THE IMPACT OF LAWN CARE PRACTICES ON SUBURBAN STREAMS IN METROPOLITAN ATLANTA, GEORGIA

by

SUSAN HERBERT

(Under the Direction of Judith Meyer)

ABSTRACT

Pesticides and fertilizers frequently detected in suburban streams could have an impact on leaf breakdown rates and fungal biomass in streams. In a field study comparing leaf breakdown rates and fungal biomass among suburban streams that differed in neighborhood socioeconomic status, significant differences in leaf breakdown rates were detected and related to stream velocity. Nutrient and fungicide (chlorothalonil and 4-hydroxy-chlorothalonil) concentrations did not differ among streams. Relationships between nutrient and fungicide concentrations and leaf breakdown rates were confounded by relationships with velocity. While breakdown rates are a good integrator of physical, chemical, and biological processes, it can be difficult to decipher which factor is having the greatest influence on differences in breakdown rates among streams. In a laboratory experiment, where the physical and chemical environment were controlled, the fungicides chlorothalonil, 4-hydroxy-chlorothalonil, and flutolanil inhibited biomass and sporulation rates of aquatic hyphomycetes and breakdown rates of tulip-poplar leaves (*Liriodendron tulipifera* L.).

INDEX WORDS: leaf breakdown rates, fungal biomass, ergosterol, tulip-poplar leaves, fungicides, chlorothalonil, hydroxychlorothalonil, flutolanil

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Thank you to my family who always encouraged my curiosity of the sciences and love of the outdoors. I think they now understand what a watershed is and why fungi and leaves are a good thing. Most importantly, thank you to my wonderful husband Andy who has been my sanity throughout my graduate career. Thank you for supporting me in every way and for your encouragement and desire for me to succeed. Lastly, I would like to include a contribution made by my brother, Bob, inspired after reading parts of my thesis; a poem to the tune of "Yesterday" by the Beatles:

"Poplar-Leaves, floating in my streams today
decaying slowly as they drift away
Oh, I believe they behave that way.

Suddenly, some started acting haphazardly,
tearing apart seemingly randomly
as if natures plan, was a comedy.

Why this Change, I don't know, but I will unhide

I suspect that the cause is some Fungicide.

Flutolanil, your destroying too much of our fungi

just so people will have some golf to play

why, oh, why does man behave this way?”

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CHAPTER 1

INTRODUCTION

Background

The United States Environmental Protection Agency (USEPA) reported that 77 million U.S. households used pesticides in 1999, spending $2 billion on pesticides for use in homes, lawns, and gardens (Donaldson et al. 2002). Although pesticides and fertilizers are applied to targeted areas, they can be transported to other parts of the environment via overland flow from impervious surfaces and groundwater recharge. Lawn care products have been detected in streams draining urban and suburban areas at greater concentrations than in streams draining agricultural areas (Gilliom 2001). Lawns were the largest source of total phosphorus and dissolved phosphorus in two urban basins in Madison, Wisconsin (Waschbusch et al. 1999). In a national study conducted by the United States Geological Survey (USGS), more than one pesticide was detected on every sampling date at 20 study units in urban and agricultural areas across the U.S.; concentrations of total phosphorus in streams were generally higher in urban areas than in agricultural areas (USGS 1999). In the southeastern U.S., pesticides have been detected in urban and suburban streams more frequently and at higher concentrations than in agricultural streams (USGS 1994). Thus, it is becoming increasingly important that the primary sources of pesticides and fertilizers contaminating suburban streams are identified. Reasons for pesticide and fertilizer usage in suburban areas differ from

reasons for use in agriculture because the intended benefit is most often something other than economic, and more likely for aesthetics or recreation.

This thesis was part of a larger study that integrates the physical, chemical, biological, and social sciences, to understand usage patterns and effects of residential lawn care chemicals on suburban streams in metropolitan Atlanta, Georgia. The study was funded by the USEPA Science to Achieve Results (STAR) program. The two objectives were to:

1) Measure the loading to streams and temporal trends in concentrations of turf care products and biological indicators of stream ecosystem health in streams receiving stormwater drainage from residential neighborhoods of different socioeconomic status.
2) Compare the cultural models of lawns and lawn care held by experts (i.e. lawn care professionals, environmental custodians, and horticultural writers) and homeowners in order to determine their points of commonality and divergence.

We hypothesized that lawn care practices of homeowners and lawn care experts in neighborhoods that differ in socioeconomic status will determine the loads of lawn care chemicals (pesticides and fertilizers) and subsequent ecological impacts in suburban streams. More specifically, in this thesis, I am examining the impacts of lawn care practices on leaf breakdown and fungal biomass in streams.

We carried out this study in Peachtree City, a planned community, located in Fayette County, Georgia, thirty miles south of downtown Atlanta in the Piedmont physiographic province (Figure 1.1). The streams are part of the Flat Creek watershed, a

tributary to the Upper Flint River, which merges with the Chattahoochee River to form the Apalachicola River before it empties into the Gulf of Mexico. Development of Peachtree City started in the late 1950s. Neighborhood design was inspired by Ebenezer Howard's English Garden City Movement of the 1890s, updated for the automobile and based on the concept of a superblock (M. Reinberger, University of Georgia, pers. comm.). The garden city movement called for an ideal, self-contained community of predetermined area where the population was surrounded by a greenbelt (Lagassè 2000). The superblock concept consisted of a neighborhood-sized unit penetrated only by cul-de-sacs, in which vehicular traffic was separated from pedestrian traffic, and greenspace was at the center of each superblock (Radburn Association 2003). In this model, neighborhoods share a village center and are connected by cart paths. The superblock was first implemented in Radburn, New Jersey, by Clarence Stein and Henry Wright, and other famous examples include Reston, VA and Columbia, MD (M. Reinberger, University of Georgia, pers. comm.). Early development in Peachtree City was based on this superblock model and was best implemented in the first neighborhoods constructed. The village center notion began to fail in the 1980s during a housing boom, when development shifted back to typical suburban sprawl. At this time, development changed from having a variation in property value within a neighborhood to a variation in property value between neighborhoods (M. Reinberger, University of Georgia, pers. comm.).

This shift enabled us to identify neighborhoods that differed in socioeconomic status. We selected four suburban sites: two streams draining high property value neighborhoods built in the late 1980s, one stream draining an intermediate property value neighborhood built in the 1970s, and one stream draining a lower property value

neighborhood built in the early 1980s (Table 1.1). We measured and compared biological, chemical, and physical aspects of these four suburban streams to two reference streams draining mixed-use areas of forest and agriculture, with some industrial and rural residential development. Reference streams were chosen to represent pre-development conditions in these watersheds.

While pesticides and fertilizers are often part of a lawn care program, the spatial and temporal usage patterns by homeowners and experts in urban and suburban areas are not well documented. Social scientists collaborating on this project are investigating whether homeowners in these four neighborhoods have different attitudes about lawn care and different product usage patterns and lawn care practices, one of which is to hire lawn care professionals (T. Gragson, A. Keeler, University of Georgia). Interviews are being conducted with homeowners and experts (e.g. lawn care professionals, horticultural writers, environmental custodians) in the suburban watersheds to determine points of commonality and divergence. Variations in lawn care practices among the neighborhoods could influence the concentrations of pesticides and nutrients detected in streams, which in turn have the potential to impact the biota and ecosystem processes.

Aquatic chemists participating in the study measured nutrient, metal, and pesticide concentrations in the six streams (K. Armbrust, L. Shuman, University of Georgia-Griffin). Entomologists/environmental toxicologists assessed the health of the macroinvertebrate community using the USEPA's Rapid Bioassessment Protocol (Plafkin et al. 1989), and assessed the effects of pulsed insecticide exposures on black fly larval growth, survival, and population rates (J. Overmyer, R. Noblet, University of Georgia). Environmental toxicologists deployed filter-feeding bivalves (*Corbicula fluminea*) for

biological monitoring. Biomarkers in these clams were measured, providing a means to assess the physiological impacts of toxicant exposure on individuals (D. Conners, M. Black, University of Georgia).

Focus of this study

Allochthonous leaf inputs are an important food source in streams, especially in those where opportunities for photosynthesis are small (Vannote et al. 1980). Upon entering streams, leaves are broken down by leaching and physical fragmentation, microbial conditioning, and invertebrate feeding (Petersen and Cummins 1974). Consequently, leaf breakdown rates integrate the physical, chemical, and biological processes working to decompose organic matter in streams and can be a useful overall indicator of stream ecosystem health (Webster and Benfield 1986) (see model in Figure 1.2.). Natural or anthropogenic alterations to basal resources in detrital food webs can have repercussions at higher trophic levels (Vannote et al. 1980, Meyer 1994). We investigated possible alterations in the food webs of these suburban streams by examining rates of leaf breakdown and fungal biomass accumulation.

Aquatic fungi are a vital link in detrital food webs through their role in conditioning leaf litter that falls into streams. This conditioning softens the leaves, making them more palatable to aquatic invertebrates consuming the leaves (Arsuffi and Suberkropp 1984, Suberkropp and Arsuffi 1984). Studies have shown that insects prefer leaves colonized by microbes to those not colonized (Cummins 1974). Fungal activity is stimulated or inhibited by physical and chemical factors such as nutrients, temperature, and anoxic conditions created by deposited sediments (Field and Webster 1983, Suberkropp 1995, Chauvet and Suberkropp 1998) (Figure 1.2). The direct impact of

lawn care fungicides on aquatic hyphomycetes and the possible secondary or indirect effects on ecosystem processes such as leaf breakdown have not previously been explored. This paucity of research prompted the inclusion of an investigation into the effect of lawn care fungicides on aquatic hyphomycetes and leaf breakdown in streams. Vertebrates are the organisms most often studied and only the direct toxicological effects (e.g. loss of sensitive species) are examined (Gilliom 2001). Hence, the objectives of this thesis were to:

1) Investigate the impacts of lawn care practices on the breakdown of tulip-poplar leaves, (*Liriodendron tulipifera* L.) a common riparian tree species, and on leaf-associated fungal biomass in suburban streams.
2) Determine whether fungicide concentrations typical of suburban streams affect rates of fungal biomass accumulation on leaves and leaf decay rates of tulip-poplar leaves in stream-simulating microcosms.

Chapter 2 includes the results of an experiment in which leaf packs were placed in Peachtree City streams to measure leaf breakdown rates, as well as fungal biomass and insects colonizing the leaf packs. Physical and chemical parameters that stimulate or inhibit leaf breakdown rates were simultaneously measured, including temperature, stream velocity, nutrient, metal, and fungicide concentrations and sediment accumulation in leaf packs (Figure 1.2). Leaf breakdown rates were compared among streams and relationships between leaf breakdown rates and physical, chemical, and biological parameters were examined.

Chapter 3 describes a laboratory microcosm experiment designed to help interpret the results from the field experiment. The concentrations of three fungicides typically

found in suburban streams were tested as to whether there was an effect on the biomass and sporulation rates of aquatic fungi and decay rates of tulip-poplar leaves. Known concentrations of flutolanil, chlorothalonil, and its main degradation product, 4-hydroxy-chlorothalonil, were added to chambers containing filtered stream water, tulip-poplar leaf disks, nitrogen and phosphorus, and a fungal inoculum. The physical and chemical environment was controlled to isolate the direct effect that fungicides have on aquatic hyphomycetes and their resultant indirect effects on leaf decay.

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Tables & Figures

Figure 1.1. Site map of six study streams: four suburban watersheds and one reference watershed located in Fayette County, and one reference watershed located in Coweta County, Georgia. The Peachtree City boundary is orange, suburban watersheds are outlined in red and reference watersheds are outlined in green.

