EFFECTS OF CONTROL OF THE INVASIVE PLANT, *PHRAGMITES AUSTRALIS*, ON MICROBES AND INVERTEBRATES IN DETRITUS

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Wetland Function and Decomposition

Wetlands serve many important roles in global ecosystem structure and function. Some important ecosystem-level processes that are regulated by environmental attributes in wetlands are: primary production, decomposition, energy flow, export of organic materials, sedimentation and maintenance of nutrient budgets (Mitsch and Gosselink, 2000). These processes provide local and global ecosystem value by mitigating storm flooding, recharging groundwater, maintaining water quality, supporting terrestrial and aquatic food webs, and adding aesthetic value. Specifically, wetlands are highly productive habitats with net primary production of 1,000-6,000 g m^{-2} yr^{-1} (Květ and Husak, 1978) and provide a rich food base for wetland invertebrates, fish and wildlife. Furthermore, wetland habitats are essential components in global biogeochemical cycles, particularly the nitrogen, sulfur and carbon cycles. Changes to these cycles are of particular concern considering the increases in pollution load and concurrent decreases in wetland acreage.

Organic matter input from plants follows one of three paths: 1) respiration and mineralization, 2) loss by export to other habitats, or 3) loss to geological processes through sedimentation (Day et al., 1998). The majority of organic matter follows the path of respiration and mineralization through decomposition. Decomposition is defined as the changes in organic matter following senescence (Brinson et al., 1981). Factors such as litter quality, environmental conditions, (Godshalk and Wetzel, 1978) microbial activity, and invertebrate feeding (Varga, 2001) influence the rates of decomposition. The process

of decomposition initiates with a period of rapid leaching of soluble abiotic materials (e.g., proteins, organic acids, polysaccharides) and minerals (e.g., potassium, calcium, magnesium and manganese) over several days after senescence. This is followed by microbial conditioning of the detritus and, later, fragmentation by mechanical means or invertebrate feeding (Webster and Benfield, 1986). This cascade of events is strongly determined by the litter quality and the ability of organisms to process the litter.

Food Web Dynamics

Decomposition plays a central role in structuring wetland communities and serves as the major source of energy and carbon in wetland ecosystems (Bayo, 2005). Microbes (i.e., bacteria and fungi) play significant roles in wetland detrital pathways (Baldy et al., 2002). Several studies have shown that bacteria and fungi antagonistically breakdown leaf material and also control decomposition rates (Mille-Lindblom and Tranvik, 2003). Overall, however, fungi appear to play the most significant roles in the recycling of detrital material (Kominkova et al., 2000; Kuehn et al., 2000).

Since fungi exude extracellular enzymes, which penetrate the leaf surface, they are more efficient at colonizing coarse particulate organic matter than bacteria (Sinsabaugh, 2005) and typically have a much higher biomass than bacteria (Mille-Lindblom and Tranvik, 2003). For example, Kominkova et al. (2000) found that fungi accounted for up to >90% of microbial biomass on decomposing leaves. Fungi can utilize organic sources unusable to bacteria so they are able to initiate microbial colonization on detrital material. Saprophytic fungi initiate the degradation of leaf material by utilizing sugars and starches,

which are both available to fungi and bacteria, and by also degrading recalcitrant leaf materials, such as cellulose, hemicellulose, lignin and other polymers (e.g., chitin) that bacteria are unable to readily process (Sinsabaugh, 2005). Fungal species within the hyphomycetes, coelomycetes and ascomycetes groups have been observed to frequently degrade surface marsh litter (Findlay et al., 2002). As fungi continue to breakdown the detritus, it is made available for bacterial consumption. The processing of detritus by fungi and bacteria microbially condition the leaf litter material and create nutritious food sources for other consumers.

Microbial conditioning of decaying material creates rich food materials essential for higher trophic level consumers. Many wetland food webs are thought to be detrital based (Nelson et al., 1990) due to large amounts of plant litter input. This input provides successive sources of food primarily from the degradation of plant material. The rate of decomposition can determine energy transfer to successive trophic levels and thereby control secondary production (Findlay et al., 1990).

Invertebrates are usually the dominant consumer of the highly nutritious detritus conditioned by microorganisms in wetlands (Úlehlová, 1998). Common detritivorous invertebrates in freshwater wetlands environments are Amphipoda, fly larvae, (especially Chironomidae) Ephemeroptera, beetles, (such as Hydrophilidae) Gastropoda, and Oligochaeta (de Szalay and Cassidy, 2001). Since microbially conditioned detritus is high in labile nutrients, invertebrates preferentially ingest this material over freshly abscised and unconditioned leaves (Graça, 2001). Invertebrates also gain substantial nutrients by digesting the attached biofilm (i.e., assemblages of algae, microbes, protozoa and other

microorganisms) on the detritus (Burns and Ryder, 2001). In addition, aquatic invertebrates impact litter breakdown rates by mechanically fragmenting litter during feeding (Brinson et al., 1981; Graça, 2001).

Both functional feeding groups (FFG) and trophic group (TG) classifications are used to assess the feeding mechanism and level of consumption in the food chain that invertebrates use (Merritt, 1996). The functional feeding groups in wetland environments utilizing detritus are shredders, collector-gatherers, collector-filterers and scrapers. Shredders feed by tearing coarse particulate organic matter (CPOM) and the attached biofilm into smaller pieces (fine particulate organic matter or FPOM). Collector-gatherers use FPOM that has settled out of the water column, and collector-filterers feed by sifting through suspended FPOM. Scrapers feed mostly on the biofilm layer and some mostly consume periphytic algae on the dead plant tissue. Other invertebrate functional feeding groups, predators and parasites, can indirectly influence the breakdown of detritus by feeding on other detritivores or organisms.

The trophic group classification places invertebrates in the food web according to their position in the food chain as herbivores, detritivores or predators. Herbivores directly consume living plant material while detritivores consume decaying materials and predators consume living animal tissue. As carbon and energy is transferred from microbes to invertebrates, it then becomes available to higher trophic level feeders such as predaceous invertebrates, fish, birds and wildlife. Therefore, factors that influence decomposition will likely impact the entire wetland ecosystem through trophic changes in the food web (Findlay et al., 1990; Chambers et al., 1999; Gratton and Denno, 2005).

Litter Quality

The patterns of plant decomposition are determined by litter quality and habitat characteristics. The rate of decomposition of standing and fallen plant litter can be categorized in four ways: 1) both standing dead and fallen litter break down rapidly, 2) only fallen litter breaks down rapidly, 3) only standing dead breaks down rapidly, or 4) neither standing dead or fallen litter break down rapidly (Day et al., 1998). For example, in some wetlands (e.g., bogs) standing dead culms and fallen leaves may accumulate within the system because decomposition processes are slow. This creates a stable nutrient source for detritivores throughout the entire year (Pieczynska et al., 1984).

Microbial breakdown rates are strongly determined by leaf structure and chemical composition. Soluble, labile compounds, such as sugars, starches and proteins, are used first and quickly decline in concentration (Dinka et al., 2004). Following this, recalcitrant materials, such as lignin, lignocellulose, cellulose waxes and tannins, are broken down over longer time periods (Godshalk and Wetzel, 1978; Dinka et al., 2004). Some aquatic hyphomycetes produce enzymes able to breakdown pectin, hemicellulose and cellulose (Webster and Benfield, 1986); comparatively few have the capacity to breakdown lignin or lignin bound structures (e.g., lignocellulose) and hemicellulose and cellulose are mineralized 2-3 times faster than lignin (Dinka et al., 2004). As a result, the proportion of lignin, cellulose and hemicellulose in the plant cell walls determines both the breakdown rates and the microbial community composition.

The leaf cuticle may inhibit penetration by microorganisms and effect degradation of the detritus (Kerstiens, 1996). The cuticle is a waxy waterproof substance that inhibits the loss of water. Many plants in arid environments have thick cuticles, but some plants in aquatic environments have a thin cuticle because water loss is not a problem. However, the waxy cuticle and thick epidermal cell walls create a barrier to microbes and therefore may slow decomposition (van Ryckegem et al., 2007).

Two simple metrics that estimate litter quality and relate to its potential for decomposition are the amount of organic matter in the detritus and the carbon to nitrogen ratio (C:N) . Inorganic and organic material content are important indicators of the litter quality (Larcher, 1995). Ash weight of a plant indicates the amount of nitrogen, phosphorus, calcium, magnesium, sulfur and silica contained in the plant. Organic matter content is the remaining portion of the leaf composed of carbon containing molecules, those utilized as food energy. The organic matter of leaves can be measured as ash free dry weight (AFDW).

The C:N ratio can indicate suitability for microbial decomposition (Larcher, 1995); material with lower ratios (i.e., higher nitrogen content per unit carbon) are utilized faster than those with higher C:N ratios, which are poorer in nitrogen (Godshalk and Wetzel, 1978; Smock and Harlowe, 1983). For example, *Spartina alterniflora* with a lower C:N ratio had higher bacterial and nematode numbers and faster decomposition rates than *Phragmites australis*, which had a higher C:N ratio (Huili et al., 2007). Overall, detritus with C:N ratios >16:1 usually decomposes more slowly (Fellerhoff et al., 2003) and microbes tend to favor C:N ratios between 10:1 and 30:1 (Larcher, 1995). Plant species

with a lot of lignin in the tissue have even lower nutrition and break down extremely slowly. Plants with higher C:N ratios (i.e., straw or lignified leaf litter) are broken down much slower unless an additional nitrogen source can be utilized (Larcher, 1995). The C:N ratio changes as the litter breaks down and often decreases as nitrogen increases from the buildup of microbial biofilms and as carbon decreases through mineralization (Kelly and Jackson, 2002; Fellerhoff et al., 2003).

Abiotic factors such as water temperature, dissolved oxygen, pH and salinity can also influence microbial metabolism and colonization and ultimately decomposition rates. At higher temperatures, dissolved oxygen and pH, microbial biomass accumulates much quicker, facilitating rapid breakdown of leaf matter (Larcher, 1995). Lower temperature, moisture and pH inhibit the mineralization of by microorganisms.

Study Organism: *Phragmites australis*

Many invasive plant species have been introduced into North American wetlands that out compete native species and change community structure (Richardson et al., 2000). An invasive strain of the common reed, *Phragmites australis*, (Cav.) Trin. Ex. Steudel (subsequently referred to as *Phragmites*), can alter community structure by decreasing native diversity, shading out competitors, and reducing habitat quality for wildlife (Chambers et al., 1999; Meyerson et al., 2000). It is highly productive in wetlands and its biomass often exceeds 1 kg of dry mass per m^{-2} (Whigham et al., 1978; Květ and Westlake, 1998). This grass (Graminaceae) grows 1-4 m high and is found in riverbanks, marshes, ditches, roadsides and brackish waters throughout the United States. *Phragmites*

spreads asexually by rhizomes and rapidly forms dense monotypic stands. Although small native populations have been located in the Midwest and western US for thousands of years (Chambers et al., 1999), the exotic haplotype introduced from Europe or Asia (Chambers et al., 1999; Saltonstall, 2002) is blamed for current declines in wetland birds and wildlife in North America (Marks et al., 1994; Meyerson et al., 2000). Ironically, *Phragmites* stands blamed for much of the ecological disruption in North American wetlands are an important and protected ecological resource in Europe (Tscharntke, 1992).

Ecological problems caused by the success of the exotic *Phragmites* in North America's wetlands are habitat destruction, pollution, alteration of hydrologic patterns, eutrophication and increased disturbance (van der Putten et al., 1997). Through the production of prolific detritus and the ability to capture and retain sediments, *Phragmites* is capable of altering the physico-chemical environment of its surroundings and subsequently alters habitats for insects, fish, birds and wildlife (Weinstein and Balletto, 1999).

The high biomass productivity of *Phragmites* can impact wetland biogeochemical cycles (Meyerson et al., 2000). *Phragmites* leaves decompose relatively quickly but the culms remain several years because they are highly lignified. Stands of *Phragmites* may retain up to double or triple the nitrogen compared to the native plants it replaces (Meyerson et al., 2000). Within these stands, the aboveground tissue contains, an average N content of 2-4% in the leaves and 0.5-1%N in the culms; by comparison, two other wetlands plants, *Spartina* and *Typha*, contain 1-4%N and 1-2%N respectively (Meyerson et al., 2000*).* These plants are plants often displaced by *Phragmites* (Meyerson et al.,

2000). *Phragmites* has been shown to sequester much more nitrogen and other nutrients than several wetland plants and has the potential to alter nutrient cycling (Meyerson et al., 2000) and decomposition. In addition to the ability to sequester more nitrogen, *Phragmites* uses aerenchyma to oxidize its rhizosphere (Armstrong et al., 1999), which can cause other nutrients, such as phosphorus, to bind to the litter and become unavailable to other plants.

Control of *Phragmites australis*

Studies have shown that *Phragmites* can reduce wetland plant diversity (Cowie et al., 1992; Ailstock et al., 2001) and alter physical and chemical properties of wetlands through the production of large quantities of detritus and by capturing sediments (Weinstein and Balletto, 1999). These alterations can influence invertebrate, fish and bird habitat and food resources. Wetland managers often work to control stands of *Phragmites* because of these ecosystem altering traits.

Phragmites stems and leaves are low in carbohydrates, high in lignin, and provide a poor quality food most invertebrates, wildlife and fish (Chambers et al., 1999; Meyerson et al., 2000). A few species such as muskrats (*Ondantra zibethicus*) and song sparrows (*Melospiza melodica*) utilize parts of the plant (rhizomes and seeds respectively), but no species rely on it exclusively in the United States (Meyerson et al., 2000). Furthermore, muskrats prefer carbohydrate rich rhizomes of *Typha* species usually found in similar habitats as *Phragmites* (which have silicaceous rhizomes that are less appealing to muskrats). Among the 50 avian species that nest in *Phragmites* stands, (e.g., marsh wren

(*Cistothorus palustris*), red-winged blackbird (*Agelaius phoeniceus*) yellow-headed blackbird (*Xanthocephalus xanthocephalus*), all nest in many other graminoid, forb and shrub communities (Meyerson et al., 2000) and are not exclusive to *Phragmites*.

The most common method to control *Phragmites australis* in wetlands is by herbicide application followed by burning or mowing (Turner and Warren, 2003). Timing of these control events is essential because the plant translocates nutrient resources, such as carbohydrates, to its rhizomes during late autumn and winter for use during the next growing season (Moreira et al., 1999). Control during late spring, summer or early fall, is therefore, the most successful because nutrient reserves are in the aboveground biomass.

A follow-up treatment of burning can be more successful than mowing, because it causes greater reduction in *Phragmites* litter and colonization by more diverse plant species following control treatments (Cowie et al., 1992). But burning alone, without the use of herbicide, may favor the regrowth of *Phragmites* through reduction of pests and diseases (Květ and Westlake, 1998). Burning also leads to early emergence of new *Phragmites* shoots in springtime, promoting new growth (Květ and Westlake, 1998). Mowing *Phragmites* during the growing season reduces biomass but repeated mowing treatments are usually needed to suppress new shoots (Květ and Westlake 1998). A combination of mowing and burning without herbicide has been shown to alter invertebrate colonization. It can increase abundance of some invertebrates (Scatopsidae) and decreased abundance of others (Chironomidae, Thripidae and Corixidae) (Ditlhogo et al., 1992). However, single treatments of mowing or burning do not influence the total number of individuals, species richness, diversity or evenness (Ditlhogo et al., 1992).

Phragmites invasions can also be controlled by alternating periods of flooding with drawdown (Ditlhogo et al., 1992 and Cowie et al., 1992). A study by Bedford (2005) documented that breakdown rates were the fastest following summer drawdown when *Phragmites* litter is well oxygenated, warm and damp. However, *Phragmites* tolerates long periods of inundation, and this is not the most effective means of control. Furthermore, improper use of flooding can have negative impacts on invertebrate communities (Ditlhogo et al., 1992). A combination of flooding with mowing and burning can reduce reed beds, but this is less effective than using herbicide alone (Ditlhogo et al., 1992).

Another method of control, biocontrol, through the use of insect herbivory, has been suggested and experimented with but not used in North America (Tewksbury et al., 2002). In Europe, insects specific to *Phragmites*, maintain *Phragmites* populations. But in North America, these specific insects do not occur and this contributes to the allowance of the invasive haplotype of *Phragmites* to become successful in North America. The impacts of the absence of herbivorous insects specific to *Phragmites* in North America reflects the Enemy Release Hypothesis (ERH) (*sensu* Elton, 1958). Over 150 species of herbivorous insects are known to attack *Phragmites* in Europe (Tewksbury et al., 2002), but few species utilize *Phragmites* in North America. Biocontrol via the introduction of insects has been tested and has been shown to be somewhat successful. In one study, stem-boring noctuid moths of the genera *Archanara* and *Arenostola* were examined; the stem borer, *Archanara geminipuncta,* was the most successful at reducing *Phragmites* biomass (Häfliger et al., 2006)**.** Tests on multiple species releases on *Phragmites* appear to be more

successful than single species introductions (Häfliger et al., 2006). However, past biological control efforts have sometimes had negative ecological effects (Häfliger et al., 2006). Since both native and exotic subspecies of *Phragmites* exist in North America, the use of biological controls has been questioned and is not currently implemented as a control mechanism (Tewksbury et al., 2002).

For the above reasons, herbicide application continues to be the widely used method to control *Phragmites*. Glyphosate (N-phosphonomethylglycine) is a broad spectrum, post-emergence herbicide used in terrestrial and aquatic systems (Franz et al., 1997). Glyphosate, the active ingredient in Roundup®, is currently used on many agricultural fields to reduce unwanted plant or weed growth. Genetically modified organisms (GMO) able to withstand Roundup®, compose a large majority of seeds planted for food crops. These include Roundup Ready™ soybeans (*Glycine max*), Roundup Ready™ corn (*Zea mays*), Roundup Ready™ cotton (*Gossypium hirsutum*) and Roundup Ready™ sugar beet (*Beta vulgaris*) and have resulted in worldwide increases in use of glyphosate application and concentration (Hatzios, 1998; Woodburn, 2000). A similar chemical, Rodeo® (Monsanto Company, St. Louis, MO) is a widely used herbicide for controlling unwanted aquatic plants, such as *Phragmites* (Hellings and Gallagher, 1992; Teal and Peterson, 2005). Timed, seasonal treatments with Rodeo® are effective in suppressing *Phragmites* and promoting growth of native plant species (Ailstock et al., 2001). Aerial spraying is most effective at dispensing the chemicals over a large area, while smaller areas can be readily covered using a backpack sprayer to dispense the herbicide.

