leaves may have had a higher initial nitrogen content compared to the sedge. The most common element found to affect breakdown rates in detritus is nitrogen. Generally, the higher the initial nitrogen concentration, the higher the break down rate (Enríquez et al. 1993, Webster and Benfield 1986). Other studies have found that fresh sedge has an initial nitrogen content of 3.83%, while red maple leaves have 0.7%, not supporting this leading theory (Federle et. al., 1982, Melillo et. al., 1982).

Another factor known to have an effect on break down rate is the initial lignin concentrations of the detritus; the more lignin a plant species initially contains, the slower the rate of decomposition (Cromack, 1973, Enríquez et al., 1993, Webster and Benfield, 1986). While sedge does not contain lignin, it contains 1-5% of amorphous silica by weight (Struyf & Conley, 2009). If this is comparable to lignin and its relationship with decomposition rate, this could contribute to the slow breakdown of sedge.

Detritivore effect

By the end of the last sample date in March, I found no evidence for a detritivore effect on the decomposition of either type of detritus. There are several explanations for the lack of detritivore effect. Detritivores have been found to have a positive effect on detritus breakdown in multiple other studies and systems (Klemmer et al., 2012, Anderson & Sedell, 1979), so it can be

assumed that there is a possibility detritivores have an effect on detrital decomposition in wetlands.

An alternative explanation is that detritivore activity is low during the winter months at this latitude. This study was done from November to March in Northwestern Pennsylvania; the temperatures in this area during these months (winter) are cold, creating slower breakdown rates. Perhaps not surprisingly, after five months, the detritus masses were still greater than half their original weight. This can be explained by the seasonal rate of decomposition. It is known that, generally, the breakdown rates of detritus increase with increased temperatures (Bärlocher & Schweizer, 1983, Webster and Benfield, 1986). The cold temperature may also be another reason for the lack of detritivore effect. The detritivores found in this wetland are poikilotherms, and therefore cannot regulate their own body temperature; the cold temperatures during this study would slow the activity of the detritivores and slow their effect on detrital decomposition (Mellanby, 1939).

Both red maple leaves and sedge have a relatively long half-life, approximately 150 days and 200 days respectively (Webster and Benfield, 1986); this study, having lasted less than 150 days, indicating that an effect may present itself with a longer duration of study. This experiment will be continued further into the spring and early summer months to account for the half lives of the detritus, along with seasonal effects. This further study in warmer months will provide evidence for whether the lack of detritivore effect through March 2017 was due to seasonal influences.

Another contributor to overall effect of detritivores is the density ratio of detritivores in comparison to detritus available in the wetland. If there was a low density of detritivores in comparison to detritus, a detritivore effect on decomposition of detritus may not be seen. Further

study at this wetland would give more insight on the ratio, providing more understanding of the lack of effect the detritivores had. Also, replications of this study in sites of differing detritivore densities would determine if the lack of effect was solely due to density ratio.

One last explanation of why this study did not result in detritivores having an effect on detrital decomposition, besides detritivores not actually having an effect, is that the study simply did not have enough power to see a trend, statistically. Creating more replicates in an identical study might produce enough data, resulting in a different effect seen overall.

Conclusion

The major results in this study were that (1) red maple detritus had a greater decomposition rate in comparison to sedge detritus, and (2) detritivores did not have an effect on the decomposition rate of detritus in this wetland. My finding that decomposition rate differs between detritus type, adds to the little knowledge we have on the holistic view of wetlands. Further studies should be conducted in different wetlands, in different seasons, and with an increased number of replicates in order to truly discern if detritivores matter in wetlands.

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