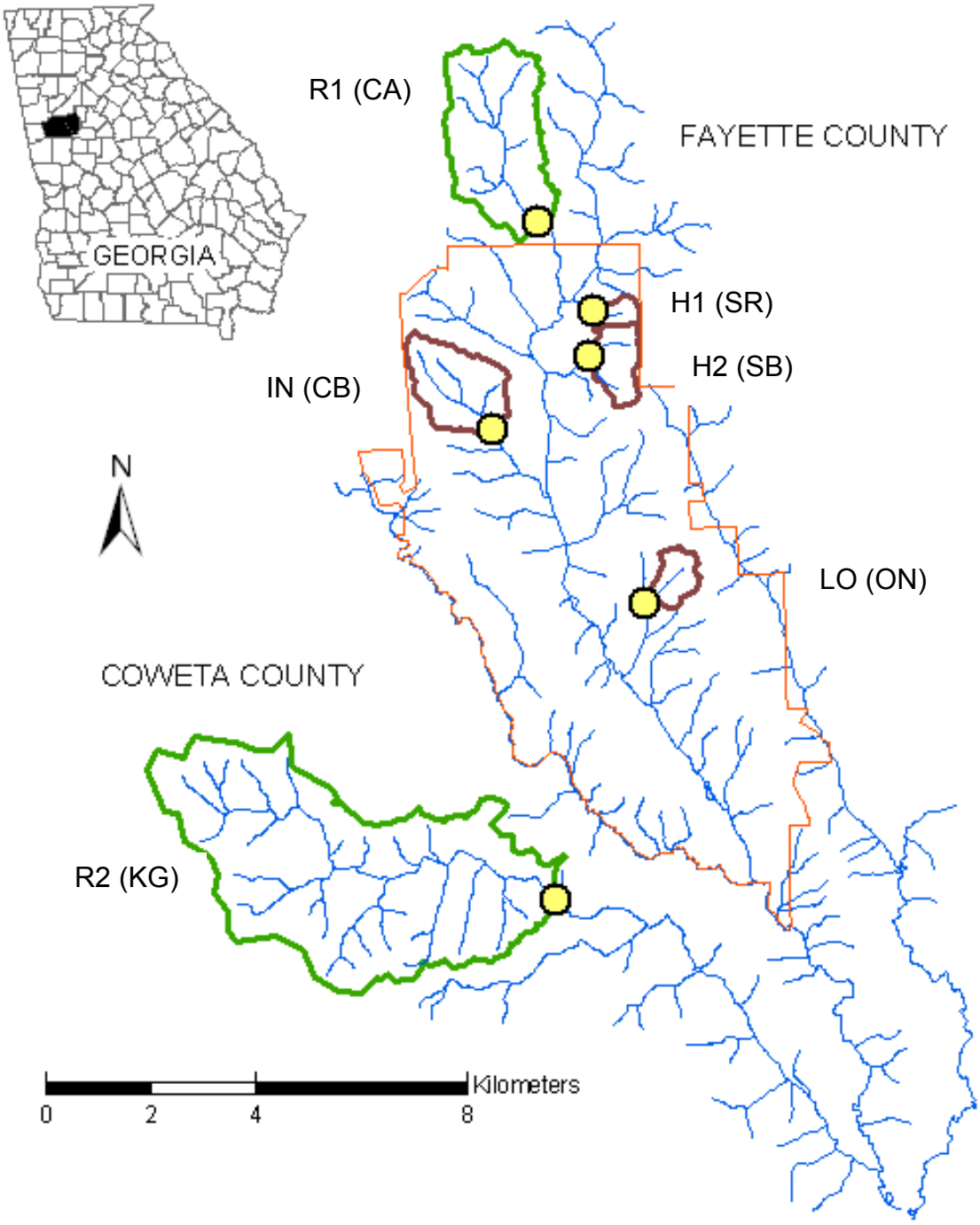
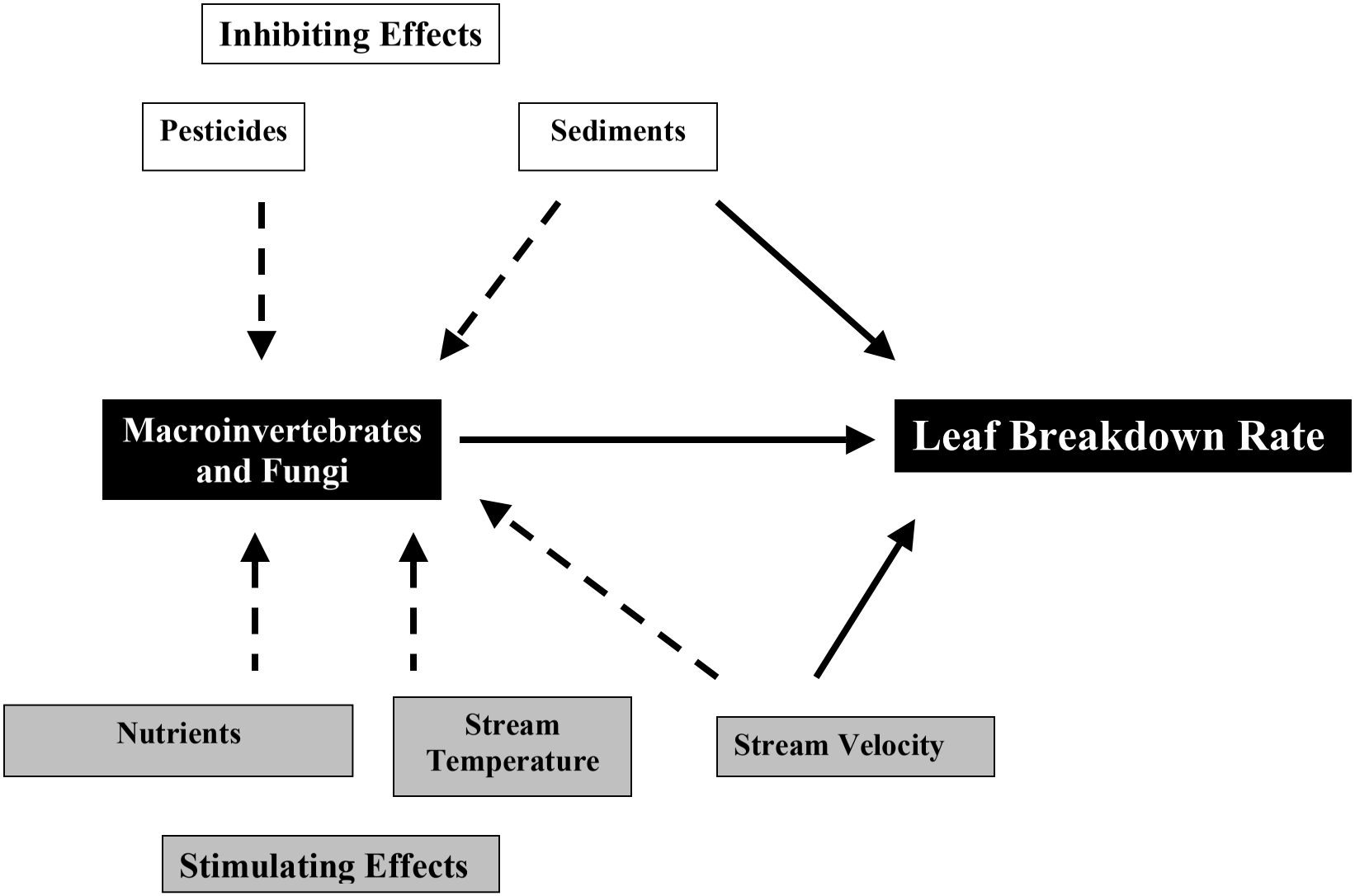

GEORGIA
R1 (CA)
FAYETTE COUNTY
H1 (SR)
H2 (SB)
IN (CB)
N
LO (ON)
COWETA COUNTY
R2 (KG)
Kilometers
0
2
4
8

Table 1.1. Stream names and codes and neighborhood descriptions of study watersheds (property values derived from 1999 Fayette County tax digest, N. Piekielek and M. Reinberger, University of Georgia).

Stream	Property Value Category	Stream Code	Year of neighborhood construction	Mean Property Value
Smoke Rise	high (H)	H1 (SR)	late 1980s	$388,900
Stoneybrook	high (H)	H2 (SB)	late 1980s	$326,200
Creekbed	intermediate (IN)	IN (CB)	1970s	$187,700
Oak Newell	lower (LO)	LO (ON)	early 1980s	$136,900
Crabapple	reference (R)	R1 (CA)	---	---
Keg	reference (R)	R2 (KG)	---	---

Figure 1.2. Physical, chemical, and biological factors that inhibit or stimulate leaf breakdown rates. Gray boxes represent the factors stimulating leaf breakdown and white boxes represent inhibiting factors. Solid arrows denote direct effects, and dashed arrows denote indirect effects.


Inhibiting Effects
Pesticides
Sediments
Macroinvertebrates and Fungi
Leaf Breakdown Rate
Nutrients
Stream Temperature
Stream Velocity
Stimulating Effects

CHAPTER 2

THE IMPACT OF LAWN CARE PRACTICES ON LEAF BREAKDOWN AND FUNGAL BIOMASS IN SUBURBAN STREAMS[1]

[1]Herbert, S., J.L. Meyer, K.L. Armbrust, L. Shuman, and N. Piekielek. To be submitted to the Journal of the North American Benthological Society

Abstract

Pesticides and nutrients are frequently detected in suburban streams, where stormwater runoff from residential lawns is routed directly to streams. Adverse effects of lawn care products on basal food resources and ecosystem processes in streams are not well understood. The impact of lawn care practices on the breakdown of tulip-poplar leaves (*Liriodendron tulipifera* L.) and fungal biomass (measured as ergosterol) was investigated in four suburban watersheds and two mixed-use watersheds near Atlanta, Georgia. Temperature, velocity, nutrient, metal, and fungicide concentrations were measured during the leaf pack experiment, and sediment accumulation and insect colonization in leaf packs were quantified. Leaf breakdown rates were compared among streams and related to physical, chemical, and biological parameters. These correlations were related to variations in human lawn care practices in the watersheds. Nutrient and fungicide concentrations were low (mean concentrations of NO_3-N <0.2 mg l^{-1}, mean concentrations of NH_4-N <0.01 mg $l^{-1}$and soluble reactive phosphorus <0.01 mg l^{-1}), with slight differences in nitrate-N among streams. There was significant fungal growth in four of the streams, but maximum ergosterol concentrations were low. There were significant differences in leaf breakdown rates among streams, with rates ranging from 0.0006 in a suburban stream to 0.0024 per degree day in both reference streams. Breakdown rates were significantly positively correlated with stream velocity (r^2=0.65, p=0.05), which differed significantly among streams. No clear relationship existed between breakdown rates and nutrient and fungicide concentrations, since these relationships were confounded by relationships between nutrient and fungicide

concentrations and velocity. Stream velocity appears to explain the variation in leaf breakdown rates among streams more than chemical or biological factors.

INDEX WORDS: leaf breakdown rates, suburban streams, tulip-poplar, fungal biomass, Georgia, fungicides, nitrogen, phosphorus, ergosterol, chlorothalonil

Introduction

There are many sources of pesticides and fertilizers in urban and suburban watersheds. Pesticide and fertilizer application to lawns in residential areas is one source that can result in contamination of streams when these compounds are exported in surface runoff. Runoff from impervious surfaces is transported directly to streams via stormwater conveyance systems that by-pass riparian buffers. In a national study, the United States Geological Survey (USGS) found that urban and suburban streams have higher loads of pesticides and pesticide metabolites than agricultural and forested streams (USGS 1999). Concentrations of nitrogen and phosphorus are also higher in urban watersheds than forested watersheds (USGS 1999, Paul and Meyer 2001).

Urban areas cover less than 5 percent of land in the U.S. (USGS 1999), but the majority of the U.S. population lives in urban areas, and the streams draining these areas are becoming more polluted. In the year 2000, 77% of the U.S. population (i.e. 200 million people), and 47% of the world population (i.e. 3 billion people) lived in urban areas. In 2030, 85% of the U.S. population is predicted to be urban and 60% of the world population is predicted to be urban (United Nations 2001). In residential areas of the U.S., homeowners invest varying amounts of time and money into lawn care and employ different lawn care practices in order to achieve a desired goal. Pesticide and fertilizer

application is often part of a lawn care program, but the patterns and amounts of use in urban and suburban areas are not well documented, especially when compared with agricultural areas. In addition, lawn care professionals are hired by homeowners and are often the managers of industrial park lawns and recreational fields; their lawn care practices may be different from homeowner practices.

Lawn care products inevitably enter streams through groundwater recharge, overland flow, or aerial deposition, and those most frequently detected are those most heavily applied in agricultural and urban areas (USGS 1999). In six streams in Peachtree City, GA, one fungicide, chlorothalonil, and its degradation product, 4-hydroxy-chlorothalonil, both had a frequency of occurrence greater than 94% in monthly samples (K. Armbrust, University of Georgia-Griffin, unpublished data). In a national study, the USGS detected more than one pesticide on every sampling date at 20 National Water Quality Assessment (NAWQA) study units of urban and agricultural areas across the U.S. (USGS 1999).

Pesticides are repeatedly being detected in non-target organisms. Concentrations of at least one pesticide exceeded U.S. and Canadian guidelines for protection of aquatic life in more than half of agricultural and urban streams sampled (USGS 1999). Urban streams had the highest frequencies of occurrence of DDT, chlordane, and dieldrin in fish and sediment (USGS 1999). Despite their widespread occurrence, there is little information on the effect of pesticides and fertilizers on stream ecosystem processes such as breakdown of allochthonous leaf litter that falls into streams. Leaf breakdown is a good indicator of ecosystem function because it integrates microbial processes, invertebrate feeding, and hydraulic flow into a single response variable (Webster and

Benfield 1986). Fungicides applied to lawns to treat terrestrial fungi could also affect non-target organisms, such as aquatic hyphomycetes, but we have found no studies that have examined this. This paucity of research prompted the inclusion of an investigation of the effect of lawn care fungicides on aquatic hyphomycetes and leaf breakdown in streams. Vertebrates are the organisms most often studied and only the direct toxicological effects (e.g. loss of sensitive species) are examined (Gilliom 2001).

Increased concentrations of nutrients can stimulate microbial processes leading to faster leaf breakdown rates (Meyer and Johnson 1983, Suberkropp 1995), while pesticides inhibit the activity of organisms and thus can slow down the conditioning of leaves by fungi and bacteria and the shredding of leaves by insects (Cuffney et al. 1984, Chung et al. 1993), creating slower leaf breakdown rates. At the same time, physical factors such as stream velocity and sediment accumulation can alter breakdown rates and may be the overriding factors in more developed watersheds (Figure 2.1).

Our objectives were to investigate the effects of lawn care practices on leaf breakdown and fungal biomass in suburban streams. In a laboratory microcosm study (Chapter 3), we detected an effect of fungicides on both leaf breakdown and fungi when leaves were exposed to low fungicide concentrations comparable to those in suburban streams. We hypothesized that lawn care practices by homeowners and lawn care professionals in neighborhoods that differ in socioeconomic status will affect fungi and leaf breakdown in streams.

Methods

Neighborhood descriptions

Peachtree City, Georgia, is located thirty miles south of downtown Atlanta, and has a population of 36,000 (Figure 2.2). It is a planned community consisting of neighborhoods that differ in socioeconomic status, with 70 miles of paved cart paths connecting the neighborhoods, which are used for running, biking, and driving electric golf carts. Planning for the city began in the 1950s when the landscape was rural farmland, and the first neighborhoods were constructed along with an industrial park in the 1960s (M. Reinberger, University of Georgia, pers. comm.).

Neighborhoods constructed in the 1960s to early 1980s were built with the concept of a village center that was to be shared by neighborhoods, which were connected with cart paths and isolated from through traffic. A housing boom in the 1980s brought with it a weakening of the village center idea. There was a shift in neighborhood development from a variation in property value within a neighborhood to a variation in property value between neighborhoods. By the mid-1980s, the neighborhoods started to resemble typical upscale suburban development in Atlanta with larger houses and lot sizes, lower housing density, disconnected cart paths, and a lack of greenways (M. Reinberger, University of Georgia, pers. comm.).

Four suburban streams receiving stormwater drainage from neighborhoods that differ in socioeconomic status were chosen for this study and classified into three property value categories: high (mean property value >$325,000), intermediate (mean property value <$190,000) and lower (mean property value <$140,000). Lawns in the high property value watersheds generally have a more formal lawn design with a high degree of symmetry, a loop driveway and elaborate gateposts and mailboxes (M.

Reinberger, University of Georgia, pers. comm.). Social scientists collaborating with the authors on this project are investigating if homeowners in these high property value neighborhoods have different attitudes about lawn care and different lawn care practices, including use of lawn care professionals, and are comparing these to lawn care practices by homeowners in the intermediate and lower property value neighborhoods. Homeowner education about lawn care will affect decisions on when, how, and what to apply to lawns. Differences in lawn care practices among the property value classes could influence the concentrations of pesticide and nutrients detected in suburban streams, which in turn have the potential to adversely affect the biota.

We chose two streams that drain high (H) property value neighborhoods, designated as H1 (SR) and H2 (SB), which were newer developments built on the periphery of Peachtree City, with more homogeneous property values (Table 2.1a). These neighborhoods lack the connectivity present in neighborhoods built earlier. We chose two additional streams draining neighborhoods of lesser property value designated as intermediate, IN (CB), and lower (LO) property value neighborhoods (Table 2.1a). Housing density in the high property value watersheds was lower than in the intermediate and lower property value watersheds and mean lot sizes were largest in the high property value watersheds (Table 2.1a). These suburban streams were compared with two streams draining less developed areas just outside of Peachtree City, which were selected to represent pre-development conditions and referred to as reference streams, R1 (CA) and R2 (KG) (Figure 2.2). All property value data and lot sizes were obtained from the 1999 Fayette County tax digest, which was compiled into a GIS database by N. Piekielek (University of Georgia). Watershed areas were derived from the Fayette County USGS

1979 30-meter digital elevation model and microstation files from the Peachtree City Engineering Department.

Stream descriptions

The six streams are located in the headwaters of the Upper Flint River drainage in the Piedmont physiographic province of Georgia (Figure 2.2). The intermediate property value stream, IN (CB) is the largest suburban stream with a watershed area of 2.6 km^2 (Table 2.1a). H1 (SR) is the smallest stream draining a 0.4 km^2 area. The reference streams, R1 (CA) and R2 (KG), are larger than the suburban streams with more forested watersheds, but have a history of farming, as was the case at the suburban streams prior to development. Some of the forested areas in R1 (CA) and R2 (KG) have been recently burned or cleared, and pine trees and shrubs have begun to recolonize; pesticides are most likely being applied in these areas. Impervious surface cover ranged from a low of 2.4% in H2 (SB) to a high of 15% in the lower property value watershed (Table 2.1a). R1 (CA) and R2 (KG) have a low percent impervious surface cover that is similar to H2 (SB).