Glyphosate's mode of action has been well-studied. It penetrates the leaf cuticle and is translocated to above and below ground meristems (Sherman et al., 1996; Franz et al., 1997). It inhibits photosynthesis, and symptoms of effective herbicide treatment are foliar chlorosis followed by necrosis and plant death in approximately 6 days (Franz et al., 1997). Glyphosate is the only known herbicide that targets the EPSP (5-enolpyruvoylshikimate-3-phosphate) synthase in the shikimic acid pathway, which disrupts production of aromatic amino acids (e.g., tryrosine, phenylalanine and tryptophan) needed for protein synthesis, cell wall formation, defense mechanisms, hormone production and energy transduction compounds (Sherman et al., 1996). The shikimic acid pathway is only found in plants and certain microorganisms, but not animals (Haslam, 1974; Franz et al., 1997; Solomon and Thompson, 2003). The few plant species that breakdown glyphosate process it very slowly, and the herbicide builds up in their tissues, eventually causing plant death.

Glyphosate is a highly soluble, polar chemical that absorbs to the soil (Forlani et al., 1999). It is then quickly degraded to aminomethylphosphonic acid (AMPA) by soil microbes (*Anthrobacter*, *Agrobacterium*, *Actinomyces*, *Flavobacterium*, *Rhizobium* and *Pseudomonas*) (Moorman and Keller, 1996; Franz et al., 1997) as well as aquatic microbes (Zaranyika and Nyandoro, 1993). The AMPA is broken down further to non-toxic phosphate, glyoxylate and methylamine. Another secondary metabolite, sarcosine, is broken down into glycine then to ammonia, inorganic phosphate and carbon dioxide (Ruppel et al., 1977).

Glyphosate has been promoted with statements such as 'low toxicity to man and minimal harmful effect on wildlife' and 'virtually nontoxic to mammals, birds, fish, insects and most bacteria' because of its specificity to the plant pathways it targets (Caffrey, 1996; Franz et al., 1997). The strong absorption of glyphosate in soils, rapid breakdown, and relatively benign end products, render the chemical non-reactive in aquatic environments. Although glyphosate-based herbicides do not adversely affect aquatic vertebrates (Dow AgroSciences, 2004), less is known about its effects on microbes and invertebrates. A short three month study by Fell et al. (2006) documented that macroinvertebrate communities and the dominant fish species (*Fundulus heteroclitus*) showed no differences when comparing them in environments with herbicided and non-herbicided *Phragmites*. However, since microbes and invertebrates are intimately linked to the decay of the leaf material, there may be important repercussions in litter mineralization if it affects these organisms.

Studies of glyphosate use in wetlands show increased diversity of plants after application on dense *Phragmites* stands (Ailstock et al., 2001), and also an increase in invertebrates (e.g., arthropods) (Gratton and Denno, 2005). Since decomposition rates are affected the quality of detritus, factors altering the chemical composition of the plant material can impact decomposition. Glyphosate may decrease the quality of plant litter, because it causes early leaf death through the increase in nutrient transport from stems and leaves to roots (Monteiro, 1999; Gessner, 2001) and thereby might cause an increase in C:N ratios.

Only a few studies have evaluated glyphosate-treated *Phragmites* litter utilization in wetland food webs, specifically looking at litter quality and utilization by fungal and invertebrate communities (Kulesza and Holomuzki, 2006; Kulesza et al., 2008). There were significant differences in ergosterol needed for estimation of fungal biomass, between herbicided *Phragmites* compared to non-herbicided *Phragmites* but herbicided *Phragmites* had similar ergosterol concentrations compared to *Typha angustifolia* (Kulesza and Holomuzki, 2006). In both studies, glyphosate did not influence invertebrate colonization (Kulesza and Holomuzki, 2006; Kulesza et al., 2008). No studies have simultaneously evaluated carbon and nitrogen content, bacterial biomass, fungal biomass and invertebrate communities in herbicided and non-herbicided *Phragmites* litter compared to a native wetland plant. These factors may provide insight into possible changes in detrital food webs by invasion of *Phragmites* or by altering litter quality through herbicide application. Depending on the results, changes in management efforts may need to be made if microbial communities, invertebrates and decomposition rates are altered by herbicide treatment.

Hypotheses

In this study, I tested how the control of *Phragmites* with glyphosate herbicide affected detritus quality, microbial and invertebrate communities, and decomposition rates in flood pulsing riparian wetlands. I also compared the same factors using litter from a non-herbicided native sedge, *Scirpus cyperinus* (woolgrass). Evaluation of microbial and

invertebrate communities on different types of detritus provided data on the potential impacts of using glyphosate on food quality and energy transfer in managed wetlands.

The hypotheses I tested are:

1. Initial C:N ratios will be higher in herbicided *Phragmites* leaf litter than non-herbicided litter because glyphosate causes nutrient translocation to roots and inhibition of microbial pathways. This will cause the herbicide-treated leaf litter to breakdown slowly compared to non-herbicided *Phragmites* litter.

2. There will be more microbes, more microbial biomass and more abundant and diverse invertebrate communities on non-herbicided *Phragmites* than the herbicided *Phragmites* because of the lower C:N ratios in the non-herbicided *Phragmites* litter and inhibition of microbial pathways by glyphosate.

3. Microbial biomass and invertebrate diversity and abundance will be highest on *Scirpus* litter than both *Phragmites* treatments because *Scirpus* has higher quality of detritus and lower C:N ratio.

METHODS

Study Site Description

Phragmites australis and *Scirpus cyperinus* litter for this experiment was collected from an emergent marsh wetland in Ravenna, Ohio (Portage Co., U.S.A.). The wetland is approximately 12.41 hectares and drains into the Mahoning River drainage system, which eventually drains into the Ohio River flowing into the Mississippi River watershed. The wetland's plant community is dominated by native emergent species, but it has two stands of invasive *Phragmites* on the northern and southern borders of the wetland close to roads and residential areas. On the eastern edge of the wetland, *Scirpus cyperinus* dominates the vegetation.

Plant litter decomposition was examined at the Art and Margaret Aquatic Ecology Research Facility (HAERF). This facility includes ten excavated earthen wetland mesocosms (10 m X 20 m) that are flooded by a small perennial creek that flows through the Kent State University campus. Five mesocosms were used in this experiment; each had relatively variable water levels of ~120 cm that varied from 80 to 140 cm depth. Water levels of the mesocosms were measured biweekly prior to the initiation of the study period (July 2006) and throughout the experiment (August 2006 through May 2007) (Figure 1). Water level depths recorded during the sampling period were expressed relative to the stable water level (above or below).

Each mesocosm is individually fed from a dammed pool in the perennial creek and during storms, overflow water drained back into the stream (Figure 2). Therefore, we considered each mesocosms as an independent unit. Aquatic plant and invertebrate communities in the mesocosms are diverse. The plant community is dominated by submerged vegetation, such as *Ceratophyllum* (coontail), and emergent vegetation, such as *Saggitaria* (arrow head), *Juncus effusus* (common rush) and sedges. *Scirpus cyperinus* occurred in all of the mesocosms. A few invasive plants occur at the study site, such as *Typha angustifolia* (narrow-leaved cattail) and *Lythrum salicaria* (purple loosestrife). *Phragmites australis* is absent from at HAERF. However, *Phragmites* occurs in surrounding perennial stream banks and ditched channels.

Experimental Design

Plots of 20 m x 20 m areas were established as the herbicide and non-herbicided treatment areas (one each) in the northern *Phragmites* stand located within the emergent marsh wetland in Ravenna, Ohio. I also designated a 20 m x 20 m area in the *Scirpus* stand as a non-herbicided area. The *Phragmites* herbicided treatment area was sprayed with Rodeo® herbicide (a 2 % solution containing with 480g L^{-1} glyphosate and Dawn dish detergent as a surfactant) with a backpack sprayer on 25 July 2006. The sprayed plants were left until the leaves showed signs of dying approximately 6 days post herbicide application. Herbicided *Phragmites,* non-herbicided *Phragmites*, and non-herbicided *Scirpus cyperinus* leaves were collected by the same method on the same date.

Leaves were selected at random locations on each plant, and 2-4 leaves were removed per plant. The leaves were removed by hand on 1 August 2006.

Air-dried leaves of each treatment type (herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus*) were weighed and divided into 5 g (± 0.05 g) portions. Fifty 5 g portions of each treatment type were placed into individual litterbags (150 litterbags total). The litterbags were created following a modified bag design (Bedford, 2004). They included a 5 mm nylon mesh front panel and a 1 mm flexible polymer mesh body sewn together with fishing line to create 25 cm x 10 cm bags. The 5 mm mesh allowed larger invertebrates to enter the litterbag, and the 1 mm mesh reduced loss of fragmented litter. Each litterbag was sealed and identified with a colored and numbered plastic zip-tie.

After examining hydrologic data from the three previous years, it was determined that the water levels in the mesocosms rarely dropped lower than 10 cm below the stable water line. Therefore, the leaf litter bags were placed 20 cm below the stable water line in each of the five flood pulsing wetland to ensure the bags would be were submerged at all times during the study. One litterbag of each treatment was randomly selected and bound with a zip-tie to a numbered tent stake. On 4 August 2006, stakes were randomly chosen and ten stakes were placed in each of the five mesocosms at HAERF.

One stake from each of the five mesocosms was retrieved on days 0, 3, 13, 15, 29, 61, 110, 231, 266, and 293 (5 litterbags per treatment per date). After retrieval of the stake, the three litterbags were disconnected and placed in separate, labeled plastic bags, and held on ice until they were processed in the laboratory.

Environmental conditions (water level, pH, water temperature, dissolved oxygen, conductivity and salinity) were measured in each mesocosm on each date. pH and water temperature were measured using an Oakton meter (Oakton Instruments, Vernon Hills, IL). Dissolved oxygen, conductivity and salinity were measured using a Y.S.I. meter (Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Water samples from each mescosm were collected on each date and stored in acid washed Falcon tubes for nutrient analysis. The water samples were filtered through 0.45 μm nitrocellulose filters (Whatman, Maidstone, UK). Filtered samples were analyzed using a Lachat QuikChem 8000 FIA instrument (Milwaukee, WI) for NO_2/NO_3, NH_4 and soluble reactive phosphorus (SRP).

Sample Processing

All litterbags were processed within 24 hours of collection. The litter was removed and gently rinsed with distilled water over a 250 μm sieve to remove invertebrates. As decomposition of the leaves progressed, leaf material began to breakdown; in these cases, the leaf pieces were placed in a container of distilled water to rinse off the invertebrates. The litterbags were also rinsed into a sieve to remove any attached invertebrates. Invertebrates retained on a 250 μm sieve were stored in 70 % ethanol until they were processed.

Following rinsing, the leaf material was weighed to the nearest 0.001 g. After weighing the rinsed material, three sets of twenty leaf disks and one set of 4-5 disks were punched from leaves at random using a 9-mm cork borer. The three sets of 20 leaf disks

were weighed to determine wet weight to nearest 0.001 g, and then were used to measure either organic content, microbial numbers, or ergosterol content. The final set of 4-5 leaf disks was used for percent carbon (%C) and percent nitrogen (%N) analysis. The first set of twenty leaf disks was dried at 70 °C in a drying oven for 24 hours in ashed tin pans and weighed for dry weight (final DW). The material was then burned in a muffle furnace at 550 °C for 5 hours and reweighed to measure ash weight (AW). Percent organic matter (% OM) was estimated as:

$$\% \text{ OM} = ((\text{final DW} - \text{AW})/\text{final DW}) * 100$$

Decomposition of leaf litter was determined by the exponential decay equation (Olson, 1963):

$$DW_t/DW_o = e^{-kt}$$

where k = the decay constant (loss per unit time), DW_t = dry weight at time t and DW_o = the initial dry weight. The half life of the detritus or the time it takes for 50 % of the litter to decay and the time it takes for 95 % of the litter to decay were calculated with the decay constants 0.693 k^{-1} and $3k^{-1}$, respectively (Olson, 1963).

C:N Analysis

The 4-5 leaf disks (~3-4 mg) reserved were used to determine the carbon and nitrogen content and the C:N ratio. The leaf disks were dried at 70º C for 24 h and placed in GenoGrinder tubes (OPS Diagnostics LLC, Bridgewater, NJ) with steel balls. The material was milled into a powder with the GenoGrinder set at 1700 RPM for 2 minutes. 1-2 mg samples were placed in 3.5 mm x 5 mm tin capsules (Leco Corporation, St.

Joseph, MI.), closed and shaped into rectangular logs. The samples were analyzed using an automated Elemental Combustion System 4010 (Costech Analytical, Valencia, CA). Percent organic carbon (% C), percent organic nitrogen (% N) and the carbon to nitrogen ratio (C:N) were recorded.

Bacteria Analyses

The second set of twenty leaf disks were placed in Falcon tubes containing 12 mL of 1 % sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_20$). The tubes were then sonicated (2210 Branson, Branson Ultrasonics Co., Danbury, CT) for 5 min to dislodge the bacteria. 36 mL of a preservative solution containing a 1:1 ratio of phosphate buffered saline (1xPBS) at pH 7.2 and 8 % paraformaldehyde (8 % PFA) solution was added to the tubes. The tubes were then stored at 4 °C until the bacteria were filtered, stained, counted and photographed. To stain the bacteria, the Falcon tubes were vortexed for 10 s to uniformly distribute the bacteria in the solution. Afterwards, a 0.25 mL - 1.00 mL portion of the solution was concentrated onto black 0.2 μm polycarbonate filters (Poretics, Livermore, CA). The filters were stained with 4 drops (15 μg/μL) of DAPI (4',6-diamidino-2-phenolindol) for 3 min and rinsed with 0.2 μm filtered, sterile water (Porter and Feig, 1980). The filters were placed on glass slides and bacteria were counted under an epifluorescent microscope (Nikon, Nikon Corporation, Tokyo, Japan). On the first three collection dates, 1.00 mL of the solution was filtered to obtain between 25-75 bacteria per field of view. On later collection dates, smaller aliquots of solution were used for staining because of the greater number of cells present.

Biomass estimation of bacterial cells was performed by viewing the DAPI stained slides under an epifluorescent microscope. Four to six black and white photographs of ~200 cells per sample were taken using Metamorph image software (Molecular Devices Corporation, Downington, PA). Total area, fiber length and breadth were measured to determine cell biovolume and biomass was estimated using:

$$CC = 435 \text{ X } V^{0.86}$$

(CC = carbon content, and V = cell volume in μm^3) (Loferer-Krößbacher et al., 1998).

Ergosterol Content

Ergosterol is found within the fungal membrane and it does not occur in multicellular plants or animals (Newell et al., 1988). Therefore, a relationship between concentrations of ergosterol in the fungal cell wall and fungal biomass can be calculated. The procedure for ergosterol extraction was taken from Newell et al. (1988) and amendments from Tank and Webster (1998). Reverse phase-high pressure liquid chromatography (RP-HPLC) was used to determine the amount of ergosterol in each sample.

The third set of twenty leaf disks was preserved in a scintillation vial with 2 mL of HPLC grade methanol and stored in a -4 ° C refrigerator in complete darkness until extraction. The vials were heated in a dry bath block heater for 1.5 h and saponified with 1 mL alcoholic KOH solution, then heated for 30 min. Following removal from the dry bath, the tubes were centrifuged at 4000 rpm for 5 min. The liquid (supernatant) was decanted into additional centrifuge tubes. 2 mL of methanol was added to the initial tubes

containing the detritus and centrifuged a second time. The second supernatant was combined with the first supernatant. 1 mL of distilled water was added to each centrifuge tube to create a barrier between the methanol and pentane in the following step. 2 mL of pentane was added to extract the ergosterol, and the tubes were vortexed to encourage mixing of the methanol/ergosterol complex and pentane. The supernatant, which included only ergosterol and pentane, was transferred into shell vials, and the pentane was evaporated overnight in a fume hood. The residual with the ergosterol was rehydrated with HPLC grade methanol and filtered through 0.2 μm membrane filters (Fisher Scientific, Pittsburgh, PA). 10 μL of the extracted fluid was injected into the RP-HPLC (Waters 7171 Plus Autosampler) in a HPLC C18 column (Prevail Analytical Column 25cm X 4.6 mm, 5μm, Alltech Associates, Inc., Deerfield, IL) and combined with 2.0 ml min^{-1} of HPLC grade methanol. Unlike other plant sterols, only ergosterol absorbs UV light at a maximum of 282 nm (Dawson-Andoh, 2002), therefore, absorbance at 282 nm was monitored (Waters 2487 Dual lambda Absorbance Detector) at a retention time of approximately 10 min. The fluorescence detector (Waters 2475 Multi lambda Fluorescence Detector) was set to 252/465 for excitation/emission. Only the absorbance reading was used in determining the ergosterol peak area. Standards consisted of 0.25 mg L^{-1}, 0.5 mg L^{-1}, 1.0 mg L^{-1} and 5.0 mg L^{-1} ergosterol (Sigma-Aldrich, Seelze, Germany) in isopropanol and peak areas of samples were compared to those of the standards. Peak area was used to determine ergosterol concentration based on linear regression of standards. Ergosterol concentration was converted to biomass based on a conversion factor of 5.5 mg g^{-1} of fungal biomass (Mille-Lindbloom and Tranvik 2003). 43 % of the

fungal biomass is carbon (Gessner and Chauvet, 1993), therefore ergosterol concentration was also used to estimate mg of fungal C g^{-1} dry leaf mass.

Invertebrate Analysis

Invertebrates preserved in 70% ethanol were viewed under dissecting microscopes and identified to family using taxonomic keys in Merritt and Cummins, 1996 and Peckarsky et al., 1990. Organisms were counted and placed in functional feeding groups (shredders, scrapers, collector-gatherers, collector-filterers and predators), and trophic groups (herbivores, detritivores or predators) based on information in Merritt and Cummins (1996) and Thorp and Covich (1991).

Statistical Analyses

Differences among treatments were examined based on % leaf litter remaining, % organic matter, %C, %N, C:N ratio, bacterial numbers, bacterial biomass, ergosterol content and fungal biomass, invertebrate total number, invertebrates g^{-1} of dry mass taxa richness, number of detritivores, number of collector-gatherers and dominant invertebrate taxa. All analysis was performed in Statistical Package for Social Sciences (SPSS) version 14.0 (SPSS Incorporated, Chicago, IL).

Data were tested for normality and equal variance, and it was necessary to log transform data bacterial numbers, bacterial biomass, invertebrate total numbers, invertebrates g^{-1} of dry mass, taxa richness, number of detritivores and number of collector-gatherers. On the last sampling date, 25 May 2007, one set of bags was not

found and this date was not included in the repeated measures ANOVA analysis. On the remaining nine sampling dates, repeated measures ANOVAs were used to test the Date*Treatment interactions. If the interaction was significant ($p<0.05$), one-way ANOVAs were run on each date to test for differences among treatments. If it was not significant, the treatment effect of the repeated measures ANOVA was examined. When there were significant treatment effects, Tukey's tests were used to compare the treatment means.