Mean depth of the stream reaches ranged from 14 to 19 cm at the suburban streams and 20 to 40 cm at the reference streams (Table 2.1b). Mean wetted width of the stream reaches ranged from 1.3 to 2.6 m at the suburban streams and from 3.1 to 5.7 m at the reference streams (Table 2.1b). IN (CB) is the shallowest and widest suburban stream. All streams have mostly intact, forested riparian buffers, but the suburban streams have stormwater conveyance systems that by-pass the buffer, conveying runoff from impervious surfaces directly into the streams so that many of the benefits typically provided by buffers (e.g. decreasing erosive power and removing contaminants) are not

occurring. Three of the suburban streams, H1 (SR), IN (CB) and LO (ON), have incised channels and one side of the riparian zone is disrupted by a paved cart path that runs through it. The streambeds of all six streams are composed mostly of sand; however, H2 (SB) has large amounts of bedrock and cobble. R2 (KG) is the only stream with significant amounts of woody debris available for habitat.

Sampling and processing

Tulip-poplar trees (*Liriodendron tulipifera* L.) are a common riparian tree species in the Georgia Piedmont. The percent of tulip-poplar in the leaf standing stock in these streams ranged from 3% to 20% when assessed in December 2001 (Table 2.1b); this was the tree species used to construct leaf packs. Senescent leaves were collected from tulip-poplar trees, or freshly fallen leaves were picked off the ground in October and early November 2000. Fifty air-dried, 8-g leaf packs were assembled in coarse-mesh bags (mesh size of 2.4 cm by 1.4 cm) using standard leaf pack methods (Benfield 1996), tied to bamboo stakes, and anchored to the streambed in pools in the six streams on November 10, 2000. Three packs were removed from each stream on days 3, 7, 15, and 22 and five packs were removed on days 33, 51, 77, 105, 147, and 193 using a Nytex bag to minimize loss of sediments and macroinvertebrates. Six packs were processed on day 0 to account for leaf mass loss due to handling.

Depth and velocity measurements were taken at the location of leaf packs each day that packs were removed. It was recorded whether or not a pack was buried on the day it was sampled. Temperature readings were taken hourly throughout the experiment with Onset Corporation HOBO® temperature loggers and used to calculate daily mean temperatures as well as cumulative degree days above 0°C. Daily precipitation data were

acquired from the Southeast Regional Climate Center for the nearby Atlanta airport (SERCC 2002). Water samples for stream concentrations of nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), and soluble reactive phosphorus (SRP) were taken monthly and each day leaf packs were removed. Samples were filtered immediately after sampling and frozen until later analysis. Analyses were performed by automated colorimetry using a flow analyzer (EPA methods 351.1, 353.2, 365.1, and EPA 600/R-93/100) (APHA 1992) (analyses by T. Maddox and L. Shuman, University of Georgia). Dissolved inorganic nitrogen (DIN) concentrations were determined by summing measured NO_3-N and NH_4-N concentrations.

Water samples for pesticide concentrations were taken monthly during baseflow conditions. Samples were analyzed by K. Armbrust (University of Georgia-Griffin), by injecting 1 ml aliquots into a Waters 2690 Alliance high performance liquid chromatograph (HPLC) and separated on a C-8 column manufactured by Alltech Associates using an acetonitrile : water gradient. Analytes were detected by ultraviolet absorbance at 220 nm using a Waters 996 photodiode array detector. The limit of quantification for the analytes was 0.1 ng l^{-1}. All reagents were obtained from Fisher Scientific. Chlorothalonil standards were purchased from ChemServ, and 4-hydroxy-chlorothalonil standards were a gift from Syngenta Agrochemicals.

Water samples for metal analyses were collected and placed in new (or washed and rinsed with distilled water) plastic bottles, transported to the laboratory in ice chests, acidified as soon as they were returned to the laboratory with equal parts of HNO_3 and water in acid-rinsed bottles to a pH<2, and kept at 4°C until analyzed by L. Shuman (University of Georgia-Griffin). Samples were filtered through a membrane filter (0.45-

μm pore size) so that the analyses were for soluble elements. Membranes were first rinsed with an aliquot of acidified sample and the sample discarded to prevent contamination by the membrane. Zinc and copper were analyzed using graphite furnace atomic absorption spectrometry (EPA Method 200.9 and EPA 600/4-93/0100). Arsenic analysis was by the same method using a special power supply for the arsenic lamp.

Leaf packs were brought back to the laboratory on ice where sediment and macroinvertebrates were rinsed from packs and preserved for later identification and quantification. Leaf disks were cut using a cork borer (18-mm diameter) for ergosterol extraction and ash-free dry mass (AFDM) determination. The remainder of the leaves was used to determine AFDM of the pack. Leaves were dried at 60°C for 24 h to obtain dry weight, ashed at 500°C for 3.5 h, and weighed again. Leaf breakdown rates (k) were calculated using a standard exponential decay regression model where:

$$M_f = M_o e^{-kt}$$

M_f = final mass M_o = initial mass k = leaf breakdown rate t = degree days

(Petersen and Cummins 1974). The slope of the regression of time versus the mean LN(% AFDM remaining) is the leaf breakdown rate. Time was expressed in degree days, which were determined by summing the mean daily temperature as cumulative number of degrees above 0°C throughout the leaf pack experiment (Allan 1995). Leaf disks for ergosterol (a sterol found in the membranes of living aquatic hyphomycete fungi) were stored in 5 ml of methanol at -16°C in the dark and then extracted following methods described in Weyers and Suberkropp (1996). Final ergosterol concentrations were

determined by high performance liquid chromatography (HPLC) (University of Georgia Pesticide and Hazardous Waste Laboratory).

Sediments, invertebrates, and organic matter rinsed from leaf packs were collected on a 250 μm sieve. Larger insects and crayfish were removed at this point and preserved in 6% formalin. Sediments <250 μm were collected in a bucket and brought to a known volume, subsampled, and frozen at -16°C until later analysis. After defrosting, samples were filtered onto a 1μm glass fiber filter, dried at 60°C for 24 h and weighed for fine sediments. Sediments and macroinvertebrates greater than 250 μm were preserved in 6% formalin with phloxine B dye added for easy sorting. This portion of the sample was elutriated to separate organic matter and macroinvertebrates from inorganic matter. Organic matter was sorted to pick out invertebrates; sediments and organic matter were then dried at 60°C for at least 24 h and then weighed.

Insects from leaf packs collected on days 15, 33, and 51 were identified to the lowest taxon necessary for separation into the shredder functional feeding group using Hilsenhoff (1981), Epler (1996), and Merritt and Cummins (1996) and functional feeding group designations of Merritt and Cummins (1996). Shredder abundances per leaf pack were determined and biomass (mg dry mass [DM]) was calculated using length-weight regressions derived from Benke et al. (1999). *Paraleptophlebia* was retained as a facultative shredder. All insects were used to determine family richness per pack (except Ceratopogonidae, which was not separated from Chironomidae). Crayfish abundance per pack was also determined for days 15, 33, and 51.

Leaf standing stock

On December 15, 2001, and March 29, 2002, leaves were collected from the bankfull channel at eight 0.5-m transects in all six streams. Wetted widths were recorded. Leaves were separated from other organic matter, subsampled if necessary, and dried at 60°C for at least 48 h before weighing. Tulip-poplar leaves were separated from other leaves in the December standing stock samples to determine percent composition of both tulip-polar and other leaves in each stream.

Data analyses

Physical, chemical, and biological data could not be normalized and were analyzed with the nonparametric Kruskal-Wallis rank test followed by Wilcoxon pairwise comparisons to test for differences among streams and property value classes (Zar 1984). To test for differences in ergosterol between buried and surface packs, a paired Wilcoxon test was used. Simple linear regression was used to determine which variables best explained the variation in leaf breakdown rates and to test for relationships between and among physical, chemical, and biological variables. Rate of fungal biomass accumulation (μg ergosterol d^{-1}) was determined by LN-transforming ergosterol concentrations and regressing against time. Leaf breakdown rates were calculated per degree day to incorporate temperature differences and were compared among streams by using 95% confidence intervals (CI). All analyses were conducted using $p<0.05$ as the criterion for significance. Statistical tests were performed using JMP® statistical software (SAS 2002).

Predicted mass of leaf standing stock was calculated using k (d^{-1}) from the leaf pack experiment, time (t in days) between December and March sampling dates, and the

December values as the initial mass (M_o) for each stream in the standard exponential decay regression model and solving for M_f (Petersen and Cummins 1974).

Results

Physical and chemical variables

Stream concentrations of NO_3-N, NH_4-N, and SRP during the leaf pack experiment were very low, and differences among streams were slight (Table 2.2a). Mean stream concentrations of NO_3-N ranged from 137 to 266 μg l^{-1}, while mean stream concentrations of NH_4-N ranged from <6 to 24 μg l^{-1}. Mean concentrations of SRP were all close to the detection limit of 6 μg l^{-1}. Median, minimum, and maximum nutrient concentrations are reported in Appendix A (Table A.1). There were no significant differences in NH_4-N among streams (Kruskal-Wallis p=0.23) or property value classes (Kruskal-Wallis, p=0.22) (Table 2.2a). There were significant differences in NO_3-N among streams with IN (CB) having lower concentration than LO (ON) (Kruskal-Wallis p=0.01 and Wilcoxon). The intermediate property value stream also had significantly lower DIN than the lower property value stream (Kruskal-Wallis p=0.01 and Wilcoxon). However, there were no significant differences among property value classes for either NO_3-N (Kruskal-Wallis p=0.86) or total DIN (Kruskal-Wallis p=0.53) (Table 2.2a). SRP did not vary significantly among streams (Kruskal-Wallis p=0.99) or property value classes (Kruskal-Wallis p=0.99).

The intermediate value stream had a significantly higher mean temperature than the other five streams (Kruskal-Wallis p<0.0001) and also had the highest number of cumulative degree days (Table 2.2a and 1.1b). R1 (CA) and R2 (KG) had somewhat

lower mean temperatures than the suburban streams. Mean stream concentrations of zinc were low and similar among streams and property value classes (Kruskal-Wallis $p>0.7$ and $p>0.5$ respectively) (Table 2.2b). Concentrations of copper and arsenic did not exceed the detection limit of 5 $\mu g\ l^{-1}$ at any of the streams except for one occasion at H1 (SR) in May 2001 just before leaf packs were removed from the streams.

Significant differences in velocity occurred among streams (Kruskal-Wallis $p<0.0001$), with the reference streams having the fastest velocities and the high property value streams having the slowest velocities (Table 2.2a). Both fine sediments (<250>1μm) and coarse sediments (>250 μm) in leaf packs varied significantly among streams (Kruskal-Wallis and Wilcoxon $p<0.0001$ for both) but not among property value classes. R2 (KG) and LO (ON) had significantly higher amounts of fine sediments per pack than the high property value streams. R1 (CA) had significantly higher coarse sediments per pack than the high property value streams (Table 2.3). Median, minimum, and maximum sediment accumulation values are reported in Appendix B (Table B.1). Percent of packs buried by sediments varied from 6 - 22% among streams, with the greatest percentage occurring at R1 (CA) (Table 2.3). Coarse sediment accumulation in packs was significantly positively correlated with velocity ($r^2=0.8$, $p=0.02$).

During the leaf pack experiment, fungicide concentrations were not significantly different among streams or property value classes (Kruskal-Wallis $p>0.7$ for both). Fungicide concentrations were somewhat higher at R1 (CA) and R2 (KG) than the other streams during the leaf pack experiment; however, when data for the entire year are considered, those differences disappear (Table 2.4 a and b). Median, minimum, and maximum fungicide concentrations are reported in Appendix A (Table A.2).

Biological variables

Ergosterol concentrations were low in all streams and did not vary significantly among streams (Kruskal-Wallis $p>0.8$). During the first 2 weeks of the leaf pack experiment, there was a decline in mean ergosterol concentrations in leaf packs in all 6 streams (Figure 2.3). After this initial period of dying off of terrestrial fungi, aquatic fungi began to colonize the leaves and significant rates of ergosterol mass accumulation were detected in four of the six streams from two weeks to fifteen weeks after leaf pack deployment. Significant rates of ergosterol mass accumulation ($\mu g\ g^{-1}$ leaf d^{-1}) were detected for all three property value classes, but were similar among classes (Table 2.5). Ergosterol concentrations in buried and surface packs collected from day 22 to 105 were compared across all streams and were not significantly different (paired Wilcoxon).

Insect shredder biomass and abundance in leaf packs varied significantly among streams for the days examined (Kruskal-Wallis $p<0.02$ and $p<0.001$ respectively). Mean shredder biomass ranged from 0.1 mg DM per pack at H1 (SR) to 8.8 mg DM per pack at R1 (CA) (Table 2.6). While significant differences between means are present, there was high variance in individuals per pack within each stream. Mean shredder abundance per pack ranged from 0.2 at both H1 (SR) and IN (CB) to 4.3 at LO (ON) (Table 2.6). Shredders were mostly from the genus *Leuctra*, along with insects from the families Tipulidae and Ephemerellidae, the caddisfly, *Pycnopsyche*, and the mayfly, *Paraleptophlebia*, a facultative shredder. Mean insect family richness was similar among streams, ranging from a low of 1.3 at H1 (SR) to a high of 4.1 at R1 (CA). LO (ON) and R1 (CA) were highest in shredder biomass, while H1 (SR) and IN (CB) were consistently low in shredder abundance and total insect family richness (Table 2.6) (see Appendix

Table B.2 for list of insect and non-insect taxa in leaf packs). Rate of ergosterol mass accumulation from day 15 to 105 had a significant negative relationship with insect shredder abundance (r^2=0.78 and p=0.02) across all streams (Figure 2.4) but a similar relationship was not found with insect shredder biomass (r^2=0.39 and p=0.18).

Mean crayfish abundance in packs collected on days 15, 33, and 51 ranged from 0.1 per pack at H1 (SB) to 0.4 at the intermediate property value stream with no significant differences among streams (Kruskal-Wallis p>0.9) (Table 2.6).

Leaf breakdown rates

Significant leaf breakdown occurred in all streams over 193 days. Leaf breakdown rates varied significantly (95% CI did not overlap) among streams and property value classes. Differences in leaf breakdown rates among streams were greater when temperature was taken into account by expressing rates per degree day (Table 2.7). Breakdown rates ranged from 0.0006 (dd^{-1}) at H2 (SB) to 0.0024 (dd^{-1}) at both R1 (CA) and R2 (KG). The high property value class had the slowest leaf breakdown (0.0008 dd^{-1}) while the reference class had the fastest breakdown (0.0024 dd^{-1}) (Table 2.7).

Leaf breakdown rate was significantly positively correlated with stream velocity (r^2=0.65, p=0.05) (Figure 2.5). The influence of stream velocity on breakdown rates is also apparent in the fact that breakdown rates did not differ significantly among streams until after day 105 when rainfall increased dramatically (precipitation rate = 0.7 cm d^{-1} vs. 0.3 cm d^{-1} previously) as a result of 19 storms in 6 weeks (Figure 2.6). Breakdown rates were also related to concentrations of NH_4-N and fungicides, but these relationships were confounded by correlations of these variables with velocity.

Velocity was weakly positively correlated with NH_4-N concentrations (r^2=0.70, p=0.12), and thus, with increasing NH_4-N concentrations, leaf breakdown rates increased (r^2=0.84, p=0.04). Mean fungicide concentrations were positively correlated with leaf breakdown rates (r^2=0.76, p=0.02); however, this relationship is presumably being driven by the positive correlation between fungicide concentrations and velocity (r^2=0.61, p=0.07).

Neither NO_3-N (r^2=0.23, p=0.66), total DIN (r^2=0.39, p=0.45), nor SRP (r^2=0.48, p=0.33) were related to leaf breakdown rates. The fact that NH_4-N is the only nutrient with a significant correlation with leaf breakdown rates lends support to leaf breakdown being driven by velocity rather than NH_4-N concentrations. If NH_4-N were truly limiting, one would expect a relationship with other forms of inorganic N. Coarse sediment accumulation in packs was not related to leaf breakdown rates (r^2=0.28, p=0.28). Further, at the stream with the greatest percentage of buried leaf packs, R1 (CA), there was no difference in leaf breakdown rate between buried (k = 0.0062 d^{-1}) and surface packs (k = 0.0121 d^{-1}) (95% CI did not overlap). Neither insect shredder biomass (r^2=0.31, p=0.25) nor abundance (r^2=0.002, p=0.93) was related to leaf breakdown rates nor was crayfish abundance (r^2=0.14, p=0.46) or rate of ergosterol accumulation (r^2=0.01, p=0.87).

Leaf standing stock

Leaf standing stock was assessed the year after the leaf pack experiment was performed, once during peak leaf fall in December and again during late winter in March. A significant difference was detected in leaf mass standing stock between the two dates (Kruskal-Wallis p<0.0001) (Figure 2.7). The percent of leaf mass loss ranged from 47

and 59% at H2 (SB) and H1 (SR) respectively, to 90 and 88% at R1 (CA) and IN (CB) respectively (Table 2.8, Figure 2.7).

The trend evident in percent loss of leaf standing stock among streams is similar to the pattern in the differences in leaf breakdown rates of leaf packs. R1 (CA), R2 (KG), IN (CB), and LO (ON) had faster breakdown rates and a higher percent loss of leaf standing stock than H1 (SR) and H2 (SB) (Table 2.7, 2.8 and Figure 2.7).

Predicted March values of leaf standing stock based on decay rates to day 193 (d^{-1}) were within the 95% CI of measured March values of leaf standing stock at four of the six streams (Table 2.8). The significant difference between measured and predicted leaf standing stock at R2 (KG) could be due to a greater input of leaves from upstream or greater retention. Leaf breakdown rates are based only on tulip-poplar leaves, whereas the leaf standing stock contains more than tulip-poplar leaves, but the similarities between measured and predicted standing stocks are striking.

Discussion

Comparison of leaf breakdown rates

Leaf breakdown rates have been classified along a continuum of processing from fast to slow (Petersen and Cummins 1974). In an early review of leaf breakdown rates for the Magnoliaceae family, of which tulip-poplar is a member, rates ranged from 0.015 to 0.005 (d^{-1}) (Webster and Benfield 1986), which places tulip-poplar in the medium to fast category along the continuum. Breakdown rates (d^{-1}) measured at the high property value streams fall within this range; however, rates at the remaining streams were faster (Figure 2.8). Rates measured in Peachtree City streams are somewhat similar to those

measured in subsequent studies using tulip-poplar leaves (Figure 2.8, Table 2.9). Breakdown rates in the two high property value streams are most similar to those measured in headwater streams in the Appalachian Mountains in Virginia (Rowe et al. 1996) and one second-order stream in Alabama (Weyers and Suberkropp 1996) (Figure 2.8, Table 2.9). However, when temperature is taken into account and leaf breakdown rates were expressed per degree day for both this study and the study in Virginia, the relationships were reversed: breakdown rates in Virginia streams were similar to breakdown rates at R1 (CA), R2 (KG), IN (CB), and LO (ON) and faster than breakdown rates at the two high property value streams (Rowe et al. 1996) (Figure 2.9). Leaf breakdown rates at LO (ON), IN (CB), R1 (CA), and R2 (KG) are faster than the range of breakdown rates for the Magnoliaceae family, but similar to those measured at both a first- and fourth-order stream in North Carolina (Paul and Meyer 1996), and two second-order streams in Alabama (Weyers and Suberkropp 1996) (Figure 2.8, Table 2.9).

The studies being compared in Figure 2.8 were all conducted in forested streams that are less likely to have been impacted by stormflow generated from impervious surfaces, large amounts of sediment, or increased pesticide concentrations. Therefore, differences in leaf breakdown rates between other studies and this study may be related to these factors (Benfield et al. 1991, Paul and Meyer 1996, Rowe et al. 1996, 1995, Weyers and Suberkropp 1996, Suberkropp 1995).

Breakdown rates measured at R1 (CA), R2 (KG), IN (CB), and LO (ON) were faster than the range found for the Magnoliaceae family by Webster and Benfield (1986) and faster breakdown could produce changes in the food web. Leaves are being exported quickly due to physical fragmentation rather than microbial conditioning and softening of

the leaves (as evidenced by low ergosterol concentrations and the lack of a relationship between ergosterol concentrations and leaf breakdown rates). When leaves are exported quickly, there is less time available for microbial colonization, and consequently, the food resource is of a lower quality for secondary consumers (Arsuffi and Suberkropp 1984, Suberkropp and Arsuffi 1984). This lower quality organic matter could force leaf-consuming invertebrates to have to consume more food in order to get the same nutrients. In addition, changes to leaf breakdown rates could result in a larger food resource of fine particulate organic matter for collector-gathering invertebrates, but a smaller resource of coarse particulate organic matter available for leaf-shredding organisms, and so overall invertebrate community dynamics could be affected.

Factors influencing leaf breakdown rate

After leaves enter streams, they are broken down by leaching and physical fragmentation, microbial conditioning, and invertebrate feeding (Petersen and Cummins 1974, Boulton and Boon 1991). This integration of physical, chemical, and biological variables make leaf breakdown a useful overall indicator of ecosystem function; however, the complex relationships in Figure 2.1 also make it hard to discern which variables are the driving factors in any one stream. Deciphering these relationships becomes increasingly difficult in disturbed watersheds where there can be contaminants along with significant changes to the hydrology and geomorphology of the stream. Among the factors inhibiting leaf breakdown rates are pesticides (by the reduction of macroinvertebrate and fungal biomass) and burial by sediment (by oxygen depletion and protection from current), while the stimulatory factors include increased temperature, nutrients, and stream velocity. These physical and chemical factors have indirect effects

on leaf breakdown by causing changes in invertebrate and fungal assemblages on leaves (Figure 2.1). Sediments and stream velocity have direct effects on leaf breakdown rates through physical fragmentation, abrasion, and burial by sediments.

Stream velocity

Differences in leaf breakdown rates among Peachtree City streams can be most attributed to differences in velocity in Peachtree City streams (Figure 2.5). R1 (CA) and R2 (KG) are larger streams with significantly faster velocities than most of the suburban streams and the fastest leaf breakdown rates were measured here. Higher flows in the reference streams as compared to the suburban streams are a result of the greater drainage areas; however, of the four suburban streams, the fastest velocities and fastest breakdown rates were seen at the streams with the highest percent of impervious surfaces, rather than at the streams with the greatest drainage areas. Therefore, impervious surfaces could be linked to the higher, flashier flows and could be contributing to the faster leaf breakdown.

In developed watersheds, runoff from impervious surfaces results in changes to the hydrology and geomorphology of streams, including increases in bankfull discharge, flashy flows, and channel incision, enlargement and widening (see review of Paul and Meyer 2001). These higher flows generated from impervious surfaces are forced to stay within the stream channel, rather than spread out onto the floodplain, which would release the erosive energy in storm flows. Hydrologic and geomorphologic changes result in ecological changes as well, since stream biota are subject to flashier flows, lower base flows, and higher water temperatures. Velocity is confounding the relationships we see between breakdown rates and nutrient and fungicide concentrations. Biological

variables impacting leaf breakdown rates are being overridden by physical variables such as velocity and sediments, to affect ecosystem processes.

Physical abrasion is one of the major contributors to leaf breakdown (Webster and Benfield 1986) and urban stormflows have been shown to increase leaf fragmentation, thus increasing leaf breakdown rates (D'Angelo and Webster 1992). The lower property value watershed has the largest amount of impervious surface cover in its watershed and an incised channel. Channel incision is a common response of streams to urbanization when the streams bed consists of erodible substrates (Trimble 1997, Pizzuto et al. 2000). In addition to having flashy flows, this stream also has the second highest insect shredder biomass of the six streams (Table 2.6); both of these factors are contributing to the fast leaf breakdown rates that we see in this small second-order stream. In forested second-order streams in Virginia and Alabama, breakdown rates are much slower (Figure 2.8). Faster leaf breakdown rates in several urban streams when compared with forested streams were attributed to physical factors, namely increased stormflows, since invertebrate feeding and fungal biomass in leaf packs were low or not significantly different from forested streams (Paul 1999). Similar physical effects on leaf breakdown rates were seen in the streams in this study.

Sediments

In sandy streams, leaves have a tendency to get buried. In the six streams sampled, 6-22% of leaf packs were recorded as being buried on the day they were sampled. Sediment accumulation in leaf packs in the less developed reference streams was greater than accumulation in packs at forested streams in a study conducted in Atlanta, GA (Paul 1999). Sediment associated with leaf packs in the Peachtree City

suburban streams was similar to sediment accumulation in leaf packs in suburban and urban streams in the Atlanta study (Paul 1999).

Sediments play both a direct role (i.e. burial and protection from current) and an indirect role by altering the environment where insects are feeding and microbial conditioning is occurring (Herbst 1980, Rounick and Winterbourn 1983, Webster and Benfield 1986, Mayack et al. 1989, Tillman et al. 2003) (Figure 2.1). It does not appear that sediments are the reason for the differences observed in leaf breakdown rates in Peachtree City streams, and they do not appear to affect fungal growth. Packs would become buried and subsequently resurfaced throughout the experiment, thus perhaps balancing the effects of burial on leaf breakdown and fungal biomass. Webster and Benfield (1986) concluded that decomposition generally occurs more slowly under anaerobic conditions as compared to aerobic conditions, but data from leaf breakdown studies do not always support this. Buried packs in a Michigan stream did not always have more mass left when compared with packs positioned on the surface (Tillman et al. 2003). Burial can inhibit insect feeding, and studies have shown that burial reduces invertebrate density and taxa richness (Mayack et al. 1989, Tillman et al. 2003). Slower biological processing and leaf breakdown rates of buried leaves has been found by several researchers (Herbst 1980, Rounick and Winterbourn 1983, Mayack et al. 1989) who attributed it to leaves being less palatable to leaf-shredding insects due to decreased microbial conditioning.

Pesticides

Pesticides can indirectly affect leaf breakdown rates by reducing insect and fungal populations (Suberkropp and Wallace 1992). A methoxychlor insecticide addition to a

forested stream at Coweeta Hydrologic laboratory in North Carolina resulted in slower leaf breakdown rates, shifts in the community structure of insect shredder taxa, and a decrease in mean shredder biomass per leaf pack in the treatment stream (Cuffney et al. 1984, Chung et al. 1993). Examination of the fungal spores in transport after an insecticide addition showed that fungi sporulated at a higher frequency in the treatment stream over an annual cycle, when compared to two reference streams, but the fungal species composition was similar in all three streams (Suberkropp and Wallace 1992). Fungicide concentrations were extremely low in the six Peachtree City streams and did not account for differences in leaf breakdown. However, in a laboratory microcosm experiment (Chapter 3), fungicide effects on leaf breakdown and fungal biomass were observed. Leaf breakdown rates per degree day were slower in the Peachtree City streams as compared to breakdown rates per day in the laboratory experiment in Chapter 3.

Fungicide concentrations in streams were low toxicologically speaking, and lower than concentrations tested in the laboratory microcosm in Chapter 3. The high frequency of occurrence of 4-hydroxy-chlorothalonil and chlorothalonil in all six streams appeared to be correlated to the size of the drainage area, rather than by socioeconomic class. Chlorothalonil concentrations ranged from 0.03 to 2 $\mu g\ l^{-1}$ and 4-hydroxy-chlorothalonil concentrations ranged from 1 to 450 $\mu g\ l^{-1}$ in the laboratory experiment. The highest concentrations of chlorothalonil and 4-hydroxy-chlorothalonil in the Peachtree City streams were 0.013 $\mu g\ l^{-1}$ and 0.026 $\mu g\ l^{-1}$ respectively.

Warmer temperatures accelerate invertebrate life history and metabolism (Sweeney 1984), as well as microbial processes (Chauvet and Suberkropp 1998), which in turn increase leaf breakdown rates (Webster and Benfield 1986). Generally, fungi sporulate faster at higher temperatures up to 15 to 20°C, but it depends on the particular fungal species (Chauvet and Suberkropp 1998). Temperature varied significantly among Peachtree City streams with the lowest mean temperature and number of degree days occurring at the largest, least developed streams (R1 and R2). The highest mean temperature occurred at the intermediate property value stream, IN (CB), which is the largest suburban stream, and drains a residential development with 10% impervious surfaces. Temperature can be related to impervious surface cover where watersheds with a higher percent of impervious surfaces have warmer stream temperatures than streams with a greater percentage of groundwater inputs, but this is not always the case, as evidenced in this study (and see review of Paul and Meyer 2001). When temperature was taken into account and breakdown rates were expressed per degree day, larger differences in rates were apparent (as compared to rates expressed per day). The ability to account for temperature in breakdown rate calculations was useful because other factors influencing breakdown could be better isolated.

Nutrients

Nutrients also act as an indirect stimulator of leaf breakdown by accelerating microbial processes (Figure 2.1). Fungi acquire nutrients from both the water column and the leaves and when nutrients are added to streams, leaf breakdown rates increase (Elwood et al. 1981, Meyer and Johnson 1983, Suberkropp 1995, Chauvet and Suberkropp 1998). Nutrients were low in these streams and there were only slight

differences in nitrate-N and total DIN among streams. Nitrate-N was higher in Peachtree City streams than in forested streams in NC and a stream in Alabama, but ammonium-N and SRP concentrations were comparable to forested streams being compared in Table 2.10. Numerous studies of urban and suburban streams have reported elevated nutrient concentrations, but concentrations in Peachtree City streams are very low (Paul and Meyer 2001, USGS 1999). Homeowners are applying fertilizers to lawns, which is a potential significant nutrient source to streams. One study of two urban residential and commercial drainage basins in Wisconsin found that lawns and streets were the largest sources of total and dissolved phosphorus, when compared to impervious surfaces such as roads, roofs, and driveways (Waschbusch et al. 1999). Perhaps low nutrient concentrations are a result of season since the experiment took place mostly in the fall and winter before most lawn care products are applied; higher nutrient concentrations may be seen in the late spring and summer.

The interpretation of how nutrients affected leaf breakdown rates was confounded by a correlation among factors. For example, velocity was correlated with ammonium-N concentrations, making it difficult to see the relationships between these concentrations and leaf breakdown rates.