Patterns among water temperature, water depth, pH, dissolved oxygen, conductivity, NO_2/NO_3, NH_4 and soluble reactive phosphorus (SRP) in relation to % litter remaining, % organic matter, %C, %N C:N ratio, fungi, bacteria and invertebrates were examined with a Principle Components Analysis (PCA) to extract components that accounted for the most variance among the variables. To obtain results that could be graphically interpreted, the two main components were graphed according to their values (between 1 and -1). Variables with similar coordinates according to either axis were considered correlated variables.

RESULTS

Physical and Chemical Conditions

Water levels in the flood pulsing mesocosms were variable during the period examined (August 2006-May 2007) and reached up to 60 cm above the stable water levels (Figure 1). In December 2006, all ponds froze and were continuously covered with ice until mid-March 2007. During this period, litterbags were unable to be retrieved.

Environmental conditions (dissolved oxygen, conductivity, temperature, pH, PO_4, NO_3, and NH_4) were monitored in the five mesocosms at HAERF where the litterbags were deployed (Table 1). Several environmental factors showed distinct seasonal changes. Mean conductivity (± 1 SE) was highest in September (676.6±125.9 μS cm^{-1}), decreased in November (167.7±5.4 μS cm^{-1}), and increased again during the spring at the end of April (653.5±107.9μS cm^{-1}). Temperature peaked in September (23.8±0.3 °C), then decreased to its lowest levels in November (5.1±0.2°C), then increased to a spring peak in May (16.0±0.2 °C). The other conditions fluctuated but did not show a clear seasonal pattern.

Leaf Litter Decomposition

Patterns in decomposition of all litter types were similar over the 293 days of the study (Figure 3). For all treatments, there was an initial increase in litter remaining on the first two sampling dates (4 and 6 September 2006), which was followed by rapid

loss from August 2006 to October 2006. Mass loss then slowed and there was very little decline in litter quantity from October 2006 to March 2007. Afterwards, decomposition rates increased for both *Phragmites* treatments but decreased for *Scirpus* until sampling ended in May.

Scirpus litter decayed much slower than either type of *Phragmites* litter. The non-herbicided *Phragmites* and herbicided *Phragmites* had similar decay rates (k=0.0051, k=0.0047, respectively), and non-herbicided *Scirpus* had the lowest decay rate (k=0.0029) (Table 2). At the end of the 293 day study, litterbags held only 13% and 14% of the initial herbicided *Phragmites* and non-herbicided *Phragmites* litter, respectively, but 44% of the initial non-herbicided *Scirpus* litter remained (Figure 3). The half-life (50% loss) was estimated at 136 days for the non-herbicided *Phragmites* treatment, 147 days for the herbicided *Phragmites* and 238 days for the non-herbicided *Scirpus*. Estimated 95% loss ranged from over one and a half years for herbicided *Phragmites* and non-herbicided *Phragmites* litter close to three years for non-herbicided *Scirpus* litter.

Repeated-measures ANOVAs were used to test for differences in properties of decomposing leaf litter. For dry mass remaining, the Date*Treatment interaction was not significant (Table 3), indicating that temporal changes were similar among treatments. An effect of treatment on dry mass was found; the non-herbicided *Scirpus* treatment had higher dry mass remaining than both the herbicided *Phragmites* and non-herbicided *Phragmites* treatments (Figure 3).

Percent organic matter decreased from 95%, initially, to 78-87% in all three treatments by the final sampling date (Figure 4). The Date*Treatment interaction was not significant (Table 3), however, the treatment effect was significant. Herbicided *Phragmites* and non-herbicided *Phragmites* treatments had similar % organic matter, but non-herbicided *Scirpus* litter had higher % organic matter than herbicided *Phragmites* litter.

Carbon content of litter remained about 40-50% during the study for all treatments (Figure 5), except for herbicided *Phragmites* litter on 16 September 2006 that was over 85% carbon. The Date*Treatment interaction for % carbon was significant (Table 3) and therefore, one-way ANOVAs were run on each date. However, there were no significant differences among treatments on any dates (Table 4).

Litter contained about 1% to 3% nitrogen in all treatments (Figure 6). The Date*Treatment interaction was significant (Table 3). One-way ANOVAs did not reveal any consistent pattern of differences among treatment types (Table 5). On 16 August 2006 and 21 November 2006, herbicided *Phragmites* litter had higher nitrogen levels than non-herbicided *Scirpus*. In contrast, on 3 October 2006, herbicided *Phragmites* litter had the lowest % nitrogen and non-herbicided *Scirpus* litter had the highest % nitrogen. Non-herbicided *Phragmites* generally had intermediate nitrogen levels relative to the other two treatments.

The C:N ratios ranged from 16:1 to 35:1 in the three treatments and there were no distinct changes through time (Figure 7). There was no significant Date*Treatment interaction, and the treatment effect was also not significant (Table 3).

Microbial Counts and Biomass

Ergosterol content in samples remained constant during the summer and winter, but increased during the following spring (Figure 8). There was a significant Date*Treatment interaction (Table 6). One-way ANOVAs revealed differences among treatments in ergosterol levels on 4 August 2006, 18 August 2006, 21 November 2006 and 25 May 2007 (Table 7). In general, herbicided *Phragmites* litter had the highest ergosterol content, while non-herbicided *Phragmites* and *Scirpus* litter had similar levels (Figure 8).

When ergosterol was converted to fungal biomass (μg C g^{-1} dry mass), all treatments had between 10-100 μg C g^{-1} dry mass, except the non-herbicided *Phragmites* treatment on 26 April 2007 which had elevated fungal biomass (Figure 9). The Date*Treatment interaction was not significant; however, the treatment effect was significant (Table 6). Herbicided *Phragmites* had the highest fungal biomass and non-herbicided *Phragmites* and non-herbicided *Scirpus* had similar amounts of fungal biomass (Figure 9).

Bacteria counts in all treatments ranged from ~10^8-10^9 bacterial cells g^{-1} dry mass and did not exhibit any strong variation among dates (Figure 10). The only exception was

on the 1 September 2006 when the number of cells declined to approximately 10^7 for all treatments. There was no significant Date*Treatment interaction but the treatment factor was significant (Table 6). Bacterial numbers in herbicided *Phragmites* and non-herbicided *Phragmites* treatments were similar, and both had higher bacterial counts than non-herbicided *Scirpus*.

Bacterial biomass ranged from ~0.025-0.100 μg C g^{-1} dry mass (Figure 11). Bacterial biomass showed similar temporal patterns during the sampling period in all treatments (Figure 11) and had similar patterns as bacterial counts. The Date*Treatment interaction was significant (Table 6). One-way ANOVAs revealed that bacterial biomass was different among treatments on 6 August 2006, 1 September 2006 and 25 May 2006. Herbicided *Phragmites* litter generally had the highest bacterial biomass while non-herbicided *Phragmites* and non-herbicided *Scirpus* had less bacterial biomass (Table 8).

Invertebrate Communities

Abundant and diverse invertebrate communities colonized the decomposing leaf litter and the number of invertebrates in litterbags increased over time. The Date*Treatment interaction and the treatment effect were not significant for the total number of invertebrates per sample (Figure 12, Table 9) or for total numbers of invertebrates g^{-1} dry mass litter remaining (Figure 13, Table 9). Although there were no significant differences, *Scirpus* litter generally had the lowest number of invertebrates throughout the sampling period. On the last sampling date (25 May 2007), invertebrate

numbers in herbicided and non-herbicided *Phragmites* were ~5 times greater than in non-herbicided *Scirpus* litter.

Twenty-nine invertebrate taxa were recovered from the litterbags (Appendix 1). Taxa richness increased in all treatments during the study from about 2 to 14 taxa per litterbag (Figure 14). The Date*Treatment interaction was significant (Table 9). One-way ANOVAs revealed herbicided *Phragmites* and non-herbicided *Phragmites* had higher taxa richness than non-herbicided *Scirpus* on four dates (Table 10).

Nine taxa were classified in the detritivore trophic group: Chironomidae, Ostracoda, Oligochaeta, Amphipoda (Talitridae and Gammaridae), Caenidae, Culicidae, Sphaeriidae and Hydrophilidae adults. The number of detritivores g^{-1} dry mass increased over time for all treatments (Figure 15). There was no Date*Treatment interactions or treatment effect (Table 11), but the general pattern revealed higher numbers in both *Phragmites* litters than *Scirpus* litter. The number of detritivores without chironomids showed a similar temporal trend (Figure 16), and again there was no Date*Treatment interaction or treatment effect (Table 11).

The most abundant functional feeding group was the collector-gatherer group. These included Chironomidae, Talitridae, Planorbidae, Ostracoda and Oligochaeta. Collector-gatherers showed similar temporal patterns as the detritivores. Collector-gatherers g^{-1} dry mass increased over time (Figure 17), but did not have a Date*Treatment interaction or treatment effect (Table 11).

Dominant taxa (> 5% of the total number of invertebrates) were Chironomidae (27%), Ostracoda (23%), Oligochaeta (22%), Amphipoda (Talitridae) (10%) and Gastropoda (Planorbidae) (6%) (Appendix 1). For all taxa in all treatments, numbers g^{-1} dry mass increased over time. There was no Date*Treatment interaction and no treatment effect for any taxa (Table 12).

Multivariate Analysis

The Principle Components Analysis (PCA) tested associations among the main water quality characteristics (conductivity, temperature, pH, SRP, nitrate and ammonia), leaf characteristics (% dry mass remaining, % organic matter, %C, %N), and biotic characteristics (bacterial biomass, fungal biomass and number of invertebrates g^{-1} dry mass and taxa richness g^{-1} dry mass) (Figure 18). Six principle components accounted for 72% of the total variance; the first and second components explained 24.9% and 11.6%, respectively of the variance. Fungal biomass, number of invertebrates g^{-1} dry mass and taxa richness were positively associated with the first axis and % dry mass remaining, % organic matter and temperature were negatively associated (Figure 18, Table 13). The second axis was positively correlated with phosphate, nitrate, % nitrogen and % dry mass remaining and negatively correlated with taxa richness, number of invertebrates g^{-1} dry mass, pH, conductivity and temperature. The third component (not shown) was positively correlated bacterial biomass, pH, water depth and ammonium and negatively correlated with number of invertebrates g^{-1} dry mass.

DISCUSSION

Because of the importance of litter quality to the decomposition process, I hypothesized that differences among the three treatments would affect the biotic (i.e., microbial and invertebrate communities) and abiotic factors (i.e., leaf chemistry) regulating litter decay. Specifically, it was hypothesized that herbicided *Phragmites* leaf litter would have higher C:N ratios than non-herbicided *Phragmites* litter, which would result in a slower decay rates and reduced biomass of microorganisms and invertebrate abundances. Furthermore, it was hypothesized that microbial biomass and invertebrate diversity and abundance would be higher on litter from native *Scirpus* than introduced *Phragmites* leaves. Although there were some differences among plant species in litter breakdown, effects of herbiciding on key biotic factors and litter chemical composition were limited.

Effects of Herbiciding *Phragmites*

C:N ratios are an important determinant of litter quality, bacterial colonization and invertebrate assimilation. Decay rates are generally low at C:N ratios >16:1 (Fellerhoff et al., 2003; Mille-Lindblom et al., 2006; Chen et al., 2007). *Phragmites* litter is highly lignified (Bedford, 2005), decays slowly, and is less palatable than other marsh plant species with lower C:N ratios and with less lignified tissues, such as *Nymphaea odorata*, which has ratios of 20:1-10:1 (Tidrick, 2005). I hypothesized that nitrogen

content and other aspects of litter quality might be affected by herbiciding, which would result in alteration in litter decay rates. However, C:N ratios were not greatly affected by herbiciding, and they ranged from 16:1 to 35:1 in both herbicided and non-herbicided *Phragmites* litter. Percent organic matter was also similar in both *Phragmites* treatments and ranged from 75%-82% organic matter. Although, higher nitrogen content was found in herbicided *Phragmites* than non-herbicided *Phragmites* on two sampling dates, this pattern was reversed on a third date. Therefore, these results show that herbiciding did not strongly affect litter quality. However, other leaf litter characteristics that were not measured (e.g., tannins, lignin) in this study, may have been modified by herbiciding and affected microbial use.

The most notable impacts of herbiciding were on microbial communities. Specifically, the highest microbial biomass was found on herbicided *Phragmites*. Likewise, others have shown that herbiciding *Phragmites* can increase fungal biomass on the litter (Kulesza and Holomuzki, 2006). These authors suggest that higher fungal biomass could be due to accelerated death of herbicided *Phragmites*. Following herbicide application on plant litter, overspray onto the soil has been shown to stimulate microbial activity by mineralizing carbon and nitrogen, accelerating the leaching of soluble materials and increasing the translocation of minerals to plant roots (Haney et al., 2000; Busse et al., 2001), and it may have similar effects on on microbes on above-ground detritus. Herbicide treated *Phragmites* leaves in the present experiment were left standing

for 6 days following herbicide application which allowed for some leaf-tissue breakdown and additional conditioning compared to the non-herbicided *Phragmites*. The acceleration of decay in herbicided leaf litter compared to non-herbicided litter has been noted in several other decomposition studies (Kuehn et al., 1999; Kulesza and Holomuzki, 2006; van Ryckegem et al., 2007). Another potential mechanism for the effect of glyphosate herbiciding on microbial colonization is that it can reduce lignin production in plants (Rozema et al., 1997). Likely, increases in fungal biomass on herbicided *Phragmites* are due to both glyphosate's ability to advance leaf tissue death and halt synthesis of lignin in herbicided *Phragmites*. Furthermore, the bacterial biomass was generally higher on herbicided *Phragmites* even though bacterial numbers were similar. Thus, herbiciding apparently resulted in increases in bacterial cell size rather than number. Possibly, accelerated leaching of soluble nutrients and inhibited lignin synthesis could have allowed more robust bacterial growth.

Another potential factor causing the differences are initial variation in biotic factors that may have contributed to the differences in microbial biomass between the two treatments. Fungal communities accumulate faster on attached senescing *Phragmites* leaves than on fresh leaf material (van Ryckegem et al., 2007). In this study, the application of glyphosate on *Phragmites* promoted accelerated leaf death causing faster mineralization of leaf litter. The herbiciding process facilitated fungal growth and higher biomass to occur on herbicided *Phragmites*.

Several studies have reported that litter nitrogen content is positively correlated with bacterial and fungal biomass (Kuehn et al., 1999; Kuehn et al., 2000; Gessner, 2001; Findlay et al., 2002). I also found higher nitrogen content in herbicided *Phragmites* than non-herbicided *Phragmites* on two sampling dates and higher bacterial and fungal biomass in herbicided *Phragmites*. However, the PCA analysis showed that leaf litter nitrogen content was not strongly correlated with fungal biomass or bacterial biomass. My study did not directly test the causal factors that influenced microbial community response. Therefore, this study was indeterminate as to the causes of the increases in nitrogen content on two dates; this may be caused by microbial community responding to the higher litter nitrogen content or by nitrogen immobilization from the microbial biofilms (Gessner, 2001; Kelly and Jackson, 2002; van Ryckegem 2007).

Although microbial biomass was different between herbicided *Phragmites* and non-herbicided *Phragmites*, herbiciding showed few other impacts on decay patterns. Both *Phragmites* litters showed similar decay rates (0.0047 k^{-1} day and 0.0051 k^{-1} day respectively) and 13-14% of the litter remained by the end of the 293 day study. These results are similar to those reported by Kulesza and Holomuzki (2006) and Kulesza et al. (2008) who found 21-31% of herbicided and non-herbicided *Phragmites* litter remaining after a 126 day study.

No effects of herbiciding were found for any of the invertebrate measurements including the taxa richness, total abundance, number of detritivores, number of collector-

gatherers, and dominant taxa. These results are similar to previous studies that found minimal impact of herbiciding *Phragmites* on invertebrates colonizing the litter (Fell et al., 2006; Kulesza et al., 2008). Therefore, my results suggest that use of glyphosate herbicide to control *Phragmites* may not have a strong impact on the invertebrate food web structure in freshwater wetlands. If invertebrate communities are similar in herbicided and non-herbicided *Phragmites* stands, then any observed changes in wildlife and fish communities are more likely to be due to changes in plant community diversity and physical structure.

It is interesting to note that invertebrate numbers and richness were positively correlated with fungal biomass in PCA analysis. Examination of the data indicates that the correlation occurred because invertebrate communities became more abundant and diverse as the season progressed at the same time when the leaf litter was being conditioned by microbes. Therefore, although invertebrate communities can respond to changes in microbial biomass, changes due to herbiciding were not sufficient to impact invertebrate numbers. This may be because aquatic invertebrates feed as generalists and ingest other items in the biofilm layer (i.e., epiphytic algae, settled FPOM), not only fungal and bacterial biomass (Merritt and Cummins, 1996). Additionally, there might have been impacts of microbial abundance on invertebrate biomass (i.e., body size, total biomass), but these were not measured in this study.

Native *Scirpus* Litter Compared to Non-native *Phragmites* Litter

The decay rates of both the herbicided *Phragmites* and non-herbicided *Phragmites* were approximately 2 times faster than the native non-herbicided *Scirpus cyperinus* litter. At the end of this 293 day study, the *Scirpus* litterbags held 44% of the initial litter and between 13% and 14% of the *Phragmites* litter remained. Others have also found that *S. cyperinus* breaks down very slowly. For example, 78% of *S. cyperinus* litter in a riparian wetland remained after 150 days (Kao, 2003) and between 81-91% remained after 365 days in depressional wetlands (Atkinson and Cairns, 2001). The results for *Phragmites* decay that I measured are similar to studies by Kulesza and Holomuzki (2006) and Kulesza et al. (2008) where 21-31% of herbicided and non-herbicided *Phragmites* litter remained after a 126 day study.

Decay rates are influenced by the type of organic material in the litter; recalcitrant organic matter (i.e., lignin) decays slower than easily degradable organic matter (i.e., cellulose) (Fog, 1988). *Phragmites* leaves are composed of over 80% of lignin, cellulose or hemicellulose, which are very recalcitrant (Dinka et al., 2004) and this may explain why the *Phragmites* litter had high percent organic matter (78-82%) at the end of this study. It is unclear why *Scirpus* decayed more slowly and had over 85% of organic matter remaining at the end of the study, but this may be due to an even higher content of recalcitrant organic matter than in *Phragmites* litter. Less is known about the

amounts of lignin, cellulose or hemicellulose in *Scirpus* leaves, but they are probably high because the plant has dense woody stems and tough leaves similar to *Phragmites*.

C:N ratios also influence decay rates, but these did not cause differences between plant species because they were high (16:1-35:1) in all litter types. Others have shown that *Phragmites* C:N ratios are sometimes lower than the native species it displaces (e.g., *Spartina,* 20:1), and this might lead to changes in litter quality in wetlands invaded by this plant (Weis et al., 2002). Therefore, an assessment of the chemical composition of litter from *Scirpus* and other native species may be useful in predicting changes in decomposition and litter accretion in wetlands invaded by *Phragmites*.

Although few significant differences were found in leaf chemical characteristics, *Phragmites* litter had higher bacterial counts than *Scirpus*. This implies that some leaf characteristics that were not assessed in this study affected the microbial communities. However, there were no significant differences in microbial biomass between the non-herbicided *Phragmites* and *Scirpus*.

Invertebrate richness was also lower on *Scirpus* litter, but invertebrate numbers were not significantly different. It is important to note that numbers of detritivores and collector-gatherers were markedly higher on *Phragmites* litter on the last sampling date (25 May 2007), however, these data were excluded from analysis due to a missing sample. Invertebrates showed differences only in taxa richness in response between native and non-native litter. The lack of pronounced differences in most of the measures

of invertebrate communities between plant species implies that invertebrates are not always affected when a native species is replaced by an invasive species. Instead, it is important to understand chemical and structural changes in the litter quality of the wetland environment to develop models to predict biotic responses in invaded wetlands.

Overall Trends of Litter Decay

The fungal community showed similar patterns in all litter types. In this study, ergosterol concentration ranged from ~0.1 to 0.6 g ergosterol g^{-1} DM. Other studies have reported ergosterol content of wetland plants from 0.1 – 2.5 g ergosterol g^{-1} DM (Kuehn et al., 2000; Newell, 2001; Findlay et al., 2002). Both *Phragmites* and *Scirpus* ergosterol contents are in the mid-range of these values and thus may contain leaf materials that are relatively good sources of nutrition for fungi.

The fungi:bacteria biomass ratio in this study was high (~100:1) for all three litter types in this study. Furthermore, the highest fungal biomass was found on dates when bacterial biomass was low. Other studies have found both higher (500:1 to 10,000:1; Findlay et al., 2002) or lower fungi:bacteria biomass ratios (9:1; Kuehn et al., 2000). For at least the first two and a half years, fungi are usually dominant on CPOM but later, bacteria become dominant as litter is broken into FPOM (Sinsabaugh and Findlay, 1995; Kuehn et al., 2000; Findlay et al., 2002). My research supports findings that fungi can be the major portion of microbial biomass during early decomposition stages of *Scirpus* and

Phragmites litter in wetlands, but the study duration was too short to test whether bacteria assume an increasing role as particle size decreases.

Although there were differences in decay rates between the native *Scirpus* litter and non-native *Phragmites* litter, all litter types followed similar temporal patterns: rapid leaching of soluble materials during the first few days, followed by microbial colonization and later mechanical breakdown by invertebrates (Mason & Bryant, 1975; Brinson et al., 1981; Webster and Benfield, 1986). Some studies have shown *Phragmites* to decay more quickly or more slowly than this study (Gessner, 2001; Dinka et al., 2004; Bedford, 2005). The variation in decay rates can be caused by factors such exposure to waves, macroinvertebrate utilization and life cycle patterns or seasonal changes in temperature (Brinson et al., 1981; van Dokkum et al., 2002). In a previous study of *Phragmites* decomposition, 17% of the litter was lost by leaching, 78% was lost by microbial utilization and 4% was lost due to invertebrate fragmentation and ingestion (Alemanno et al., 2007), where microbial utilization of leaf litter was found to have the greatest impact on *Phragmites* decomposition. The results of this study also support that microbial decay plays a significant role in the degradation of non-native *Phragmites* and native *Scirpus* leaf litter.

Temporal patterns of decomposition in both *Scirpus* and *Phragmites* were correlated with environmental conditions. In particular, losses of litter mass and organic matter content are generally faster in warmer water temperatures (Brinson et al., 1981).

In this study it was also found that decay was fastest during warmer water temperatures on the initial sampling dates, then slowed during the winter months (November through March) and increased in spring. However, my PCA analysis showed an unexpected negative correlation between abiotic (dry mass and temperature) and biotic factors (fungi and invertebrates). In a natural environment, a positive correlation would be expected between favorable, warmer temperature and higher numbers of fungi and invertebrates (Batzer, 1998). However, examination of the temporal patterns in this study suggest that the lowest invertebrate numbers occurred immediately after the litter bags were deployed in summer, and invertebrate numbers increased even as the weather cooled in fall.

The invertebrate communities in the litterbags were dominated by amphipods, chironomids, ostracods, oligochaetes, and gastropods, which are common taxa in decomposing plant litter (van Dokkum, 2002; Varga, 2003; Giano et al., 2004; Stanczak and Keiper, 2004; Bedford and Powell, 2006; Fell et al., 2006). These taxa feed primarily on FPOM as collector gatherers or as filter feeders. These two groups preferentially feed on microorganisms (i.e., fungi, bacteria and algae) within the detritus and are likely influenced by changes in the microbial communities.

Management Implications

A common management goal when using glyphosate herbicide is to prevent *Phragmites* stands from replacing diverse stands of native wetland plants. Since *Phragmites* sometimes replaces native species that are also well-lignified, it is important

to note that these efforts may not always substantially change food webs within marsh ecosystems. In comparing litter from non-native *Phragmites* and native species, Findlay et al. (2002) suggested that *Phragmites* litter did not greatly affect food resources for aquatic invertebrate detritivores. Others have also suggested that *Phragmites* stands can support a similar food web as that of native plant stands (Fell et al., 1998). Studies of another non-native wetland species (*Typha*) have shown that leaf litter processing and benthic invertebrate community structure was not different when replaced by either herbicided or non-herbicided *Phragmites* (Solomon and Thompson, 2003; Kulesza et al., 2008). Furthermore, if *Phragmites* replaces native plant species that break down very slowly like *Scirpus*, *Phragmites* may lead to a faster nutrient recycling in wetland food webs. Therefore, land managers concerned about invertebrate communities and their role in food webs may not have to completely eradicate *Phragmites*. However, when control of *Phragmites* is necessary (e.g., to protect native plant species), this study suggests that glyphosate herbicide may not greatly impact detritivore communities. This has further implications within the wetland food web where food sources, derived from plant detritus, for higher trophic level consumers (e.g., fish and birds) would be the same regardless of litter type (*Scirpus* or *Phragmites*) and treatment (herbicided or non-herbicided *Phragmites*). Along these same lines, no differences were found in mummichog (*Fundulus heteroclitus*) populations or their food sources (amphipods) in either herbicided or non-herbicided *Phragmites* stands (Fell et al., 2006).

Although this studies and previous others have provided evidence that *Phragmites* invasions may not greatly alter microbial and invertebrate communities on decomposing litter, it is important to note that other aspects of wetland function are altered by the replacement of native wetland species with *Phragmites*. For example, invasions by this plant can alter hydrological and nutrient regimes (Vitousek, 1990; Meyerson et al., 2000), increase accumulation of detritus (Clevering, 1998), and reduce plant (Meyerson et al., 2000) and bird diversity (Benoit and Askins, 1999). Therefore, further research should examine how indirect impacts of *Phragmites* invasion and management on wetland processes affect microbial and invertebrate communities in wetland environments.

Future Research

Leaf litter components such as percent carbon, percent nitrogen and C:N ratio did not show differences between litter treatment types, but these are relatively coarse estimates of plant litter chemistry and detrital quality. More detailed chemical analyses, such as determining tannin, lignin, cellulose and hemicellulose content, of leaf litter might provide more insight as to patterns of decomposition in comparing herbicided *Phragmites*, non-herbicided *Phragmites* and the native *Scirpus*.

Evaluation of a single native plant species (e.g., *Scirpus cyperinus*) as a comparison to *Phragmites*, limits the conclusions that can be drawn on community level impacts. Furthermore, unlike *Phragmites*, *Scirpus* does not often occur in monoculture

stands (personal observation). Therefore, further studies using a mixture of native vegetation (e.g., *Carex* spp., *Polygonum* spp., *Impatiens capensis*, *Glyceria* spp.), could be used. This might be a more realistic way to test the impacts of *Phragmites* invasion and impacts of herbicide use to control *Phragmites* in Ohio wetlands.

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Table 1. Mean (± SE) physicochemical conditions in the five mesocosms at HAERF. Mean water depth (cm), dissolved oxygen (mg L^{-1}), conductivity (µs cm^{-1}), temperature (°C), pH, PO_4 (µg L^{-1}), NO_3 (mg L^{-1}), NH_4 (µg L^{-1}) are shown for each sampling date.

Date	water depth	dissolved oxygen	conductivity	temperature	pH	PO_4	NO_3	NH_4
8/4/2006	118±9	6.8±0.2	435.2±51.5	23.8±0.3	7.65±0.50	20.70±2.09	0.0103±0.0075	135.0±19.3
8/6/2006	114±10	7.9±0.2	473.5±60.1	23.2±0.6	7.60±0.54	3.37±1.13	0.0034±0.0034	166.5±61.5
8/16/2006	112±10	7.6±0.2	644.1±128.1	20.7±0.3	7.00±0.38	2.34±0.17	0.0000±0.0000	53.2±6.7
8/18/2006	109±11	6.9±0.2	676.6±125.9	21.9±0.3	6.95±0.39	3.89±2.27	0.0077±0.0077	75.5±18.6
9/1/2006	112±11	6.7±0.2	591.8±53.0	20.1±0.1	6.97±0.06	4.22±2.55	0.0247±0.0052	34.8±15.7
10/3/2006	127±9	6.1±0.4	230.5±5.4	14.7±0.1	7.01±0.08	4.81±2.44	0.0249±0.0065	33.6±14.7
11/21/2006	127±11	8.8±0.5	167.7±5.4	5.1±0.2	6.69±0.04	7.04±1.48	0.1002±0.0152	54.3±5.0
3/23/2007	119±11	11.8±0.5	280.5±82.9	9.1±0.7	7.02±0.15	5.77±2.84	0.0755±0.0369	90.4±22.1
4/26/2007	120±8	6.4±0.5	653.5±107.9	11.7±0.2	7.37±0.07	9.57±4.41	0.1463±0.0450	95.4±13.9
5/25/2007	115±17	8.7±1.8	502.3±20.1	16.0±0.2	9.02±0.41	5.90±2.20	0.1018±0.986	89.8±15.9

Table 2. Average decay rates (k day^{-1}) for the non-herbicided *Phragmites*, herbicided *Phragmites* and non-herbicided *Scirpus* treatments. Number of days to 50% and 95% loss are estimated using the decay constants $0.693k^{-1}$ and $3k^{-1}$, respectively.

	Mean rate (k^{-1})	**50% loss**	**95% loss**
non-herbicided *Phragmites*	0.0051	136	588
herbicided *Phragmites*	0.0047	147	638
non-herbicided *Scirpus*	0.0029	238	1034

Table 3. Results of repeated measures ANOVAs comparing leaf litter characteristics (dry mass remaining, percent organic matter, percent carbon, percent nitrogen and C:N ratio) of different treatments. Bold p-values indicate significant effects ($p<0.05$).

	DM	**%OM**	**%C**	**%N**	**C:N**
Date	$F_{8, 96}$=51.963, **p<0.001**	$F_{8, 96}$ =24.745, **p<0.001**	$F_{8, 96}$=2.156, **p=0.038**	$F_{8, 96}$=2.734, **p=0.009**	$F_{8, 96}$ =0.843, p=0.567
Treatment	$F_{2, 12}$=42.213, **p<0.001**	$F_{2, 12}$=4.269, **p=0.040**	$F_{2, 12}$=0.317, p=0.734	$F_{2, 12}$=0.576, p=0.577	$F_{2, 12}$=1.155, p=0.348
Date*Treatment	$F_{16, 96}$=1.645, p=0.072	$F_{16, 96}$=0.820, p=0.660	$F_{16, 96}$=2.519, **p=0.003**	$F_{16, 96}$=2.815, **p=0.001**	$F_{16, 96}$=1.448, p=0.136

Table 4. One-way ANOVAs of percent carbon comparing treatments on each sampling date. Bold p-values indicate significant differences ($p<0.05$). Different letters indicate differences in treatments on that sampling date using Tukey's HSD *post hoc* multiple comparisons.

Date	**$F_{2, 12}$**	**P value**	**Herb. *Phragmites***	**Non-herb. *Phragmites***	**Non-herb. *Scirpus***
8/4/06	1.511	0.260			
8/6/06	0.446	0.650			
8/16/06	2.867	0.096			
8/18/06	1.314	0.305			
9/1/06	2.195	0.154			
10/3/06	1.451	0.273			
11/21/06	0.951	0.414			
3/23/06	1.395	0.285			
4/26/06	0.088	0.916			
5/25/06	0.811	0.474			

Table 5. One-way ANOVAs of percent nitrogen comparing treatments on each sampling date. Bold p-values indicate significant differences ($p<0.05$). Different letters indicate differences in treatments on that sampling date using Tukey's HSD *post hoc* multiple comparisons.

Date	**$F_{2, 12}$**	**P value**	**Herb. *Phragmites***	**Non-herb. *Phragmites***	**Non-herb. *Scirpus***
8/4/06	3.482	0.064			
8/6/06	0.567	0.581			
8/16/06	3.989	**0.047**	a	a, b	b
8/18/06	0.409	0.673			
9/1/06	0.656	0.537			
10/3/06	6.635	**0.011**	a	a, b	b
11/21/06	7.873	**0.007**	a	b	a, b
3/23/06	1.344	0.297			
4/26/06	1.704	0.223			
5/25/06	1.000	0.405			

Table 6. Results of repeated measures ANOVAs comparing the microbial communities (ergosterol content (mg g^{-1} dry mass), fungal biomass (µg C g^{-1} dry mass), bacteria numbers (g^{-1} dry mass), bacteria biomass (µg C g^{-1} dry mass) of different treatments. Bold p-values indicate significant effects ($p<0.05$).

	Ergosterol content	**Fungal biomass**	**Bacterial numbers**	**Bacteria biomass**
Date	$F_{8, 96}$=9.353, **p<0.001**	$F_{8, 96}$=9.116, **p<0.001**	$F_{8, 96}$=12.777, **p<0.001**	$F_{8, 96}$=22.975, **p<0.001**
Treatment	$F_{2, 12}$=11.495, **p=0.003**	$F_{2, 12}$=10.9685, **p=0.002**	$F_{2, 12}$=6.286, **p=0.014**	$F_{2, 12}$=7.311, **p=0.008**
Date*Treatment	$F_{16, 96}$=2.113, **p=0.013**	$F_{16, 96}$=1.448, p=0.136	$F_{16, 96}$=1.465, p=0.129	$F_{16, 96}$=5.356, **p<0.001**

Table 7. One-way ANOVAs of ergosterol content comparing treatments on each sampling date. Bold p-values indicate significant differences ($p<0.05$). Different letters indicate differences in treatments on that sampling date using Tukey's HSD *post hoc* multiple comparisons.

Date	**$F_{2, 12}$**	**P value**	**Herb. *Phragmites***	**Non-herb. *Phragmites***	**Non-herb. *Scirpus***
8/4/06	10.491	**0.002**	a	b	b
8/6/06	0.980	0.403			
8/16/06	1.259	0.319			
8/18/06	4.292	**0.039**	a	a	a
9/1/06	2.369	0.136			
10/3/06	2.708	0.107			
11/21/06	20.892	**<0.001**	a	b	b
3/23/06	1.038	0.384			
4/26/06	1.593	0.244			
5/25/06	5.433	**0.028**	a	b	a, b

Table 8. One-way ANOVAs of bacteria biomass comparing treatments on each sampling date. Bold p-values indicate significant differences ($p<0.05$). Different letters indicate differences in treatments on that sampling date using Tukey's HSD *post hoc* multiple comparisons.

Date	**$F_{2, 12}$**	**P value**	**Herb. *Phragmites***	**Non-herb. *Phragmites***	**Non-herb. *Scirpus***
8/4/06	0.902	0.432			
8/6/06	11.593	**0.002**	a	b	b
8/16/06	1.732	0.218			
8/18/06	1.550	0.252			
9/1/06	3.992	**0.047**	a	a, b	b
10/3/06	1.064	0.376			
11/21/06	0.260	0.776			
3/23/06	0.558	0.587			
4/26/06	3.041	0.085			
5/25/06	9.691	**0.006**	a	b	a

Table 9. Results of repeated measures ANOVAs comparing total invertebrate numbers, adjusted invertebrate numbers (numbers g^{-1} dry mass) and taxa richness (taxa g^{-1} dry mass). Bold p-values indicate significant effects ($p<0.05$).

	Total invertebrates	**Adjusted invertebrates**	**Taxa richness**
Date	$F_{8,96}=44.011$, **p<0.001**	$F_{8,96}=51.818$, **p<0.001**	$F_{8,96}=44.308$, **p<0.001**
Treatment	$F_{2,12}=0.187$, p=0.999	$F_{2,12}=0.355$, p=0.989	$F_{2,12}=1.097$, p=0.369
Date*Treatment	$F_{16,96}=0.094$, p=0.911	$F_{16,96}=1.052$, p=0.376	$F_{16,96}=5.059$, p=**0.026**

Table 10. One-way ANOVAs of taxa richness (number of taxa g^{-1} dry mass) comparing treatments on each sampling date. Bold p-values indicate significant differences ($p<0.05$). Different letters indicate differences in treatments on that sampling date using Tukey's HSD *post hoc* multiple comparisons.

Date	**$F_{2, 12}$**	**P value**	**Herb. *Phragmites***	**Non-herb. *Phragmites***	**Non-herb. *Scirpus***
8/4/06	0.846	0.453			
8/6/06	0.381	0.691			
8/16/06	1.734	0.218			
8/18/06	1.770	0.212			
9/1/06	7.065	**0.009**	a	a, b	b
10/3/06	5.704	**0.018**	a	a, b	b
11/21/06	6.085	**0.015**	a, b	a	b
3/23//06	1.408	0.282			
4/26/06	2.291	0.144			
5/25/06	10.790	**0.004**	a	a	b

Table 11. Results of repeated measures ANOVAs comparing detritivores (numbers g^{-1} dry mass), detritivores without chironomids (numbers g^{-1} dry mass) and collector-gatherers (numbers g^{-1} dry mass). Bold p-values indicate significant effects ($p<0.05$).