Fungi and Insects

Ergosterol concentrations were 20 to 50% of concentrations measured in other studies of fungi on tulip-poplar leaves in forested streams (Table 2.10). There was slower fungal growth in the Peachtree City streams, and peak concentration might not yet have been reached at four of the streams when the last samples were taken (Figure 2.3). In addition, rates of ergosterol mass accumulation were lower in this field experiment than

in the laboratory experiment (Chapter 3). In most studies, fungal colonization occurs rapidly after leaves fall into streams, and peak colonization takes place within the first month or so (Table 2.10). Fungal colonization was delayed in the streams in this study, and started sometime after day 15 with peak concentrations reached after day 77. The significant negative correlation between insect shredder abundance and fungal biomass accumulation suggest that the insects are eating the fungi (Figure 2.4).

Measures of insect shredder biomass and abundance in leaf packs were low in all Peachtree City streams when compared to shredders in pine and maple leaf packs in forested streams in North Carolina (Whiles and Wallace 1997) and red maple leaf packs in urban and suburban Maine streams (Huryn et al. 2002). Due to high rates of leaf pack burial in this study, this is not surprising since other researchers found lower insect abundance and diversity in buried packs versus surface packs (Mayack et al. 1989, Tillman et al. 2003). However, shredder abundance is slightly higher than in urban and suburban streams in Atlanta, GA, on chalk maple leaves where almost no shredder taxa were found in leaf packs (Paul 1999). Insect family richness in Peachtree City streams was greater than insect genus richness in urban and suburban streams in Atlanta (Paul 1999). The substrate at H1 (SR) was covered with a layer of sediment, making it poor habitat for aquatic insects. H2 (SB) and R2 (KG) had greater amounts of large substrate, but measures of the insect community did not reflect this increased habitat availability at these two streams. Abundances of shredding insects and crayfish (also a leaf shredder) did not account for differences in leaf breakdown rates.

Relationships between nutrient and fungicide concentrations and leaf breakdown rates were confounded by relationships with stream velocity. This made it difficult to link nutrient and fungicide concentrations directly to differences in leaf breakdown rates. Therefore, a connection between human lawn care practices and fungal biomass and leaf breakdown was unclear.

Biological decay of leaves leads to a softening of the leaves, making them more susceptible to physical fragmentation (Petersen and Cummins 1974). Increased nutrients have a stimulatory effect on biological agents of leaf breakdown, namely macroinvertebrates and fungi, which leads to faster breakdown rates (Meyer and Johnson 1983, Elwood et al. 1981, Suberkropp 1995, Chauvet and Suberkropp 1998). During the second half of this study, stream velocity appeared to be driving leaf breakdown and the results show that velocity explained the differences in leaf breakdown rates more so than the biological or chemical factors.

While leaf breakdown rates are a good integrator of physical, chemical, and biological processes, it can be difficult to decipher which factor is having the greatest influence on differences in leaf breakdown rates among streams. In suburban watersheds where numerous physical disturbances are occurring, we see changes in stream hydrology and geomorphology, which in turn can alter the ecology of streams. Ecological effects such as changes in food webs can disrupt stream ecosystem function and lead to overall stream impairment. Steps to restore the hydrology and geomorphology of streams are needed so that ecological rehabilitation can begin.

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Tables and Figures

Figure 2.1. Physical, chemical, and biological factors that inhibit or stimulate leaf breakdown rates. Gray boxes represent the factors stimulating leaf breakdown and white boxes represent inhibiting factors. Solid arrows denote direct effects, and dashed arrows denote indirect effects.

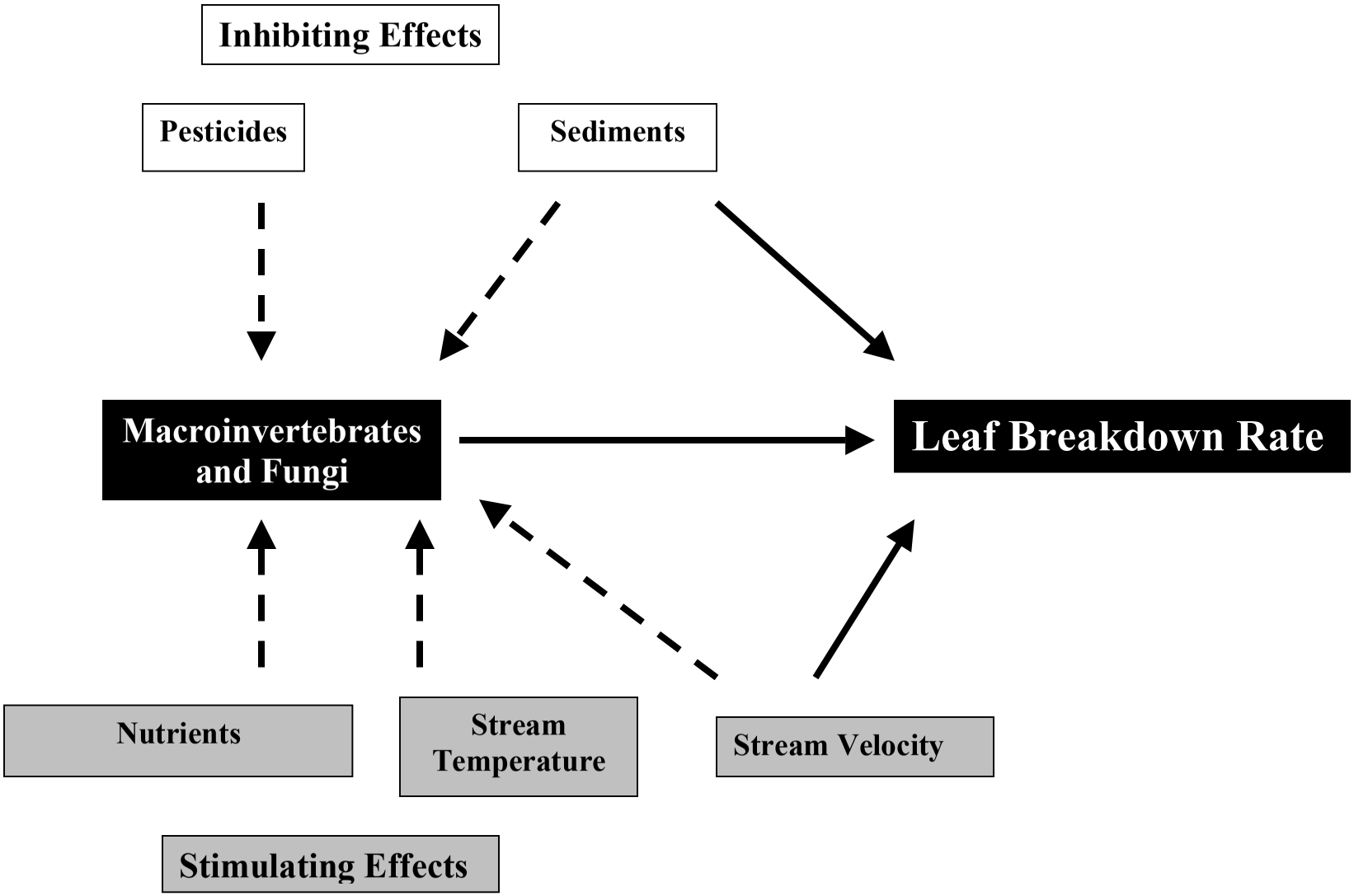
Inhibiting Effects
Pesticides
Sediments
Macroinvertebrates and Fungi
Leaf Breakdown Rate
Nutrients
Stream Temperature
Stream Velocity
Stimulating Effects

Figure 2.2. Site map of six study streams: four suburban watersheds and one reference watershed located in Fayette County, and one reference watershed located in Coweta County, Georgia. The Peachtree City boundary is in orange, suburban watersheds are outlined in red and reference watersheds are outlined in green.

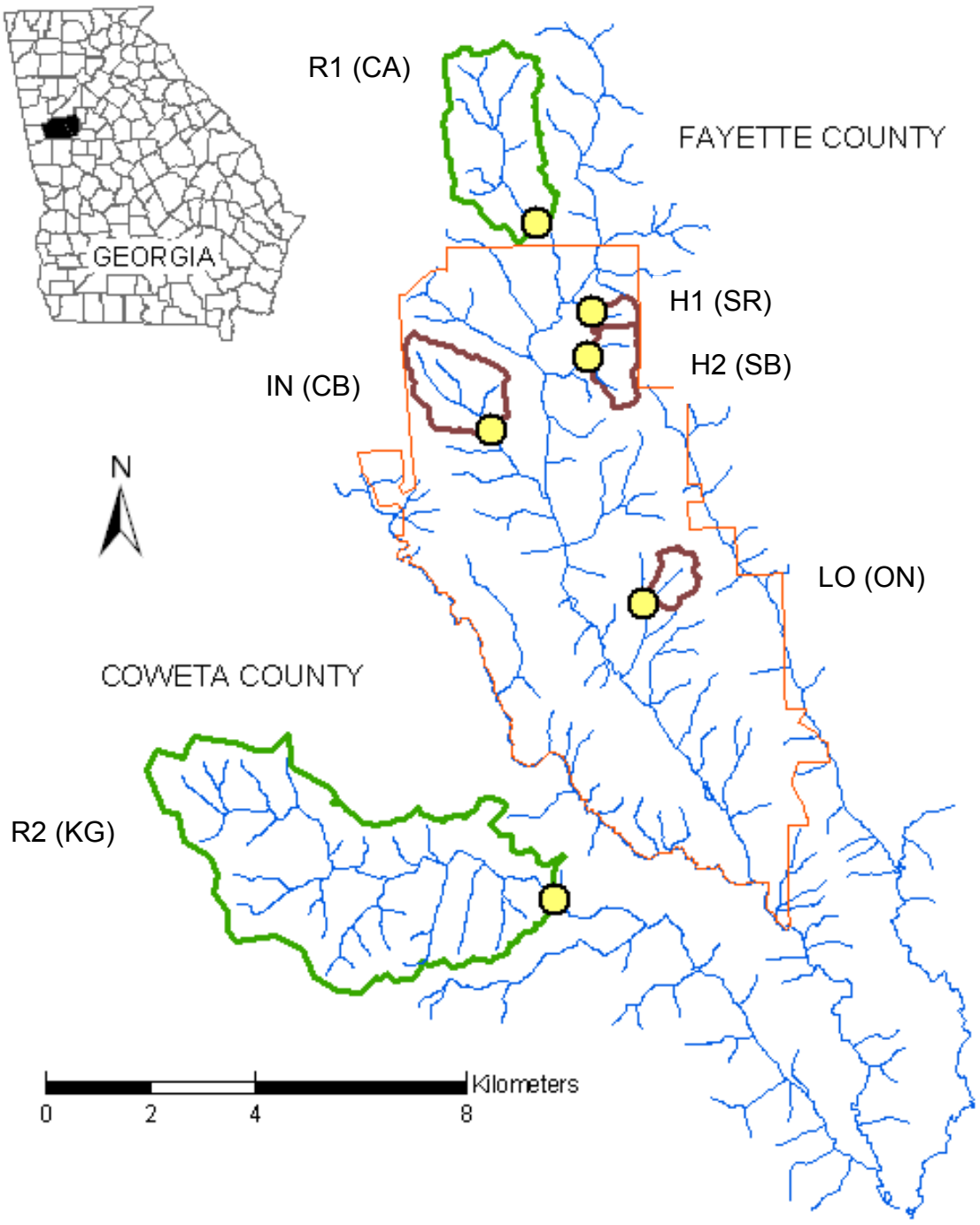
GEORGIA
R1 (CA)
FAYETTE COUNTY
H1 (SR)
H2 (SB)
IN (CB)
N
LO (ON)
COWETA COUNTY
R2 (KG)
Kilometers
0
2
4
8

Table 2.1 a and b. Watershed (a) and stream (b) characteristics during the study period for suburban and reference streams. Percent of tulip-poplar leaves is reported as mean (± 1 SE) (n=8). Property values include price of house and lot price. All property value data and lot sizes were obtained from the 1999 Fayette County tax digest, which was compiled in a GIS database. % ISC = impervious surface cover.

(a)

Stream	Watershed Area (km^2)	Stream Order	% ISC	Mean Property Value	# Residences	# Residences km^{-2}	Mean lot size (m^2)
H1 (SR)	0.39	1	7.1 [a]	$388,900	60	154	4,613
H2 (SB)	1.12	2	2.4 [a]	$326,200	71	63	5,463
IN (CB)	2.59	2	10.1 [a]	$187,700	739	285	1,861
LO (ON)	0.75	2	15.4 [a]	$136,900	266	355	2,388
R1 (CA)	5.75	3	3 [b]	-	-	-	-
R2 (KG)	21.28	4	2.4 [b]	-	-	-	-

[a] ISC determined using roads, golf cart paths, and house footprints
[b] ISC determined using roads and is most likely an underestimate

(b)

Stream	Σ degree days (> 0°C)	Mean depth [c] (cm)	Mean wetted width [d] (m)	% Tulip-poplar in leaf standing stock in Dec. 2001
H1 (SR)	2311	19	1.4	8 ± 2
H2 (SB)	2098	18	2.3	3 ± 1
IN (CB)	2630	14	2.6	7 ± 3
LO (ON)	2246	16	1.3	12 ± 3
R1 (CA)	2088	20	3.1	20 ± 4
R2 (KG)	2098	40	5.7	12 ± 3

[c] mean of measurements taken at location of leaf packs from November 2000 to May 2001
[d] mean of two dates: December 15, 2001 and March 29, 2002

Table 2.2 a and b. (a) Mean stream concentrations (± 1 SE) (µg l^{-1}) of nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), total dissolved inorganic nitrogen (DIN), and soluble reactive phosphorus (SRP) and mean stream velocity during the leaf pack experiment (cm s^{-1}). For nutrient samples, n=17 for IN (CB) and LO (ON) and n=18 for R1 (CA), R2 (KG), H1 (SR), and H2 (SB). Temperature is reported as mean daily values in degrees Celsius and velocity is reported as cm s^{-1}. Superscripts denote significant differences at $p<0.05$. Detection limits for NO_3-N, NH_4-N, and SRP were 13, 4, and 6 µg l^{-1} and nd= none detected.

(b) Mean concentrations of zinc (± 1 SE) are in µg l^{-1}. No significant differences were detected among streams or property value classes (Kruskal-Wallis $p>0.7$ and $p>0.5$ respectively).

(a)

Stream	NO_3-N	NH_4-N	DIN	SRP	Temp.	Velocity
H1 (SR)	177 ± 18 ^{ac}	nd	180 ± 18 ^{cd}	nd	12.1 ^{b}	4 ± 1 ^{c}
H2 (SB)	204 ± 22 ^{bc}	5 ± 2	210 ± 22 ^{ac}	nd	11.1 ^{bcd}	6 ± 2 ^{c}
IN (CB)	137 ± 28 ^{a}	12 ± 6	149 ± 32 ^{bd}	nd	13.8 ^{a}	8 ± 2 ^{b}
LO (ON)	266 ± 27 ^{b}	6 ± 3	273 ± 28 ^{a}	nd	11.8 ^{bc}	7 ± 1 ^{b}
R1 (CA)	222 ± 20 ^{bc}	16 ± 13	238 ± 20 ^{ac}	6	11.0 ^{d}	14 ± 3 ^{a}
R2 (KG)	187 ± 20 ^{ac}	24 ± 10	211 ± 27 ^{acd}	nd	11.0 ^{cd}	9 ± 2 ^{ab}
H1 and H2	191 ± 14	4 ± 1	195 ± 14	nd		
IN and LO	202 ± 22	9 ± 3	211 ± 24	nd		
R1 and R2	204 ± 14	20 ± 8	225 ± 17	nd		

(b)

Stream	Zinc
H1 (SR)	6.12 ± 0.23
H2 (SB)	6.44 ± 0.22
IN (CB)	6.08 ± 0.19
LO (ON)	6.03 ± 0.29
R1 (CA)	6.12 ± 0.21
R2 (KG)	6.17 ± 0.27
H1 and H2	6.28 ± 0.16
IN and LO	6.06 ± 0.17
R1 and R2	6.14 ± 0.16

Table 2.3 Mean fine (<250>1 μm) and coarse (>250 μm) sediments per leaf pack from days 22 to 77 for fine and days 22 to 105 for coarse sediments. Values are mean ± 1 SE per leaf pack. Percentage of leaf packs buried at each stream is from days 22 to 105. Superscripts denote significant differences at $p<0.05$.