	Detritivores	**Detritivores without chironomids**	**Collector-gatherers**
Date	$F_{8,96}=56.663$, **p<0.001**	$F_{8,96}=41.816$, **p<0.001**	$F_{8,96}=55.325$, **p<0.001**
Treatment	$F_{2,12}=0.397$, p=0.980	$F_{2,12}=0.317$, p=0.994	$F_{2,12}=0.439$, p=0.968
Date*Treatment	$F_{16,96}=1.138$, p=0.353	$F_{16,96}=1.495$, p=0.263	$F_{16,96}=1.074$, p=0.372

Table 12. Results of repeated measures ANOVAs comparing dominant taxa (numbers g^{-1} dry mass). Bold p-values indicate significant effects ($p<0.05$).

	Chironomidae	**Ostracoda**	**Oligochaeta**	**Talitridae**	**Planorbidae**
Date	$F_{8,96}$=6.236, **p<0.001**	$F_{8,96}$=4.703, **p<0.001**	$F_{8,96}$=46.494, **p<0.001**	$F_{8,96}$=7.174, **p<0.001**	$F_{8,96}$=9.372, **p<0.001**
Treatment	$F_{2,12}$=0.916, p=0.426	$F_{2,12}$=0.654, p=0.537	$F_{2,12}$=0.798 p=0.473	$F_{2,12}$=0.215, p=0.809	$F_{2,12}$=0.390, p=0.686
Date*Treatment	$F_{16,96}$=0.563, p=0.904	$F_{16,96}$=0.386, p=0.983	$F_{16,96}$=1.005, p=0.458	$F_{16,96}$=0.120, p=0.999	$F_{16,96}$=0.382, p=0.984

Table 13. Principle components analysis (PCA) matrix. Component 1 accounted for 24.9% of the variance, Component 2 accounted for 11.6% of the variance and Component 3 accounted for 11.1% of the variance.

	Component 1	**Component 2**	**Component 3**
Bacteria biomass g^{-1} DM	-0.161	-0.154	0.533
Fungal biomass g^{-1} DM	0.604	0.105	0.164
Number of invert. g^{-1} DM	0.780	-0.373	-0.241
Invertebrate taxa richness	0.631	-0.480	0.230
%C	-0.091	0.370	-0.039
%N	0.232	0.505	0.091
Dry mass	-0.759	0.357	0.002
% Organic matter	-0.718	0.054	-0.022
Water depth	0.305	0.298	0.459
Dissolved oxygen	0.368	0.080	0.131
Conductivity	-0.330	-0.327	-0.076
Temperature	-0.814	-0.313	0.241
pH	0.124	-0.397	0.693
Phosphate	0.001	0.493	0.495
Nitrate	0.606	0.478	0.022
Ammonium	-0.220	-0.038	0.570

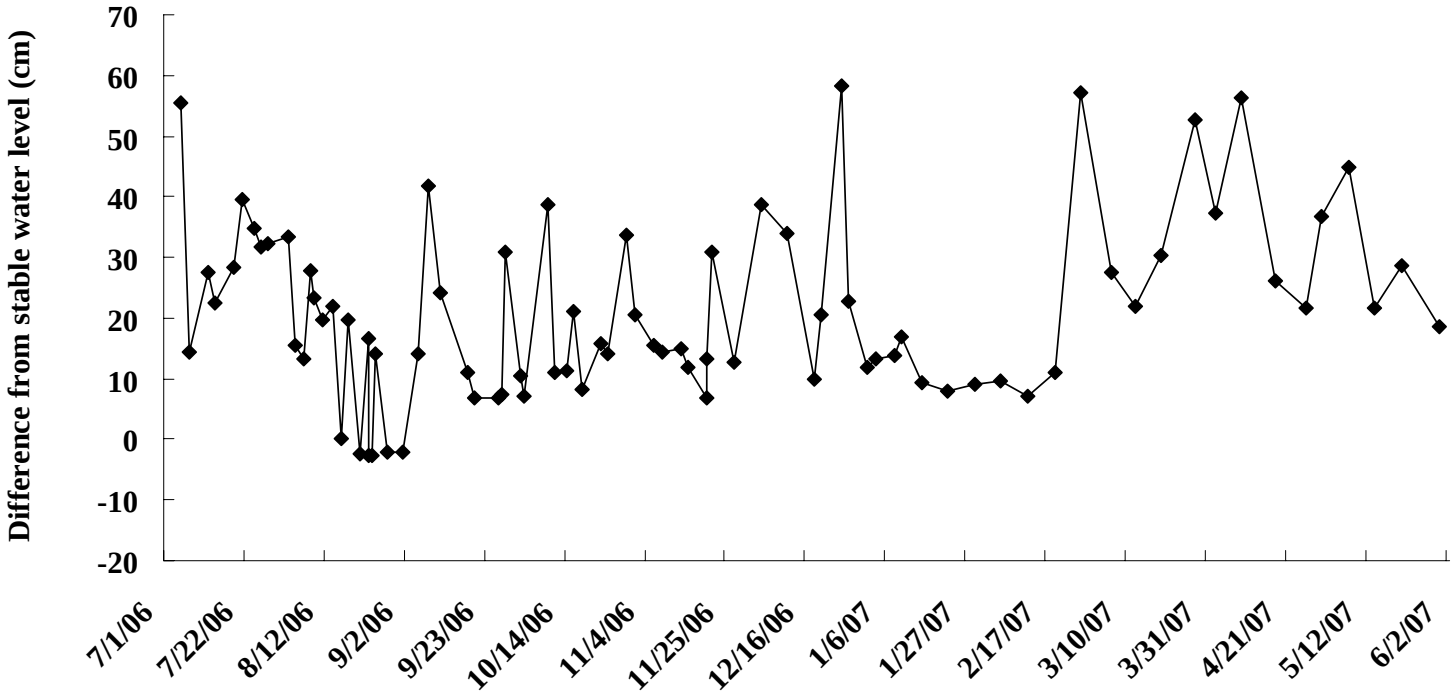

Figure 1. Hydrograph of Herrick Aquatic Ecology Research Facility (HAERF) from 1 July 2006 to 1 June 2007. The average stable water level is indicated by 0. Water levels are the mean of the five mescosms used to in this experiment.

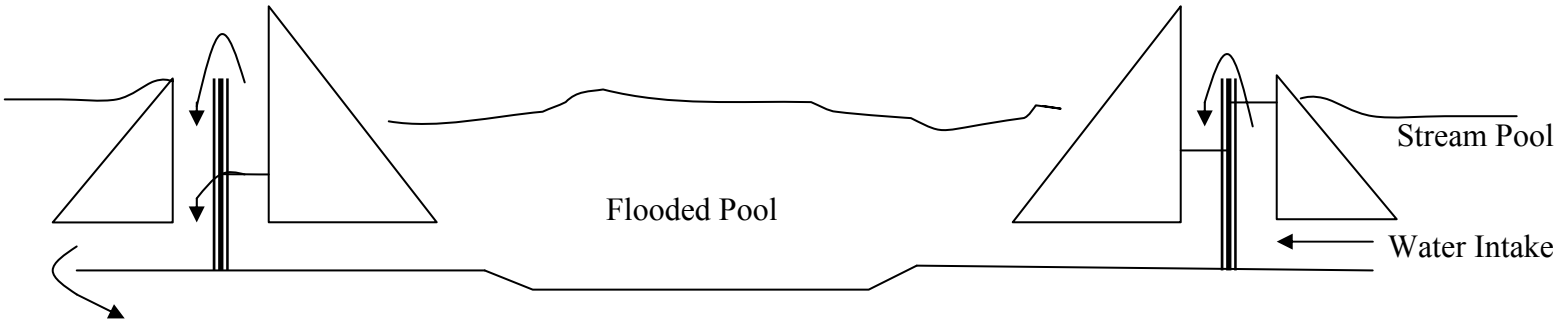

Figure 2. Mesocosm cross sectional view of a flood pulsing wetland at the Art and Margaret Herrick Aquatic Ecology Research Facility.

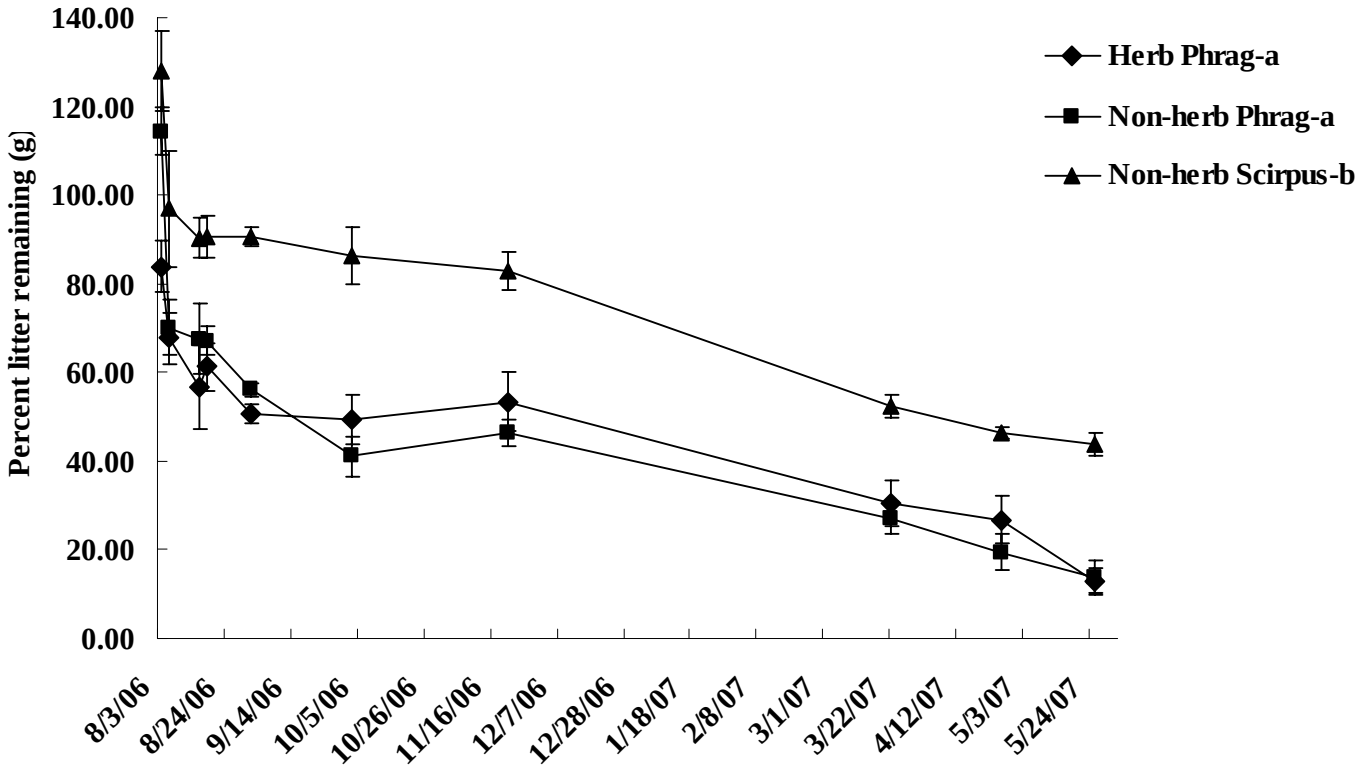

Figure 3. Percent litter remaining (g) for herbicided *Phragmites*, non-herbicided *Phragmites* and non-herbicided *Scirpus*. Different letters in the legend indicate significantly different treatments. Error bars indicate ± 1 SE.

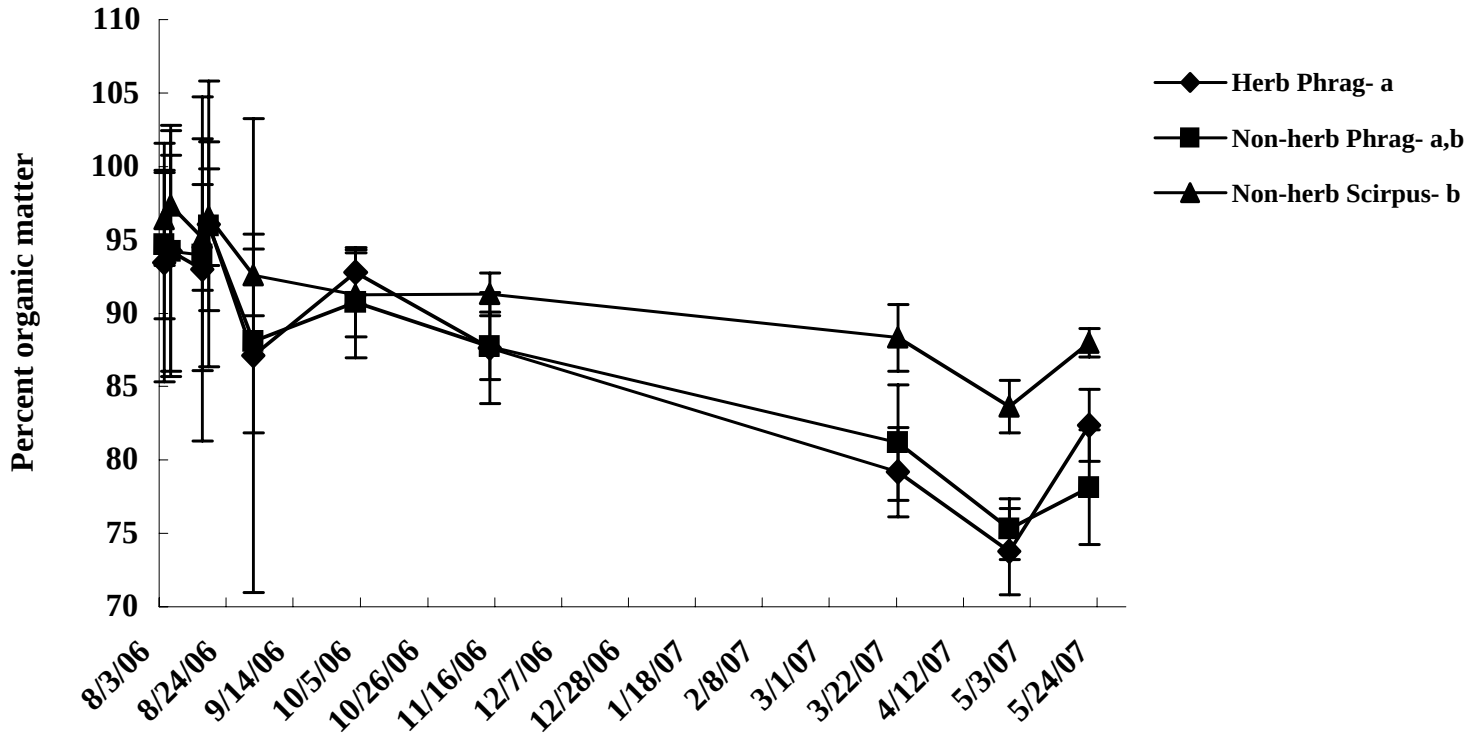

Figure 4. Percent organic matter of herbicided *Phragmites*, non-herbicided *Phragmites* and non-herbicided *Scirpus*. Different letters in the legend indicate significantly different treatments. Error bars indicate ± 1 SE.

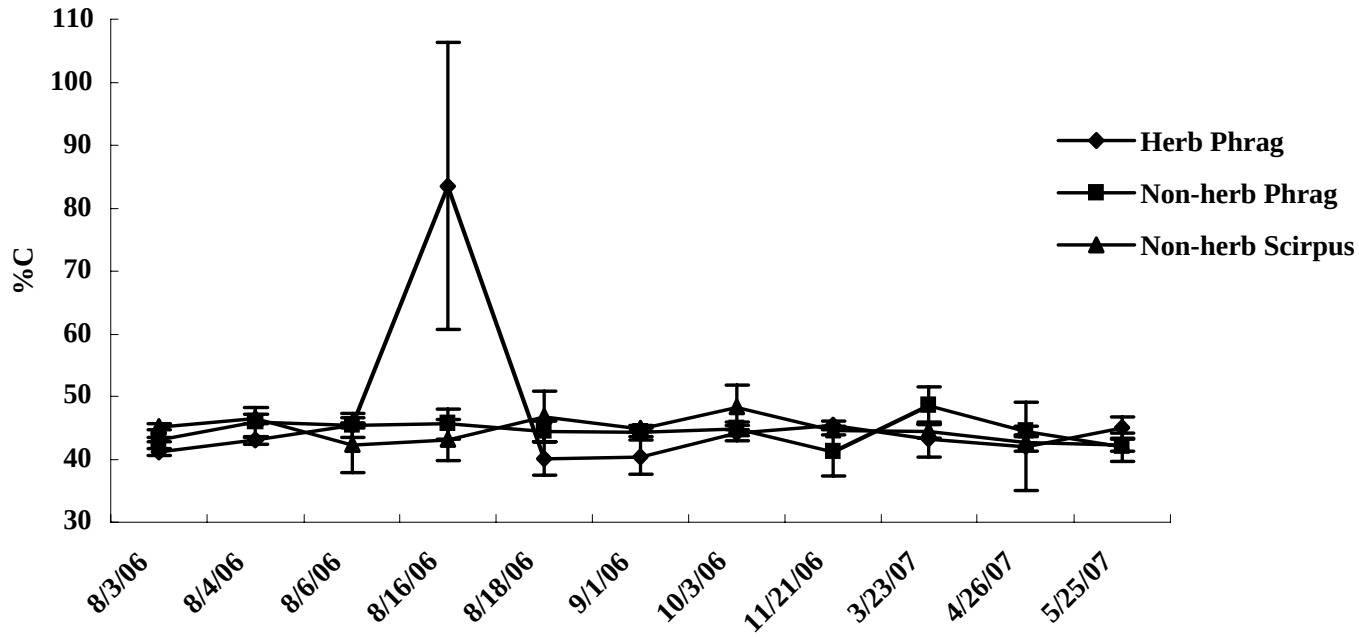

Figure 5. Percent carbon of herbicided *Phragmites*, non-herbicided *Phragmites* and non-herbicided *Scirpus*. Treatment effects were not significant ($p>0.05$). Error bars indicate ± 1 SE.

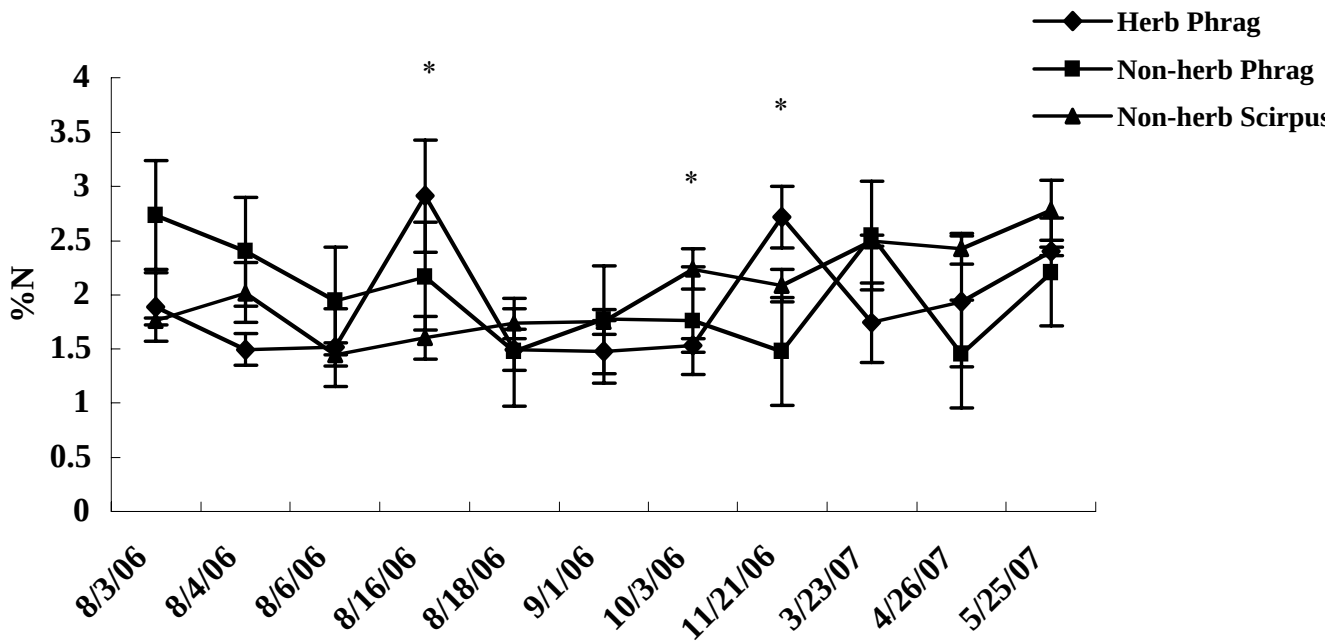

Figure 6. Percent nitrogen for herbicided *Phragmites*, non-herbicided *Phragmites* and non-herbicided *Scirpus*. Asterisks indicate significant differences among treatment on those dates. Error bars indicate ± 1 SE.

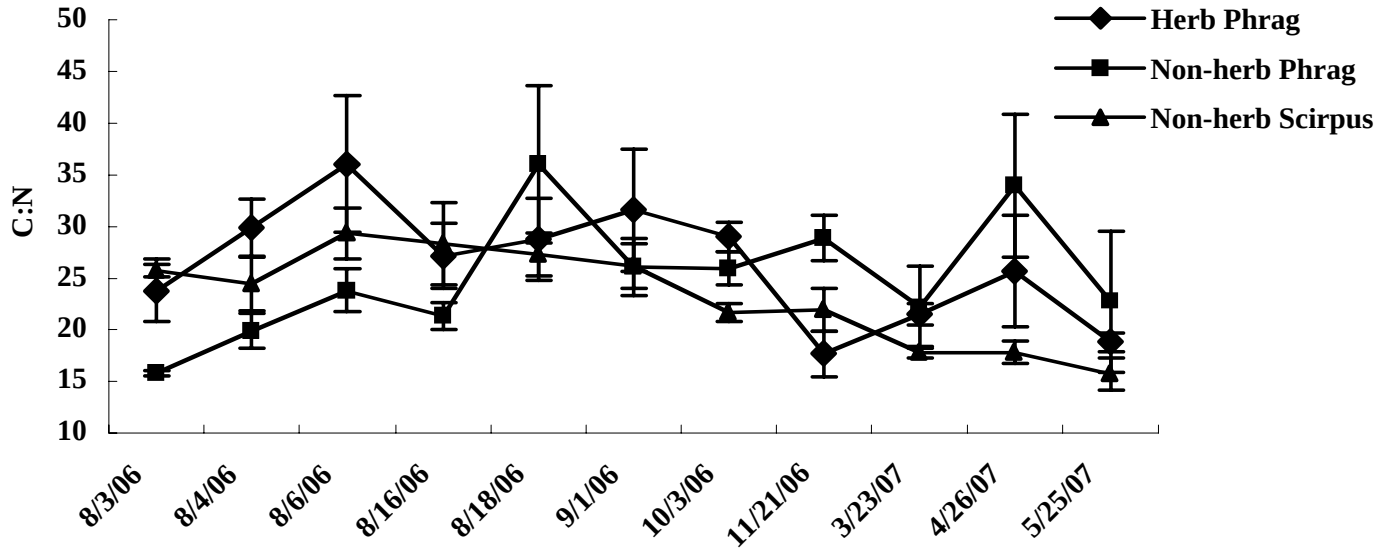

Figure 7. Carbon:nitrogen ratios for herbicided *Phragmites*, non-herbicided *Phragmites*, non-herbicided *Scirpus*. Treatment effects were not significant ($p>0.05$). Error bars indicate ± 1 SE.

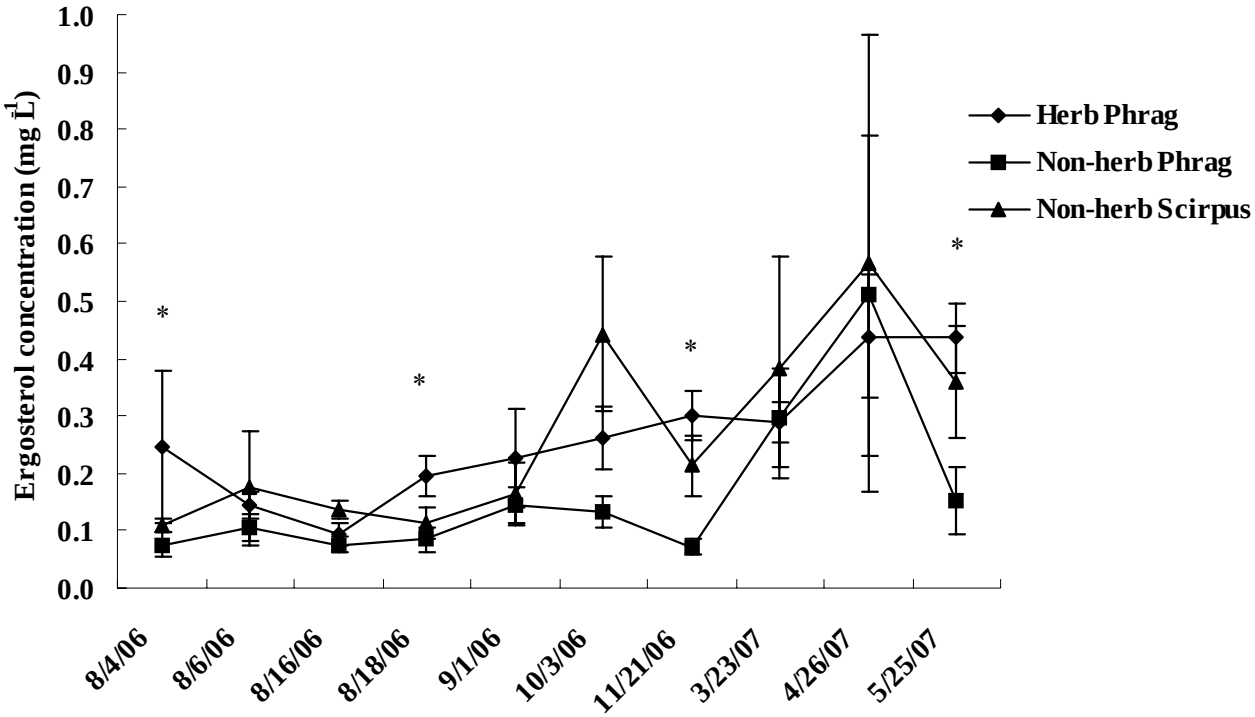

Figure 8. Ergosterol concentration on the leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Asterisks indicate significant differences among treatment on those dates. Error bars indicate ± 1 SE.

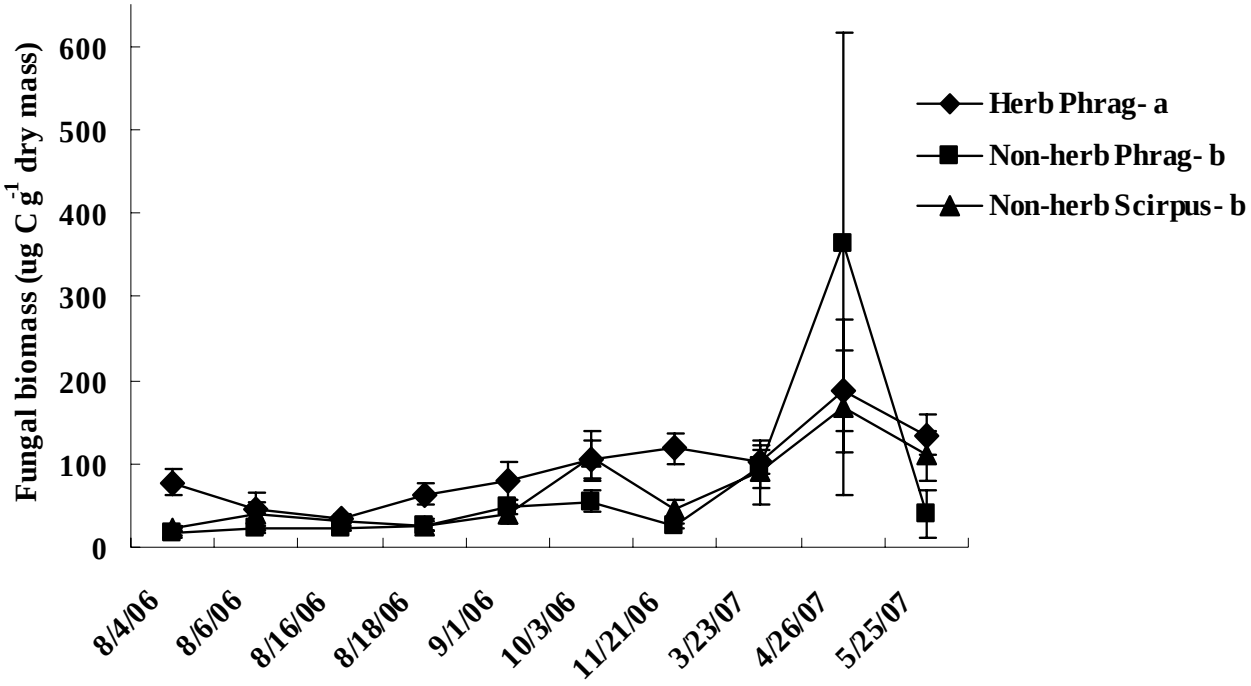

Figure 9. Fungal biomass on leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Different letters in the legend indicate significantly different treatments. Error bars indicate ± 1 SE.

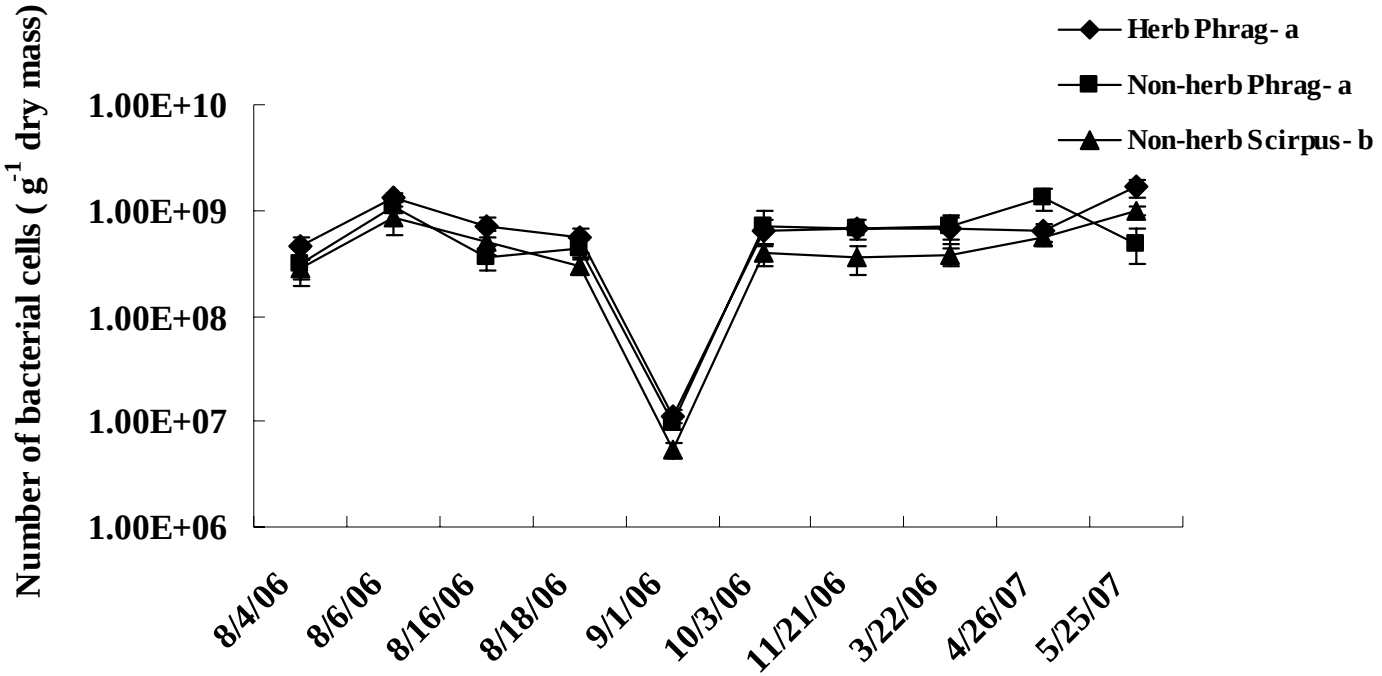

Figure 10. Bacteria counts on leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Different letter indicate significantly different treatments. Error bars indicate ± 1 SE.

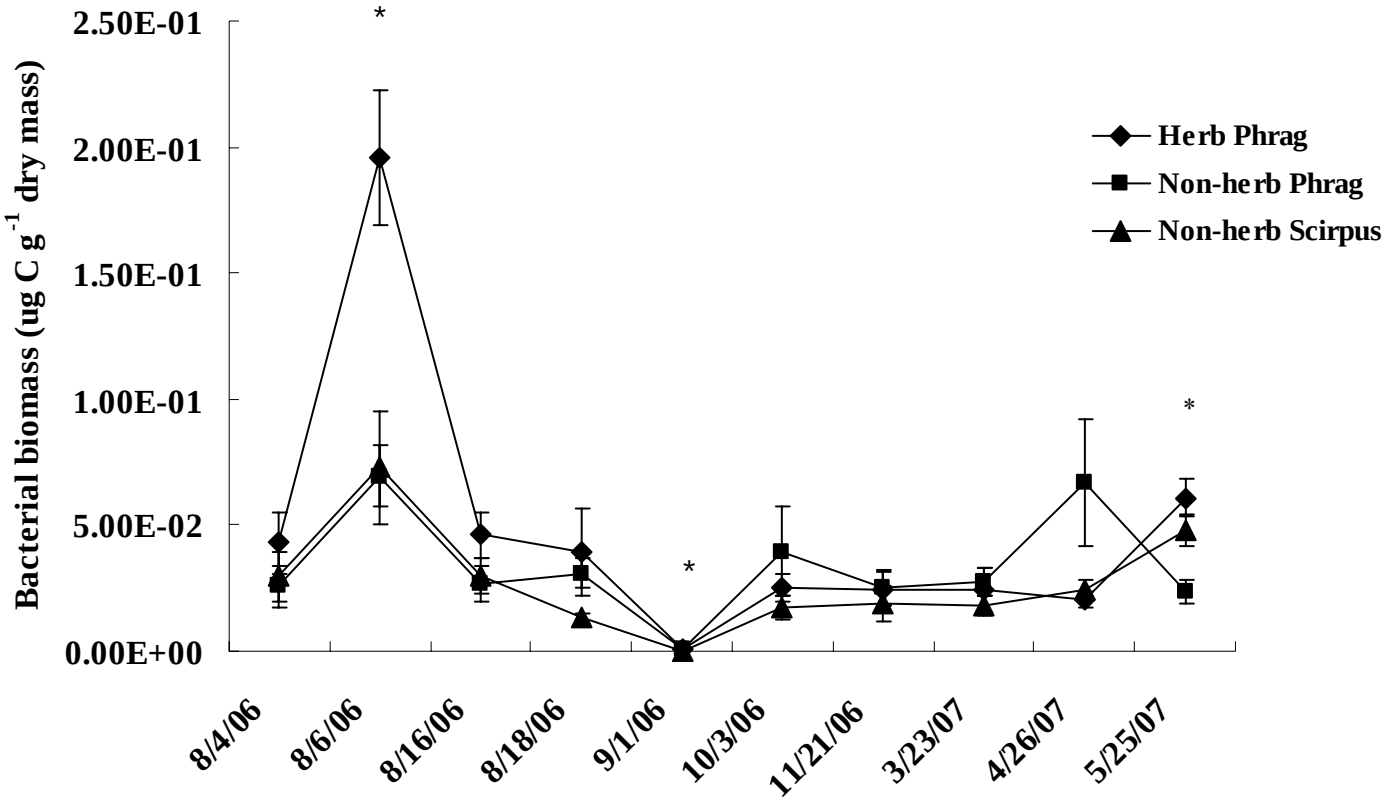

Figure 11. Bacterial biomass on the leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Asterisks indicate significant differences among treatment on those dates. Error bars indicate ± 1 SE.