Stream	Mean fine sediments (g pack^{-1})	Mean Coarse Sediments (g pack^{-1})	% leaf packs buried
H1 (SR)	0.0034 ± 0.0006^{c}	10.8 ± 4.6^{d}	9
H2 (SB)	0.0034 ± 0.0008^{c}	35.2 ± 16.8^{cd}	6
IN (CB)	0.0041 ± 0.0008^{bc}	84.8 ± 23.1^{ab}	12
LO (ON)	0.0066 ± 0.0010^{ab}	51.8 ± 16.3^{bc}	9
R1 (CA)	0.0022 ± 0.0004^{c}	131.4 ± 37.6^{a}	22
R2 (KG)	0.0104 ± 0.0018^{a}	32.8 ± 15.2^{cd}	10

Table 2.4 a and b. Mean fungicide concentrations (ng l^{-1}) (± 1 SE) for streams and property value classes during the leaf pack experiment from November 2000 to May 2001 based on 5-6 values (a) and mean annual concentrations from July 2000 to September 2001 based on 13-14 values (b). Concentrations that were detected below the limit of quantification, 0.1 ng l^{-1}, were included in calculations of means as 0.05 ng l^{-1} (APHA 1998).

(a)

Stream	Chlorothalonil	4-hydroxy-chlorothalonil	4-hydroxy-chlorothalonil + Chlorothalonil
H1 (SR)	0.72 ± 0.27	1.54 ± 0.75	2.25 ± 0.95
H2 (SB)	0.64 ± 0.32	3.87 ± 2.30	4.51 ± 2.61
IN (CB)	1.04 ± 0.69	3.04 ± 1.38	4.08 ± 1.90
LO (ON)	2.79 ± 2.65	2.29 ± 1.26	5.08 ± 3.71
R1 (CA)	0.36 ± 0.25	7.39 ± 3.99	7.75 ± 4.18
R2 (KG)	0.17 ± 0.30	8.10 ± 4.38	8.56 ± 4.41
H1 and H2	0.68 ± 0.20	2.70 ± 1.20	3.38 ± 1.37
IN and LO	1.84 ± 1.22	2.70 ± 0.91	4.54 ± 1.88
R1 and R2	0.42 ± 0.19	7.78 ± 2.85	8.20 ± 2.91

(b)

Stream	Chlorothalonil	4-hydroxy-chlorothalonil	4-hydroxy-chlorothalonil + Chlorothalonil
H1 (SR)	0.51 ± 0.17	1.99 ± 0.63	2.50 ± 0.76
H2 (SB)	0.38 ± 0.15	4.61 ± 1.25	4.99 ± 1.34
IN (CB)	0.79 ± 0.40	2.95 ± 0.96	3.75 ± 1.30
LO (ON)	1.28 ± 1.02	1.95 ± 0.62	3.23 ± 1.49
R1 (CA)	0.31 ± 0.13	4.60 ± 1.77	4.91 ± 1.85
R2 (KG)	0.31 ± 0.14	4.66 ± 2.01	4.96 ± 2.05
H1 and H2	0.44 ± 0.11	3.30 ± 0.73	3.74 ± 0.79
IN and LO	1.03 ± 0.52	2.47 ± 0.58	3.50 ± 0.97
R1 and R2	0.31 ± 0.09	4.63 ± 1.32	4.94 ± 1.36

Figure 2.3. Mean ergosterol concentrations on each sampling date measured in tulip-poplar leaf packs from day 3 to 105 (n=3 for each date and each stream). Error bars are ± 1 SE. No significant differences in ergosterol concentrations among streams were detected (Kruskal-Wallis $p>0.8$).

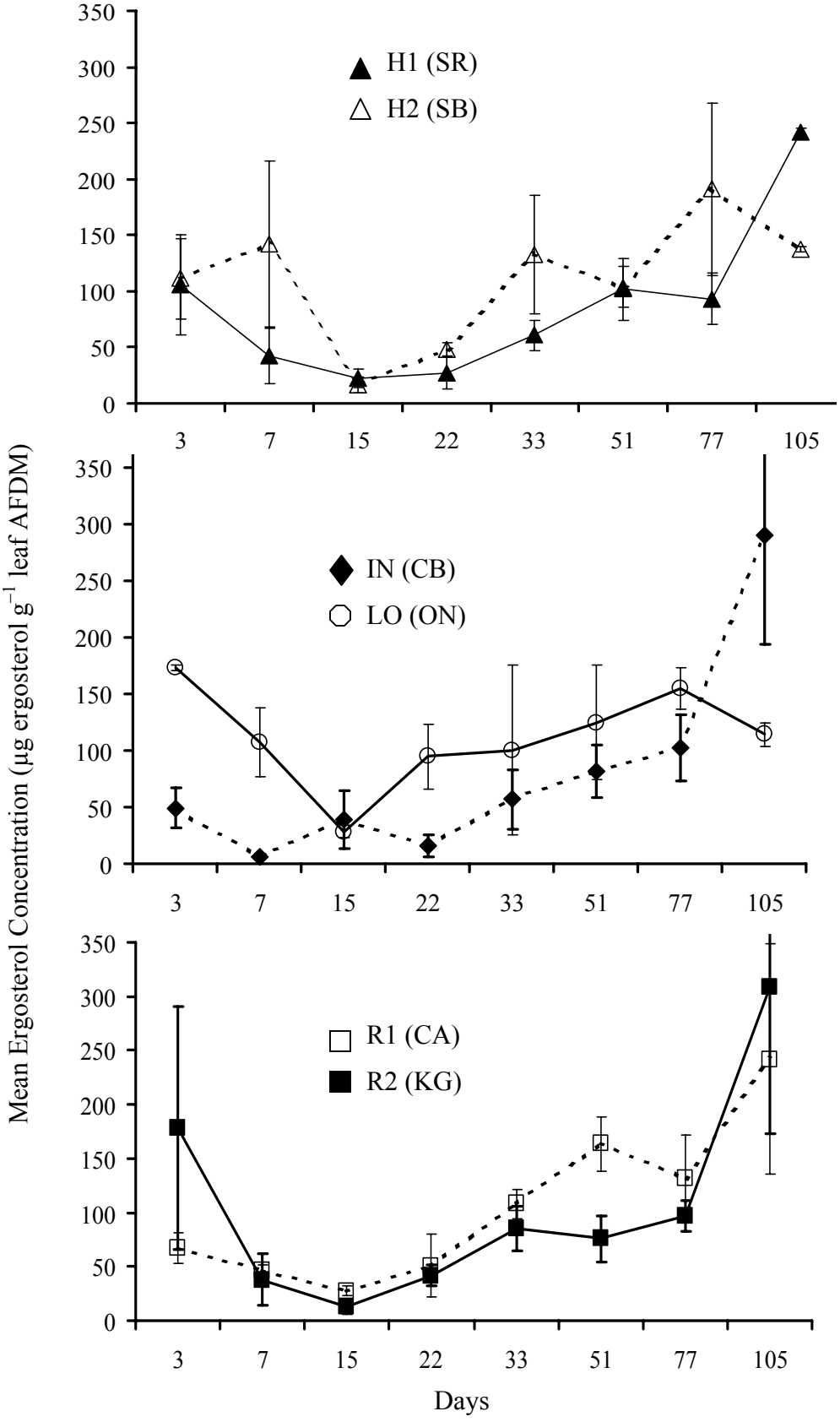
H1 (SR)
H2 (SB)
IN (CB)
LO (ON)
R1 (CA)
R2 (KG)
Mean Ergosterol Concentration (µg ergosterol g^{-1} leaf AFDM)
Days
0
50
100
150
200
250
300
350
3
7
15
22
33
51
77
105

Table 2.5. Rate of ergosterol mass accumulation (μg g leaf $^{-1}$ d^{-1}) from day 15 to 105 in suburban and reference streams and property value classes. Significant accumulation of ergosterol mass occurred at four of the six streams and at all property value classes.

Stream	Rate of ergosterol mass accumulation	r^2	p
H1 (SR)	0.024	0.88	0.006
H2 (SB)	0.019	0.52	0.105
IN (CB)	0.026	0.83	0.012
LO (ON)	0.011	0.39	0.186
R1 (CA)	0.020	0.71	0.030
R2 (KG)	0.027	0.79	0.020
H1 and H2	0.021	0.68	0.001
IN and LO	0.018	0.58	0.004
R1 and R2	0.023	0.72	0.001

Table 2.6. Insect shredder biomass (mg DM pack^{-1}) and abundance (# individuals pack^{-1}) in leaf packs at each stream. Mean insect family richness (# families pack^{-1}) and mean crayfish abundance (# individuals pack^{-1}) in leaf packs was also determined. Data are reported as mean ± 1 SE based on leaf packs collected on days 15, 33, and 51.

Stream	Mean shredder biomass	Mean shredder abundance	Insect family richness	Mean crayfish abundance
H1 (SR)	0.1 ± 0.03^{b}	0.2 ± 0.2^{a}	1.3 ± 0.3	0.22 ± 0.15
H2 (SB)	0.8 ± 0.4^{a}	3.7 ± 1.3^{bd}	3.4 ± 0.3	0.11 ± 0.11
IN (CB)	1.3 ± 1.3^{bc}	0.2 ± 0.2^{ac}	1.4 ± 0.3	0.44 ± 0.24
LO (ON)	6.6 ± 4.0^{a}	4.3 ± 1.6^{bd}	2.6 ± 0.4	0.22 ± 0.15
R1 (CA)	8.8 ± 5.4^{ac}	3.3 ± 2.5^{cd}	4.1 ± 0.7	0.33 ± 0.24
R2 (KG)	0.7 ± 0.3^{abc}	0.9 ± 0.4^{ac}	3.0 ± 0.7	0.22 ± 0.15

Figure 2.4. Regression of rate of ergosterol mass accumulation ($\mu g\ g^{-1}\ d^{-1}$) versus insect shredder abundance (# individuals $pack^{-1}$) ($y = -279.1x + 8.01$) ($r^2=0.78$, $p=0.02$).

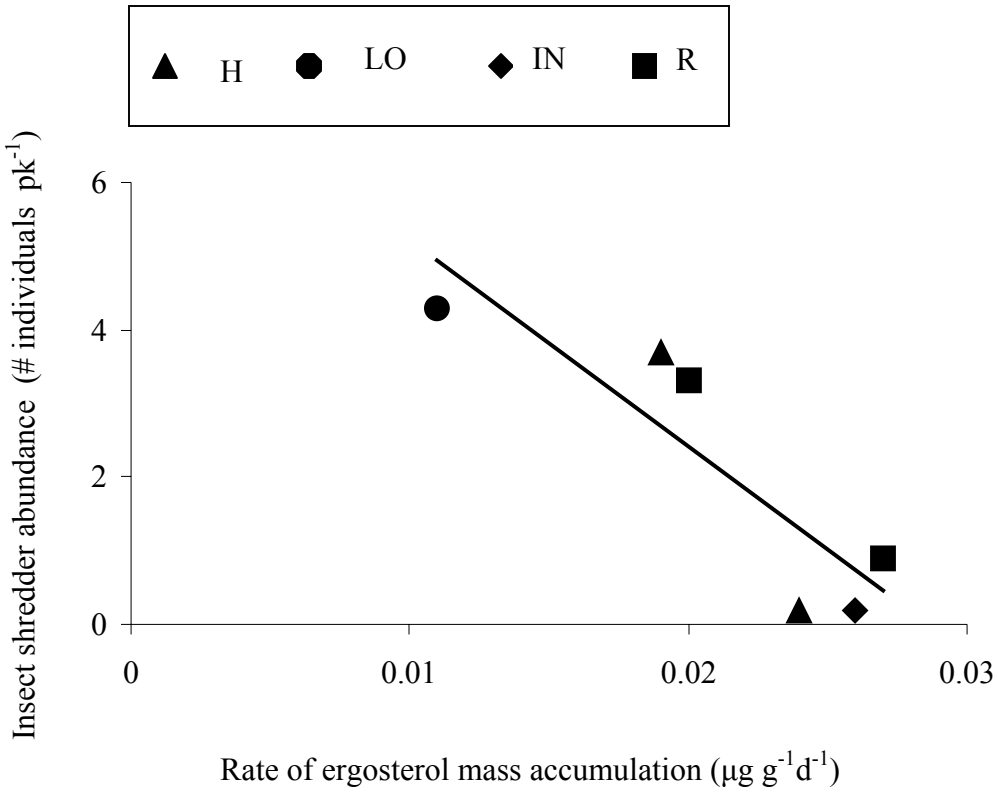
H
LO
IN
R
Insect shredder abundance (# individuals pk-1)
6
4
2
0
0
0.01
0.02
0.03
Rate of ergosterol mass accumulation (µg g-1d-1)

Table 2.7. Leaf breakdown rates (k) per degree day ± 95% confidence intervals for each stream and property value class after 193 days; different superscripts indicate 95% confidence intervals did not overlap.

Stream	k (dd^{-1})	r^2	p
H1 (SR)	0.0009 ± 0.0001^{d}	0.98	0.0001
H2 (SB)	0.0006 ± 0.0006^{cd}	0.41	0.0348
IN (CB)	0.0014 ± 0.0002^{bc}	0.97	0.0001
LO (ON)	0.0017 ± 0.0002^{ab}	0.98	0.0001
R1 (CA)	0.0024 ± 0.0007^{a}	0.90	0.0001
R2 (KG)	0.0024 ± 0.0007^{a}	0.88	0.0001
H1 and H2	0.0008 ± 0.0003^{c}	0.68	0.0001
IN and LO	0.0015 ± 0.0002^{b}	0.96	0.0001
R1 and R2	0.0024 ± 0.0004^{a}	0.89	0.0001

Figure 2.5. Regression of mean velocity (cm s^{-1}) during the leaf pack experiment versus leaf breakdown rates (dd^{-1}) (y= 0.0002x + 0.0001) (r^2=0.65, p=0.05).

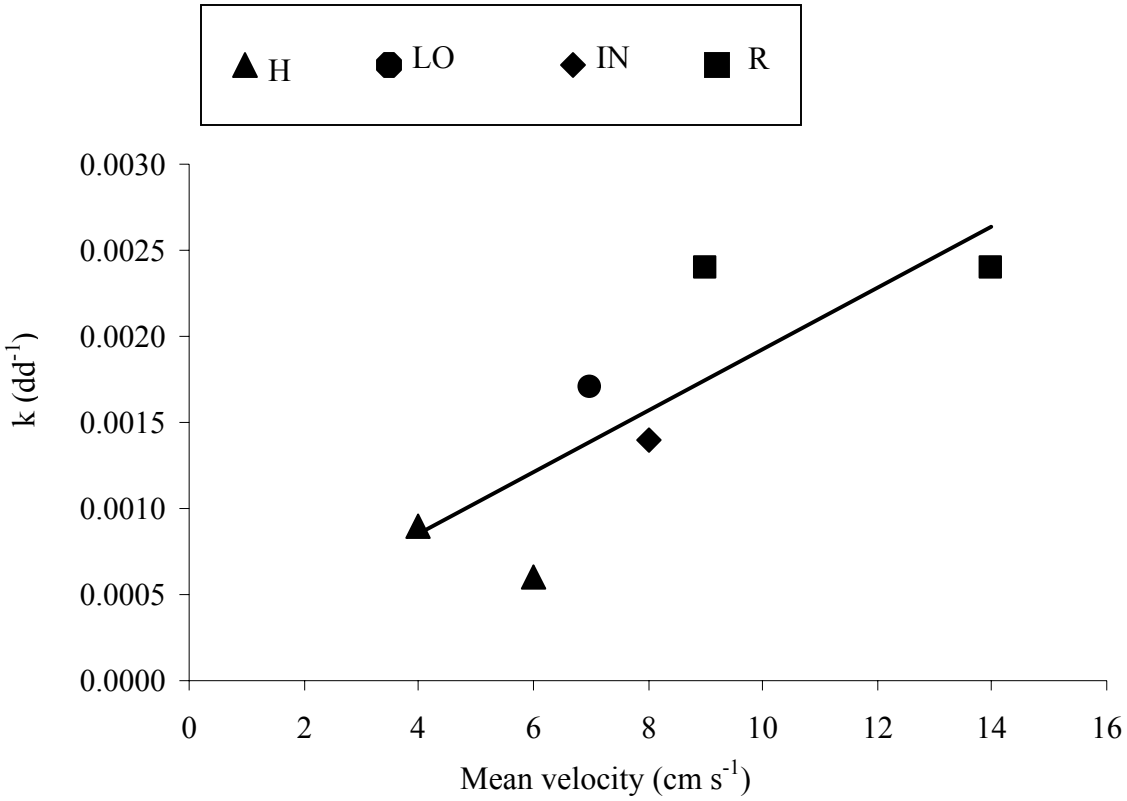
H
LO
IN
R
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
k (dd-1)
0
2
4
6
8
10
12
14
16
Mean velocity (cm s-1)

Figure 2.6. Precipitation during the leaf pack experiment. The dashed line indicates a division between the first half of the leaf pack experiment and the second half of the experiment. Boxes on either side of the dashed line indicate the rate of precipitation during that period. Note the significant amount of precipitation after February. Data courtesy of the Southeast Regional Climate Center (SERCC 2002) for the Atlanta airport, which is in close proximity to the six streams in this study.

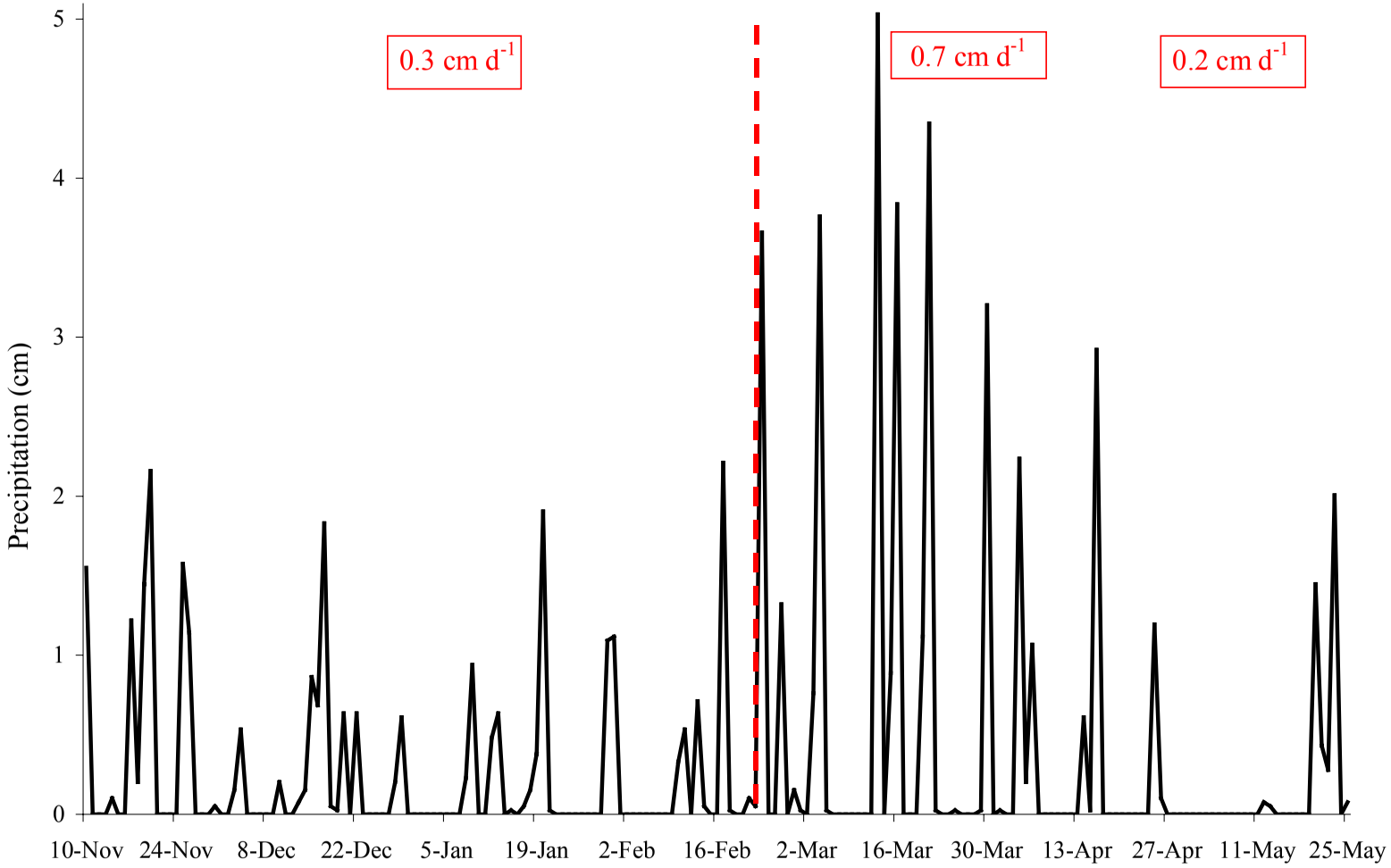
0.3 cm d-1
0.7 cm d-1
0.2 cm d-1
Precipitation (cm)
0
1
2
3
4
5
10-Nov
24-Nov
8-Dec
22-Dec
5-Jan
19-Jan
2-Feb
16-Feb
2-Mar
16-Mar
30-Mar
13-Apr
27-Apr
11-May
25-May

Figure 2.7. Mean leaf standing stock (g AFDM m^{-1} stream length) in December 2001 (solid bars) and March 2002 (open bars). Values are expressed as means ± standard error bars.

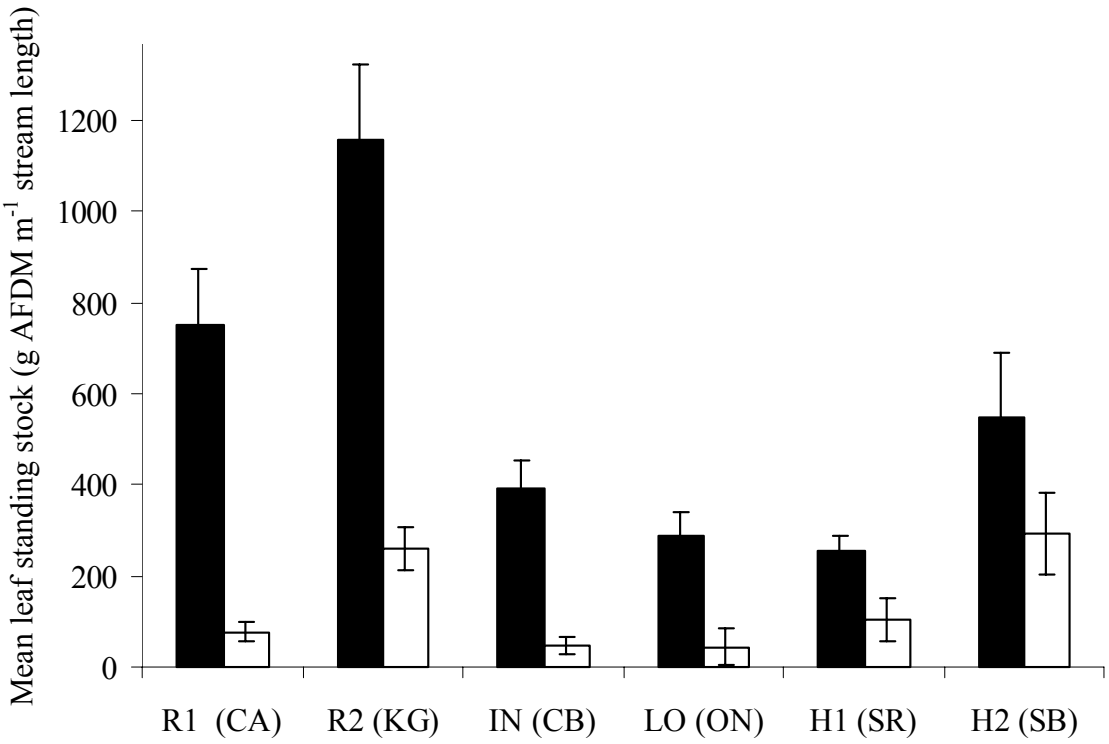
Mean leaf standing stock (g AFDM m-1 stream length)
0
200
400
600
800
1000
1200
R1 (CA)
R2 (KG)
IN (CB)
LO (ON)
H1 (SR)
H2 (SB)

Table 2.8. Percent of leaf standing stock lost from December 2001 to March 2002. Mean measured leaf standing stock (g AFDM m^{-1} stream length ± 95% CI) and predicted mean leaf standing stock (g AFDM m^{-1} stream length) based on leaf breakdown rates (k) to day 193 (d^{-1}).

Stream	% leaf mass lost	Measured mean leaf standing stock in March	k (d^{-1})	Predicted mean leaf standing stock in March
H1 (SR)	59*	104.4 ± 94.6	0.0104	85.8**
H2 (SB)	47	292.0 ± 163.1	0.0071	262.0**
IN (CB)	88*	45.4 ± 11.3	0.0180	60.5
LO (ON)	85	44.4 ± 38.0	0.0185	42.3**
R1 (CA)	90*	77.9 ± 38.6	0.0241	61.8**
R2 (KG)	78*	259.6 ± 89.2	0.0242	93.3

* % loss from these streams is underestimated because pine needles were excluded from some samples during processing of the December samples
** predicted leaf standing stock was within 95% CI of measured mean leaf standing stock

Figure 2.8. A comparison of breakdown rates of tulip-poplar leaves (day^{-1}) in studies conducted in Alabama, Georgia, Virginia, and North Carolina. The dashed box is highlighting a range of breakdown rates from 0.005 to 0.015 (day^{-1}) for the Magnoliaceae family reported by Webster and Benfield (1986). The solid bars are leaf breakdown rates from studies in forested streams reported by Weyers & Suberkropp (1996), Rowe et al. (1996), Paul and Meyer (1996), Benfield (1991), and Suberkropp (1995); the open bars are from this study. Labels on the x-axis indicate the location of the study (state) and stream order. Data are adapted from Abelho (2001).

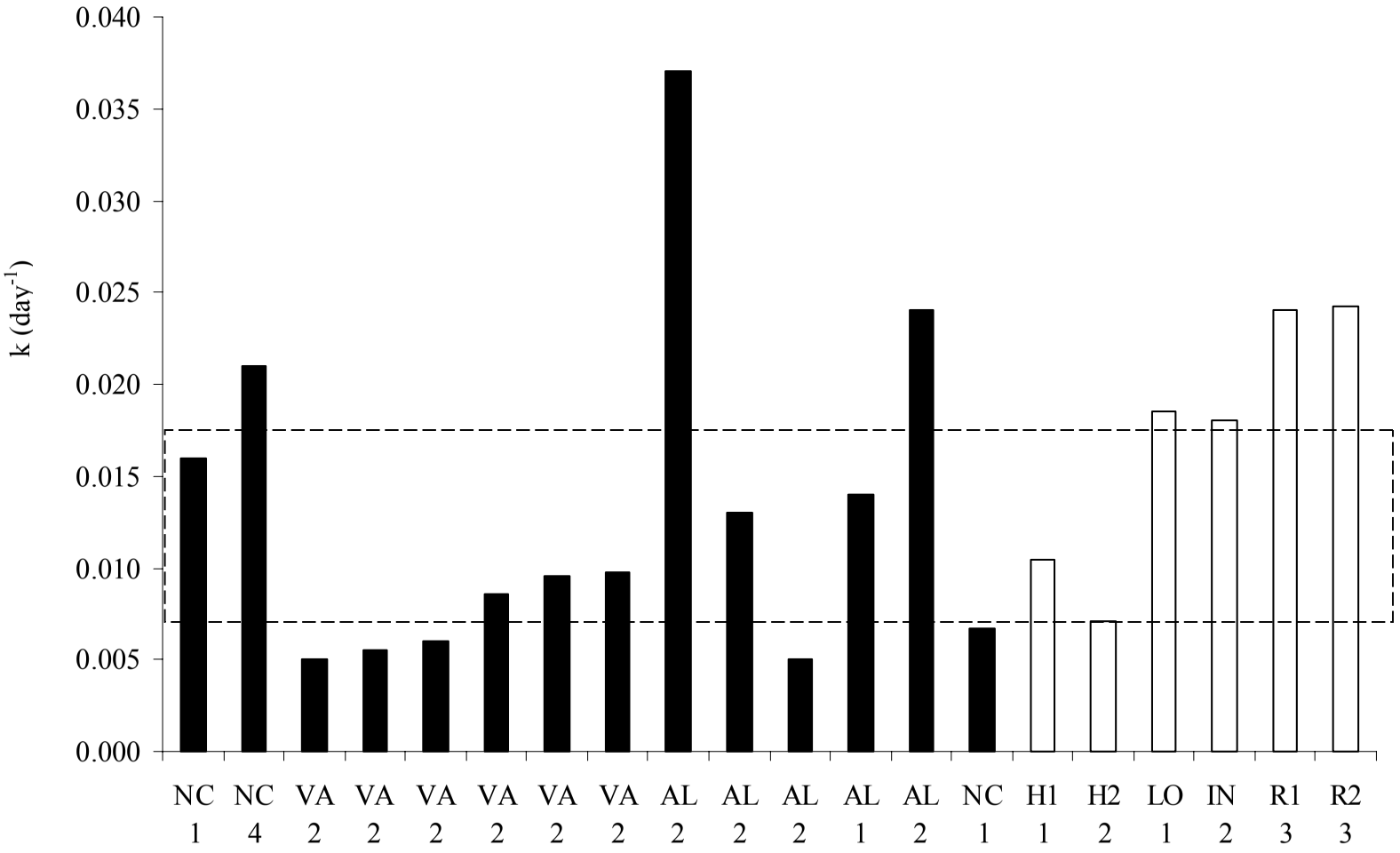
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
k (day-1)
NC 1
NC 4
VA 2
VA 2
VA 2
VA 2
VA 2
VA 2
AL 2
AL 2
AL 2
AL 1
AL 2
NC 1
H1 1
H2 2
LO 1
IN 2
R1 3
R2 3

Figure 2.9. A comparison of breakdown rates of tulip-poplar leaves (degree day^{-1}) in forested Virginia streams and Peachtree City streams. The solid bars are leaf breakdown rates in forested streams in Virginia (Rowe et al. 1996); the open bars are from this study. Labels on the x-axis indicate the location of the study (state) and stream order.