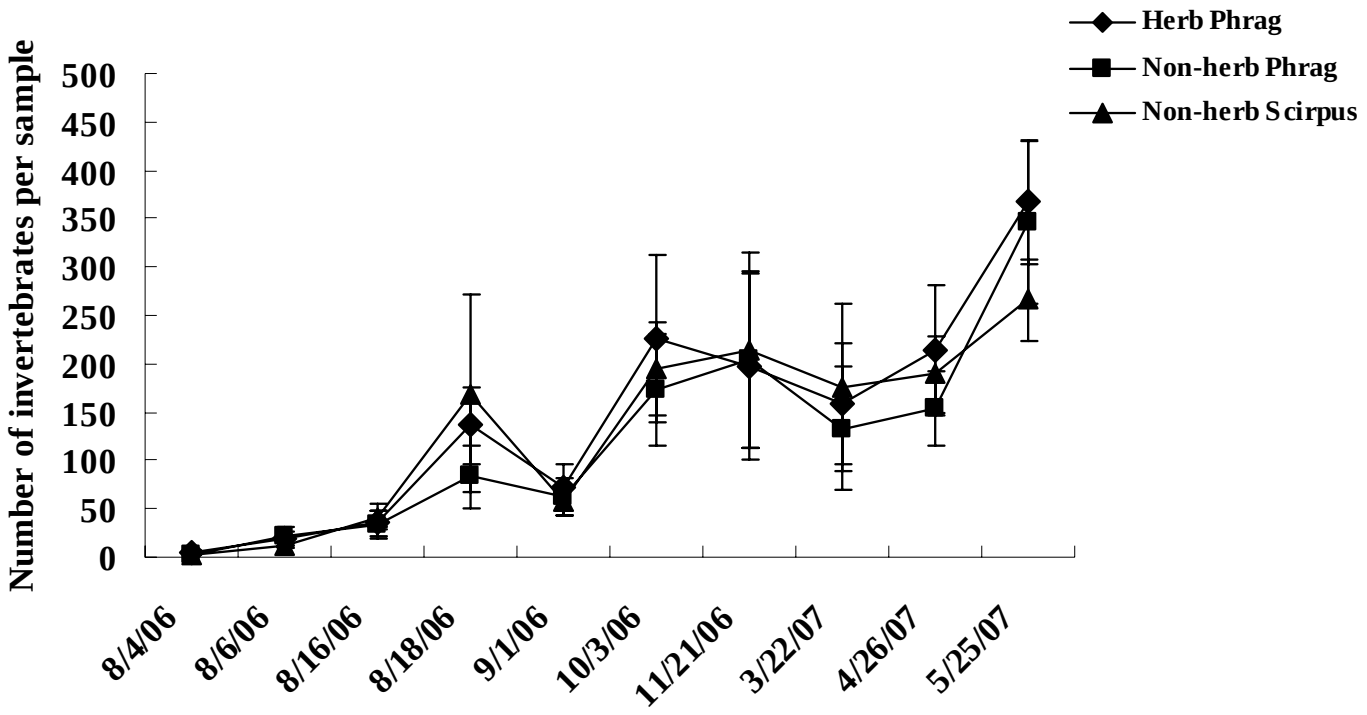

Figure 12. Total number of invertebrates per sample in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Treatment effects were not significant ($p>0.05$). Error bars indicate ± 1 SE.

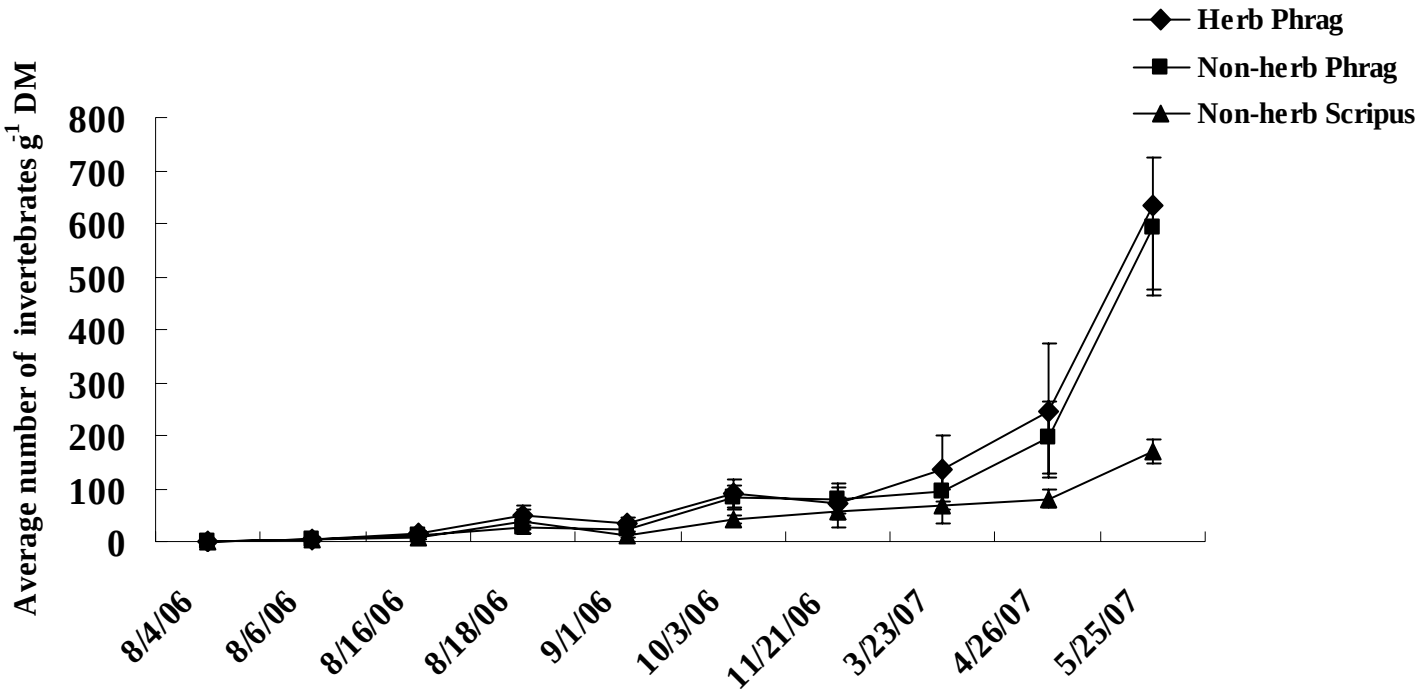

Figure 13. Average number of invertebrates per gram dry mass of leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Treatment effects were not significant (P>0.05). Error bars indicate ± 1 SE.

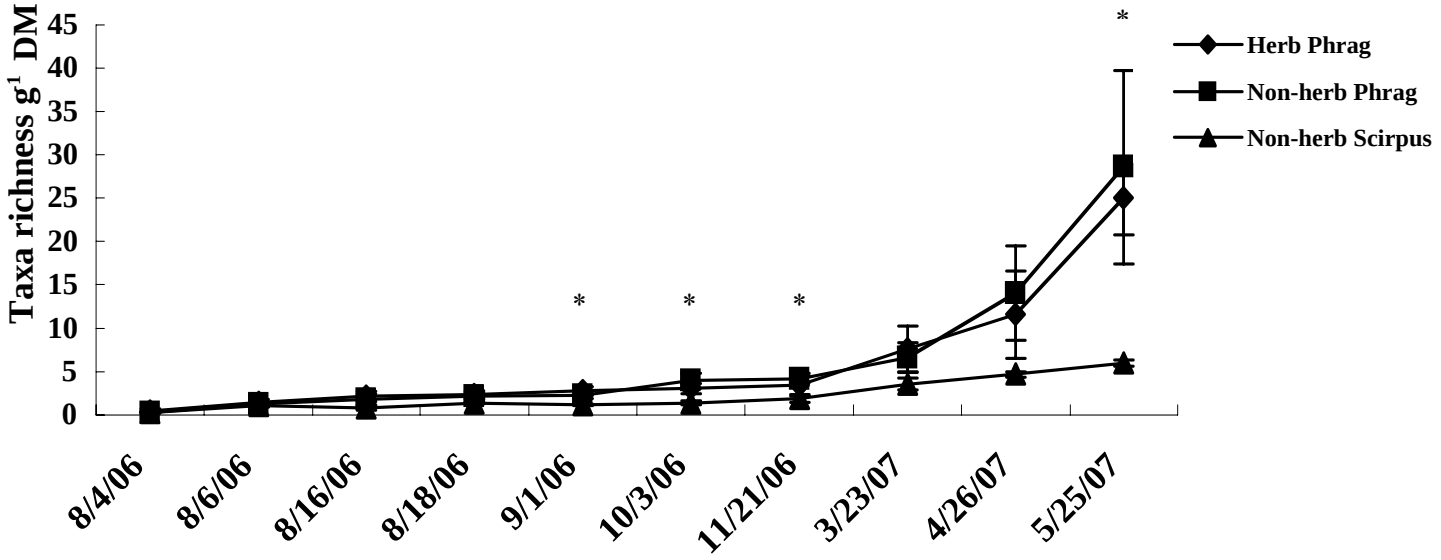

Figure 14. Taxa richness per gram dry mass of leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Asterisks indicate significant differences among treatment on those dates. Error bars indicate ± 1 SE.

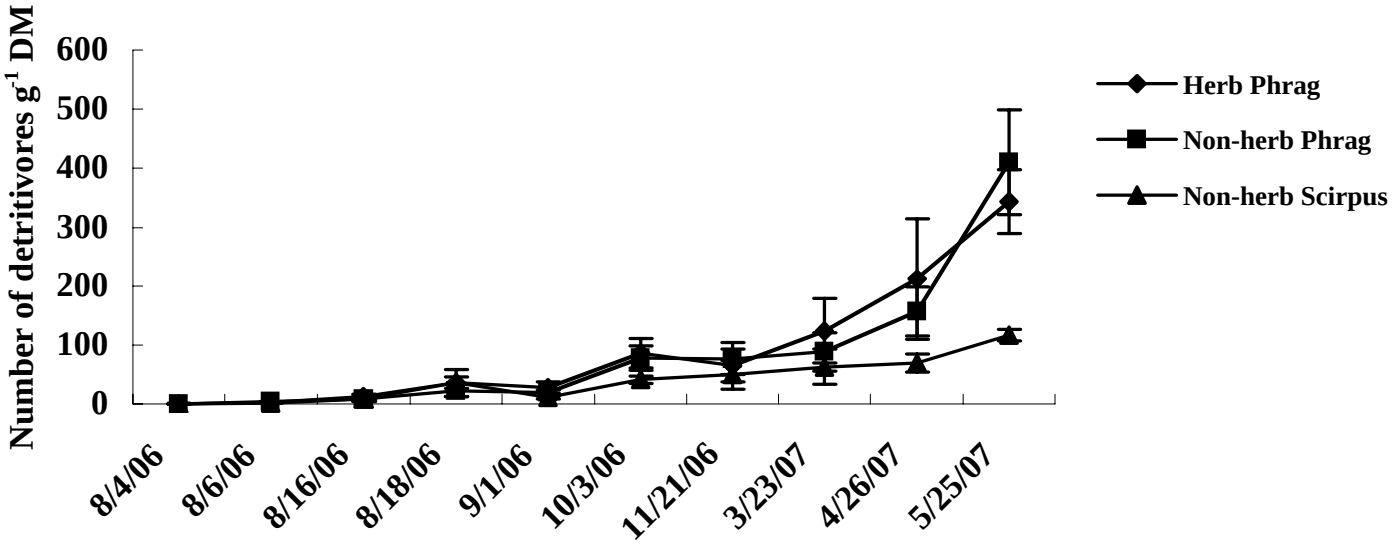

Figure 15. Number of detritivores per gram dry mass of leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Treatment effects were not significant ($p>0.05$). Error bars indicate ± 1 SE.

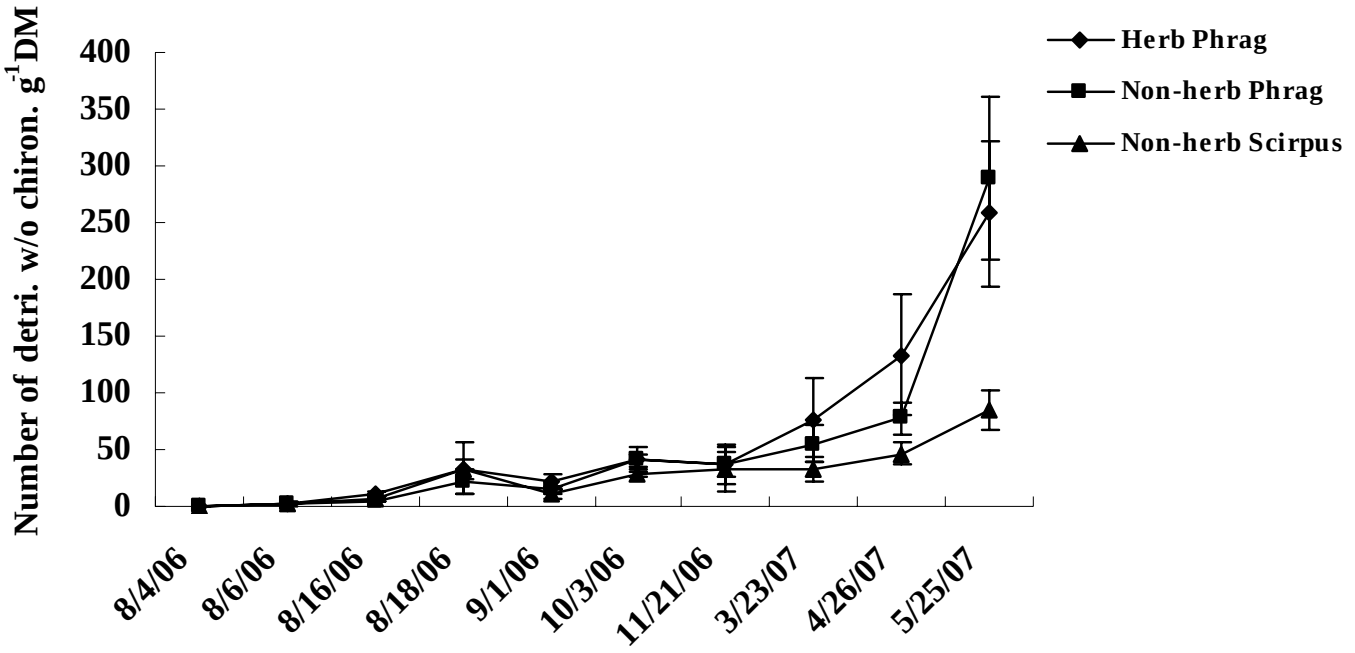

Figure 16. Number of detritivores without chironomids per gram dry mass of leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Treatment effects were not significant ($p>0.05$). Error bars indicate ± 1 SE.

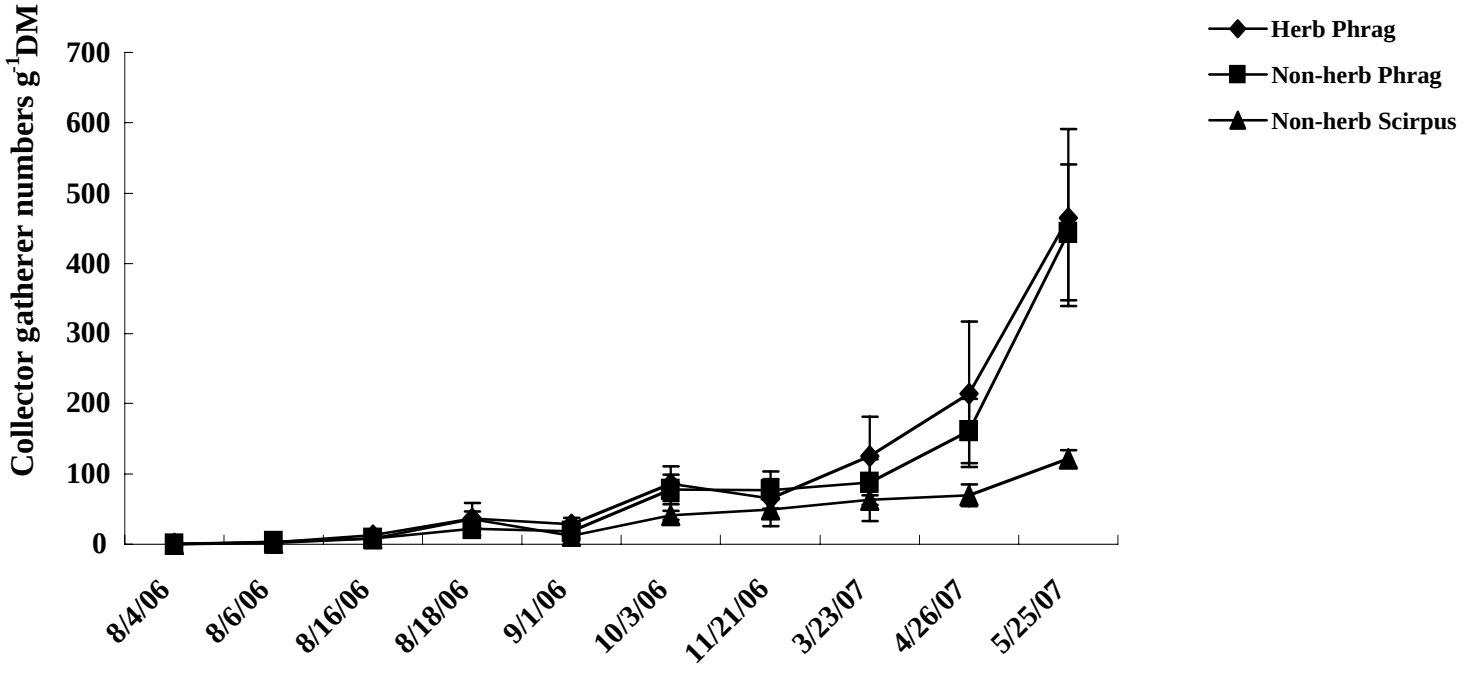

Figure 17. Number of collector-gatherer invertebrates per gram dry mass of leaf litter in the herbicided *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments. Treatment effects were not significant ($p>0.05$). Error bars indicate ± 1 SE.

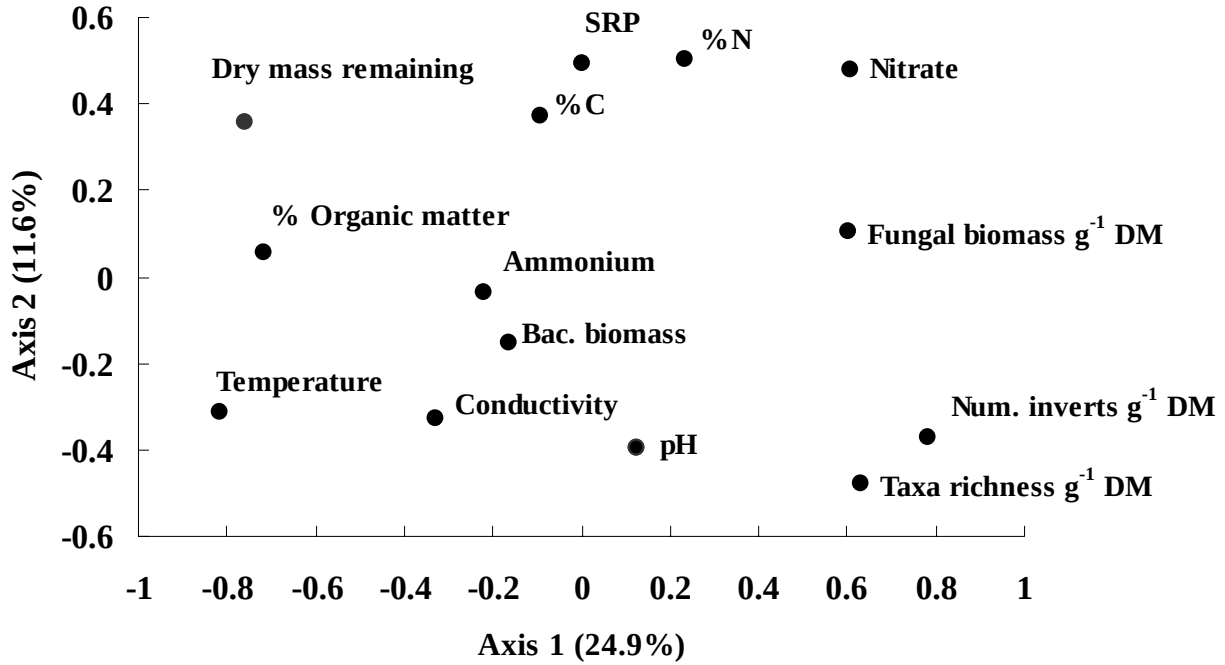

Figure 18. Principle components analysis (PCA) for physico-chemical and biotic data for *Phragmites*, non-herbicided *Phragmites*, and non-herbicided *Scirpus* treatments.

Appendix 1. Mean number (± 1 SE) of invertebrates per sample for each treatment on each sampling dates. Dashes (--) indicate none collected on that date. Invertebrate taxa were classified by Trophic Group (**TG**) as predominantly detritivores (D), herbivores (H) or predators (P). The taxa were also classified by Functional Feeding Group (**FFG**) as collector-gatherers (CG), collector-filterers (CF), scrapers (SCR), shredders (SHR), piercers (PI) or engulfing predators (EP). If taxa belonged to more than one TG or FFG, the classification noted first was used in analysis. Asterisks(*) indicate dominant taxa (>5% total invertebrates).

				Treatment		
Taxa	**Date**	**TG**	**FFG**	**Herbicided *Phragmites***	**Non-herbicided *Phragmites***	**Non-herbicided *Scirpus***
Ephemeroptera						
Caenidae		D, H	CG, SCR			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			0.60±0.60	0.40±0.24	0.20±0.20
	18 Aug 06			0.40±0.24	0.20±0.20	--
	1 Sept 06			--	0.60±0.60	0.80±0.80
	3 Oct 06			0.40±0.24	1.40±0.75	0.40±0.40
	21 Nov 06			--	--	0.50±0.26
	23 Mar 07			0.60±0.24	0.20±0.20	1.20±0.49
	26 Apr 07			0.80±0.37	0.60±0.40	1.20±0.80
	25 May 07			2.00±1.51	3.50±3.13	3.00±1.15
Siphlonuridae		P	EP			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	--	--
	1 Sept 06			--	0.20±0.20	--
	3 Oct 06			0.40±0.24	--	--
	21 Nov 06			0.60±0.40	--	--
	23 Mar 07			--	--	0.40±0.40
	26 Apr 07			--	--	--
	25 May 07			--	--	--
Odonata						
Aeshnidae		P	EP			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--

	16 Aug 06			0.20±0.20	0.20±0.20	--
	18 Aug 06			0.60±0.40	0.20±0.20	0.20±0.20
	1 Sept 06			--	--	--
	3 Oct 06			--	--	--
	21 Nov 06			--	--	--
	23 Mar 07			--	--	0.20±0.20
	26 Apr 07			--	--	--
	25 May 07			--	--	--
Coenagrionidae		P	EP			
	4 Aug 06			0.40±0.40	0.20±0.20	0.40±0.40
	6 Aug 06			1.20±0.73	1.20±0.73	0.20±0.20
	16 Aug 06			0.60±0.60	0.40±0.40	2.20±2.20
	18 Aug 06			3.40±2.27	0.20±0.20	2.00±1.26
	1 Sept 06			2.00±1.55	0.20±0.20	1.20±1.20
	3 Oct 06			1.60±0.81	0.60±0.60	0.40±0.24
	21 Nov 06			2.80±0.92	1.40±0.51	3.00±2.28
	23 Mar 07			1.00±0.45	1.20±0.58	1.00±0.55
	26 Apr 07			2.00±1.05	1.40±0.60	4.60±3.61
	25 May 07			3.50±1.13	1.00±0.52	3.75±0.99
Libellulidae		P	EP			
	4 Aug 06			--	0.20±0.20	--
	6 Aug 06			0.40±0.40	--	0.20±0.20
	16 Aug 06			0.40±0.24	0.60±0.40	--
	18 Aug 06			--	0.80±0.58	0.20±0.20
	1 Sept 06			0.20±0.20	0.20±0.20	--
	3 Oct 06			1.00±1.00	1.20±0.73	1.80±1.80
	21 Nov 06			1.00±0.77	1.20±1.20	1.00±0.45
	23 Mar 07			0.40±0.40	--	0.60±0.40
	26 Apr 07			0.20±0.20	0.60±0.60	1.40±0.87
	25 May 07			0.75±0.43	0.50±0.45	--
Hemiptera						
Belostomatidae		P	PI			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	--	--

	1 Sept 06			--	--	--
	3 Oct 06			--	--	--
	21 Nov 06			--	--	0.20±0.20
	23 Mar 07			--	--	--
	26 Apr 07			--	--	--
	25 May 07			--	--	--
Naucoridae		P	PI			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	--	--
	1 Sept 06			--	--	--
	3 Oct 06			--	--	--
	21 Nov 06			--	--	--
	23 Mar 07			--	0.20±0.20	--
	26 Apr 07			--	--	0.20±0.20
	25 May 07			--	--	--
Trichoptera						
Hydroptilidae		H	PI, SCR			
	4 Aug 06			--	--	--
	6 Aug 06			--	0.20±0.20	--
	16 Aug 06			--	--	--
	18 Aug 06			--	--	--
	1 Sept 06			--	--	--
	3 Oct 06			--	--	--
	21 Nov 06			--	--	--
	23 Mar 07			0.20±0.20	--	--
	26 Apr 07			--	--	--
	25 May 07			--	--	0.50±0.45
Limnephilidae		P, H	EP, SHR			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	0.20±0.20	0.20±0.20
	1 Sept 06			--	--	--
	3 Oct 06			--	--	--

	21 Nov 06		--	--	--
	23 Mar 06		0.20±0.20	1.20±0.73	--
	26 Apr 07		1.00±0.55	0.80±0.58	1.40±0.51
	25 May 07		--	0.50±0.26	1.00±0.37
Lepidoptera					
Noctuidae		H	SHR		
	4 Aug 06		--	--	--
	6 Aug 06		0.20±0.20	--	--
	16 Aug 06		--	--	--
	18 Aug 06		--	--	--
	1 Sept 06		--	--	--
	3 Oct 06		--	--	--
	21 Nov 06		--	--	--
	23 Mar 07		--	--	--
	26 Apr 07		--	--	--
	25 May 07		--	--	--
Coleoptera					
Dytiscidae		P	PI		
	4 Aug 06		--	--	--
	6 Aug 06		--	--	--
	16 Aug 06		0.20± 0.20	--	--
	18 Aug 06		--	--	--
	1 Sept 06		--	--	--
	3 Oct 06		--	--	--
	21 Nov 06		--	--	--
	23 Mar 07		--	0.80±0.50	0.20±0.20
	26 Apr 07		0.20±0.20	0.20±0.20	--
	25 May 07		2.00±1.00	1.25±1.12	0.40±0.24
Haliplidae		H	SHR, PI		
	4 Aug 06		0.20±0.20	--	0.20±0.20
	6 Aug 06		--	--	--
	16 Aug 06		--	--	--
	18 Aug 06		--	--	--
	1 Sept 06		--	--	--
	3 Oct 06		--	--	--
	21 Nov 06		--	0.20±0.20	--

	23 Mar 07			--	--	--
	26 Apr 07			--	--	--
	25 May 07			--	--	--
Hydrophilidae (adults)		D	CG, SHR			
	4 Aug 06			--	--	--
	6 Aug 06			--	0.20± 0.20	--
	16 Aug 06			--	--	--
	18 Aug 06			--	0.20±0.20	0.20±0.20
	1 Sept 06			0.40±0.24	--	--
	3 Oct 06			--	0.20±0.20	--
	21 Nov 06			--	--	--
	23 Mar 07			--	--	--
	26 Apr 07			--	--	--
	25 May 07			0.20±0.20	0.20±0.20	--
Diptera						
Ceratopogonidae		P	EP, CG			
				--	--	--
				--	--	--
				--	--	--
				--	0.40±0.24	--
				--	--	--
				9.40±9.40	7.80±7.55	5.20±4.95
				4.00±2.63	1.80±0.97	0.80±0.80
				1.40±0.98	0.60±0.40	12.6±7.19
				1.80±1.36	1.60±0.68	1.80±0.97
				3.00±1.22	3.25±1.25	2.25±0.85
Chironomidae*		D, P	CG, SHR, EP			
	4 Aug 06			0.40±0.40	--	--
	6 Aug 06			1.40±0.93	2.60±1.29	3.20±0.97
	16 Aug 06			7.00±3.18	8.00±4.89	6.40±1.81
	18 Aug 06			11.00±1.58	3.80±2.03	10.80±4.00
	1 Sept 06			11.80±4.77	13.00±7.06	3.20±1.59
	3 Oct 06			116.60±68.80	79.80±42.42	64.80±31.94
	21 Nov 06			73.00±38.27	100.80±48.63	66.80±18.97

	23 Mar 07			50.60±18.99	46.20±28.32	80.80±51.31
	26 Apr 07			61.00±32.82	52.20±21.99	51.60±24.52
	25 May 07			44.25±11.03	40.00±8.70	75.50±33.74
Culicidae		D, H	CG, CF			
	4 Aug 06			--	--	--
	6 Aug 06			--	0.20±0.20	--
	16 Aug 06			--	0.20±0.20	--
	18 Aug 06			1.60±1.36	1.60±1.60	0.20±0.20
	1 Sept 06			0.20±0.20	0.20±0.20	0.20±0.20
	3 Oct 06			0.20±0.20	--	--
	21 Nov 06			--	--	--
	23 Mar 07			0.20±0.20	--	--
	26 Apr 07			0.40±0.24	0.80±0.58	--
	25 May 07			1.50±0.96	0.50±0.29	0.50±0.29
Cladocera		H	CF			
	4 Aug 06			--	0.80±0.80	0.80±0.80
	6 Aug 06			--	0.20±0.20	0.80±0.58
	16 Aug 06			0.20±0.2	0.60±0.40	--
	18 Aug 06			0.60±0.60	0.40±0.40	--
	1 Sept 06			1.00±0.55	0.20±0.20	1.20±0.80
	3 Oct 06			--	--	0.20±0.20
	21 Nov 06			4.40±1.94	3.00±1.49	15.20±14.95
	23 Mar 07			4.80±4.80	1.40±1.17	1.20±0.97
	26 Apr 07			7.00±4.20	2.20±1.96	1.60±1.36
	25 May 07			15.25±7.14	6.75±2.79	3.50±0.35
Copepoda		H	CG			
	4 Aug 06			1.20±0.80	0.20±0.20	1.00±0.55
	6 Aug 06			0.20±0.20	0.20±0.20	0.40±0.24
	16 Aug 06			1.40±0.40	0.40±0.40	3.20±3.20
	18 Aug 06			0.80±0.20	0.40±0.24	2.60±2.36
	1 Sept 06			1.00±0.55	0.20±0.20	1.20±0.80
	3 Oct 06			1.40±0.98	0.20±0.20	0.60±0.40
	21 Nov 06			0.80±0.37	0.60±0.24	0.20±0.20
	23 Mar 07			2.00±1.78	0.20±0.20	0.40±0.24
	26 Apr 07			1.6±0.93	1.60±1.60	0.80±0.58
	25 May 07			44.00±36.39	25.50±14.67	10.50±4.63

Amphipoda					
Gammaridae		D	CG, SHR		
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	--	--
	1 Sept 06			--	--	--
	3 Oct 06			0.20±0.20	--	0.20±0.20
	21 Nov 06			--	--	--
	23 Mar 07			--	0.20±0.20	1.20±1.20
	26 Apr 07			0.60±0.60	0.20±0.20	1.00±0.77
	25 May 07			2.00±1.22	7.50±5.55	5.00±4.67
Talitridae*		D	CG, SHR		
	4 Aug 06			0.60 ±0.60	--	--
	6 Aug 06			3.00 ±2.07	1.20±1.20	0.60±0.55
	16 Aug 06			4.40 ±3.23	4.20±2.85	10.60±8.53
	18 Aug 06			19.00 ±10.43	3.60±2.87	11.20±5.24
	1 Sept 06			5.20 ±3.88	7.60±7.10	4.80±4.55
	3 Oct 06			19.20 ±17.00	8.60±8.60	8.60±7.13
	21 Nov 06			38.20 ±33.06	24.40±20.44	79.80±72.87
	23 Mar 07			14.40 ±6.91	10.40±5.70	12.60±7.19
	26 Apr 07			16.80 ±7.07	12.40±7.14	21.80±6.64
	25 May 07			46.33 ±21.54	14.75±6.09	15.25±7.36
Ostracoda*		D	CG		
	4 Aug 06			1.00±0.45	0.40±0.24	0.20±0.20
	6 Aug 06			3.80±1.59	7.80±2.27	3.40±1.08
	16 Aug 06			13.20±4.35	10.60±2.99	14.80±8.64
	18 Aug 06			57.20±24.22	46.00±27.60	118.80±97.54
	1 Sept 06			31.60±14.83	23.20±9.40	32.00±18.82
	3 Oct 06			23.00±3.48	11.40±1.78	22.20±2.85
	21 Nov 06			15.20±6.41	13.60±3.97	11.00±4.24
	23 Mar 07			37.20±20.36	18.60±8.93	29.00±15.53
	26 Apr 07			58.80±19.23	30.20±8.45	47.00±16.90

	25 May 07			87.50±43.59	87.75±42.70	80.50±30.54
Acari		P	PI			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	--	--
	1 Sept 06			0.60±0.60	--	--
	3 Oct 06			0.40±0.40	0.20±0.20	0.20±0.20
	21 Nov 06			0.60±0.40	0.40±0.24	0.20±0.20
	23 Mar 07			0.40±0.24	0.20±0.20	0.20±0.20
	26 Apr 07			1.00±0.77	1.00±0.63	--
	25 May 07			3.00 ±1.59	2.75±1.70	--
Gastropoda- Snails						
Ancylidae		H	SCR			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			--	0.20±0.20	--
	1 Sept 06			--	--	--
	3 Oct 06			--	0.20±0.20	--
	21 Nov 06			0.40±0.25	0.80±0.80	0.60±0.60
	23 Mar 07			0.20±0.20	--	0.80±0.80
	26 Apr 07			--	--	--
	25 May 07			2.50±1.50	1.50±1.06	6.00±4.71
Physidae		H	SCR			
	4 Aug 06			--	--	--
	6 Aug 06			0.20±0.20	--	--
	16 Aug 06			--	1.00±1.00	--
	18 Aug 06			0.20±0.20	--	0.20±0.20
	1 Sept 06			--	--	--

	3 Oct 06			--	0.40±0.25	--
	21 Nov 06			--	--	--
	23 Mar 07			1.60±0.93	3.60±3.36	3.20±2.52
	26 Apr 07			1.80± 0.86	7.40± 4.56	6.80±3.56
	25 May 07			33.25± 10.42	20.00±8.91	43.25±20.16
Planorbidae*		H	SCR			
	4 Aug 06			0.40±0.40	0.40±0.24	--
	6 Aug 06			8.20±8.20	6.20±5.70	1.40±1.40
	16 Aug 06			3.20±3.20	4.80±4.55	0.80±0.80
	18 Aug 06			26.20±26.20	8.20±7.71	1.60±1.36
	1 Sept 06			5.20±4.95	7.00±6.51	0.20±0.20
	3 Oct 06			1.20±0.90	2.40±0.87	1.20±0.97
	21 Nov 06			1.40±0.75	2.60±1.03	4.20±2.20
	23 Mar 07			3.20±1.56	2.40±0.87	2.40±0.81
	26 Apr 07			8.00±3.52	5.60±2.11	11.20±5.12
	25 May 07			48.00±10.88	37.5±11.24	50.00±15.24
Bivalvia						
Sphaeriidae		D	CF			
	4 Aug 06			--	--	--
	6 Aug 06			--	--	--
	16 Aug 06			--	--	--
	18 Aug 06			0.60±0.60	0.60±0.40	--
	1 Sept 06			0.20±0.20	0.60±0.40	0.80±0.80
	3 Oct 06			0.40±0.24	0.20±0.20	--
	21 Nov 06			0.20±0.20	--	--
	23 Mar 07			--	--	--
	26 Apr 07			--	0.40±0.40	0.60±0.60
	25 May 07			0.50±0.50	--	--
Oligochaeta*		D	CG			
	4 Aug 06			--	--	0.20±0.20

	6 Aug 06		1.20±0.80	0.80±0.58	1.20±0.58
	16 Aug 06		4.20±1.83	2.40±01.69	3.20±2.72
	18 Aug 06		14.80±9.73	13.40±7.93	19.80±9.12
	1 Sept 06		12.00±6.41	8.40±3.64	10.60±5.90
	3 Oct 06		51.20±15.44	57.80±14.03	88.80±18.60
	21 Nov 06		47.20±22.06	49.40±13.24	30.60±17.82
	23 Mar 07		41.00±22.69	45.40±17.23	39.00±14.75
	26 Apr 07		51.20±32.30	34.60±15.58	35.80±19.22
	25 May 07		41.25±16.25	93.00±24.64	75.50±35.76
Hirudinea					
Glossiphoniidae		P	EP		
	4 Aug 06		--	--	--
	6 Aug 06		0.20±0.20	--	--
	16 Aug 06		--	0.20±0.20	0.40±0.40
	18 Aug 06		0.20±0.20	0.20±0.20	0.80±0.37
	1 Sept 06		1.20±0.58	0.80±0.49	0.40±0.40
	3 Oct 06		0.40±0.40	0.60±0.40	--
	21 Nov 06		6.80±6.80	1.00±0.32	--
	23 Mar 07		--	--	--
	26 Apr 07		--	--	--
	25 May 07		0.25±0.25	0.25±0.25	--