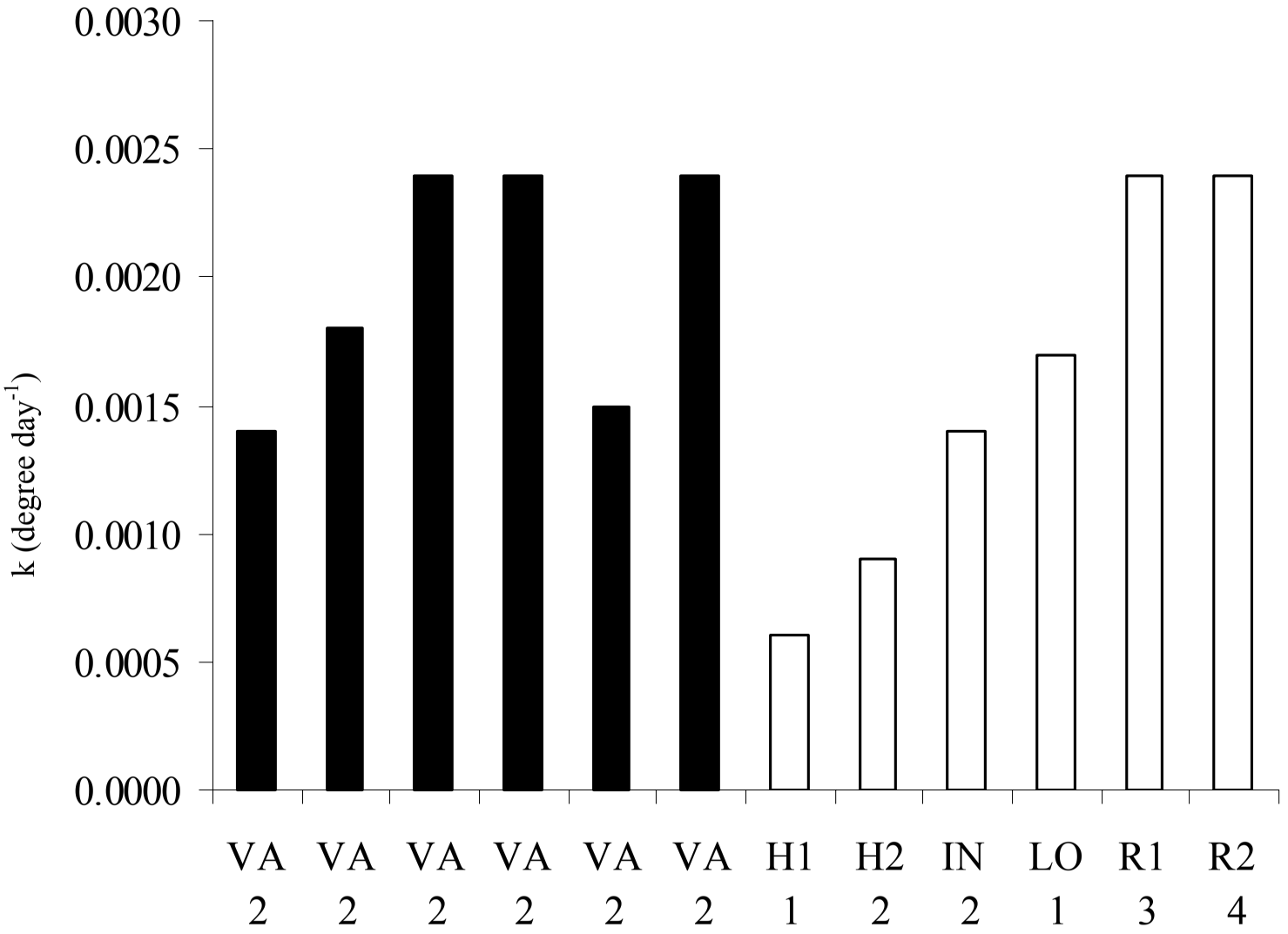
0.0030
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
k (degree day-1)
VA 2
VA 2
VA 2
VA 2
VA 2
VA 2
H1 1
H2 2
IN 2
LO 1
R1 3
R2 4

Table 2.9. A comparison of breakdown rates of tulip-poplar leaf packs (d^{-1}), the duration of the experiment, and mean temperatures in the streams. Location refers to state in the United States. Data are adapted from Abelho (2001).

Location and Stream order	Temp. (°C)	Duration (d)	k (d^{-1})	Reference
AL 2	12-17	90	0.037	Weyers & Suberkropp 1996
AL 2	9-21	90	0.013	Weyers & Suberkropp 1996
NC 1	0.5-15.8	125	0.016	Paul & Meyer 1996
NC 4	1.7-18.5	56	0.021	Paul & Meyer 1996
NC 1	-	215	0.0067	Benfield et al. 1991
AL 2	9.6(3-18)	80	0.005	Suberkropp 1995
AL 1	13.1(9-19)	80	0.014	Suberkropp 1995
AL 2	14.2(8-19)	80	0.024	Suberkropp 1995
VA 2	0-10	91-108	0.005	Rowe et al. 1996
VA 2	0-10	91-108	0.0055	Rowe et al. 1996
VA 2	0-9	91-108	0.006	Rowe et al. 1996
VA 2	0-9	91-108	0.0086	Rowe et al. 1996
VA 2	0-12	91-108	0.0096	Rowe et al. 1996
VA 2	0-12	91-108	0.0098	Rowe et al. 1996
GA H1 (SR) 1	12.1	193	0.0104	This study
GA H2 (SB) 2	11.1	193	0.0071	This study
GA IN (CB) 2	13.8	193	0.0180	This study
GA LO (ON) 2	11.8	193	0.0185	This study
GA R1 (CA) 3	11	193	0.0240	This study
GA R2 (KG) 4	11	193	0.0242	This study

Table 2.10. A comparison of maximum ergosterol concentrations on tulip-poplar leaves. The duration of the experiment and the day when maximum ergosterol concentrations were reached are also reported. Location refers to state in the United States. Data are adapted from Abelho (2001).

Location and Stream order	Mean Temp. (°C)	Duration (d)	Day of peaking	Max Ergos. Conc. (μg g^{-1} AFDM)	Reference
AL 1-2	15.5	20	11	420	Grattan & Suberkropp 2001
AL 1-2	11.9	32	32	500	Grattan & Suberkropp 2001
AL 1-2	15.5	28	15	830	Grattan & Suberkropp 2001
NC 1	0.5-15.8	21	3	600	Paul & Meyer 1996
NC 4	1.7-18.5	21	3	800	Paul & Meyer 1996
AL 2	3-18	80	35	500	Suberkropp 1995
AL 1	9-19	80	40	800	Suberkropp 1995
AL 2	8-19	80	15	1000	Suberkropp 1995
AL 1	9-21	90	56	400	Weyers & Suberkropp 1996
AL 2	12-17	90	15	800	Weyers & Suberkropp 1996
GA H1 (SR) 1	12.1	105	105	240	This study
GA H2 (SB) 2	11.1	105	77	190	This study
GA IN (CB) 2	13.8	105	105	290	This study
GA LO (ON) 2	11.8	105	77	150	This study
GA R1 (CA) 3	11	105	105	240	This study
GA R2 (KG) 4	11	105	105	300	This study

CHAPTER 3

THE EFFECTS OF FUNGICIDES ON AQUATIC FUNGI AND LEAF DECAY[1]

[1] Herbert, S., J.L. Meyer, and K.L. Armbrust. To be submitted to Environmental Toxicology and Chemistry Journal

Abstract

Pesticides are becoming more prevalent in suburban streams, where surface runoff from impervious surfaces is often routed directly to streams. Stream food webs could be altered by adverse effects of pesticides on non-target organisms, such as aquatic fungi, which play a crucial role in leaf breakdown. In this study, we tested whether the concentrations of three fungicides typically found in suburban streams had an effect on the biomass and sporulation rates of aquatic fungi and decay rates of tulip-poplar leaves (*Liriodendron tulipifera* L.). The fungicides flutolanil, chlorothalonil, and its degradation product 4-hydroxy-chlorothalonil (4OH-chlorothalonil), were investigated due to their prevalence in suburban streams in Georgia. Tulip-poplar leaves colonized by aquatic fungi were exposed to a range of fungicide concentrations in stream-simulating microcosms containing nutrients. Low fungicide concentrations were comparable to those measured in suburban streams, and high concentrations were comparable to those measured at a golf course. With increasing 4OH-chlorothalonil concentrations, three measures of fungal growth decreased. No effect of chlorothalonil or flutolanil on fungal biomass and sporulation rates was observed. Significant leaf decay occurred only in the control flasks and one of the flutolanil treatments. These low, but environmentally-realistic fungicide concentrations inhibited leaf decay, but only 4OH-chlorothalonil produced a detectable reduction in fungal biomass. Increased detection frequencies of fungicides and their degradation products in streams demonstrate the need for further research on the adverse effects of these compounds on non-target organisms and ecological processes.

INDEX WORDS: tulip-poplar, leaf decay, microcosm, fungal biomass, aquatic hyphomycetes, chlorothalonil, hydroxychlorothalonil, flutolanil, bacteria

Introduction

Background

Fungi and bacteria are biological agents of leaf decay in streams, and their productivity fuels the microbial food web (Kaushik and Hynes 1971), (Meyer 1994). Aquatic fungi perform a vital service in stream food webs by conditioning leaves, making them more palatable to secondary consumers (Arsuffi and Suberkropp 1984, Suberkropp and Arsuffi 1984). Leaf decay is faster with microbes present (Kaushik and Hynes 1971) and when nutrients are high (Meyer and Johnson 1983). Natural or anthropogenic alterations in the relative importance of these vital food resources have consequences at higher trophic levels (Vannote et al. 1980, Meyer 1994).

Pesticides are being detected in aquatic ecosystems as a result of runoff or contaminated groundwater inputs (USGS 1994). Sources of pesticides include residential lawns, golf courses, recreational fields, and agricultural areas. Pesticides have been detected in urban and suburban streams more frequently and at higher concentrations than those found in agricultural streams in the southeastern United States (USGS 1994). Most of the compounds detected in urban and suburban streams are associated with turf grass (USGS 1994). The United States Environmental Protection Agency (USEPA) reported that 80 million pounds of active ingredient were used in homes, lawns and gardens, accounting for 9% of total usage of pesticides in 1999 (Donaldson et al. 2002). Adverse

effects of pesticides on non-target organisms in aquatic ecosystems require the attention of researchers and water quality managers alike.

Most pesticide research in aquatic ecosystems has focused on agricultural streams; however, pesticides used in agricultural areas are not always the same as those used in urban areas (Gilliom 2001). Pesticides have a direct effect on the growth, survival, or reproduction of sensitive species as well as having secondary effects, which are the changes in ecosystem processes that occur as a result of the direct effects (Hurlbert 1975). The influence of pesticides on ecosystem processes, such as detritus-processing and nutrient cycling, may exceed that of direct effects on growth, survival, or reproduction of individual species (Wallace 1989) and need to be further investigated.

Fungicides: Mode of Action and Toxicity

Three fungicides were investigated in this study: chlorothalonil, its main degradation product, 4-hydroxy-chlorothalonil (4OH-chlorothalonil), and flutolanil. For purposes of this paper, all three will be referred to as fungicides, even though 4OH-chlorothalonil is a metabolite of chlorothalonil. Chlorothalonil and 4OH-chlorothalonil were chosen because of their prevalence in suburban streams near Atlanta, Georgia, and flutolanil was chosen because of its repeated detection in a stream downstream of an Atlanta golf course (Table 3.1).

Chlorothalonil is the active ingredient in fungicides that are widely used to treat fungal diseases such as brown patch and dollar spot on turf (USEPA 1999a). It is a broad-spectrum, non-systemic fungicide, which also acts as a bactericide, microbiocide, algacide, insecticide, and acaricide on turf and ornamental plants (USEPA 1999a), (USEPA 1999b). Chlorothalonil has low mobility in soils with a range in adsorption

coefficients (k_{oc}) of 790 to 14,000 and a median of 1600 in sandy and silty soils (Table 3.2) (Caux et al. 1996, Sigler et al. 2000, Tomlin 2000, Wauchope et al. 1992,van der Pas et al. 1999). Chlorothalonil was ranked the seventh most commonly used conventional pesticide in the industrial/commercial/government sector (which includes applications to homes and gardens by professional applicants) (USEPA 1999c). It was one of the top 36 pesticides commonly used to treat warm season turf grasses during the 1990s (Cohen et al. 1999) and was detected in 94% of monthly samples of suburban streams near Atlanta (Table 3.1). It has been found to be "very highly toxic" to fish and *Daphnia* (Tomlin 2000), (USEPA 1999b) (Table 3.2).

4OH-chlorothalonil, also known as 4-hydroxy-2,5,6-trichloroisophthalonitrile and SDS-3701 is the primary degradation product of the fungicide chlorothalonil (USEPA 1999a). Less information is available for the adsorptive potential of 4OH-chlorothalonil, but one study reports a k_{oc} of 460 and 430 in low-humic sandy soils, indicating a higher mobility than chlorothalonil and similar mobility to flutolanil (Table 3.2) (van der Pas et al. 1999). It was detected in 99% of monthly water samples of suburban streams near Atlanta (Table 3.1). It is "slightly toxic" to fish and *Daphnia* and is significantly less toxic than its parent compound, chlorothalonil, to bluegill and *Daphnia* (Table 3.2) (USEPA 1999a).

Flutolanil is a systemic fungicide that prevents hyphal growth of targeted fungal species (*Lepista* spp., *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Rhizoctonia cerealis*) on turf grasses. There is less information available on the sorptive characteristics of flutolanil, which has been reported to have moderate mobility in turf grass soil with a k_{oc} of 420 (Table 3.2) (Suzuki et al. 1998). Based on visual observations (and dominant

substrate assessments along stream transects), the soils in Peachtree City streams are mostly sandy with high amounts of silt and organic matter present. Chlorothalonil will most likely bind to these sediments, while 4OH-chlorothalonil and flutolanil will be more mobile. Flutolanil is not registered for use in residential areas and is mainly used on golf courses and in agriculture. It is "moderately toxic" to fish and "slightly toxic" to *Daphnia* according to the USEPA (Table 3.2) (USEPA 1999b).

There is little information on the effect of these three fungicides on aquatic hyphomycetes, which play a critical role in leaf decay in streams (Barlocher 1992). One laboratory study tested the effect of chlorothalonil-dosed culture media on physiological aspects of three of the most common aquatic oomycetes (slime molds) in the Nile River in Egypt (El-Hissy et al. 1995). Chlorothalonil concentrations similar to and higher than concentrations tested in this study (1, 30, 50, 70, and 100 mg l^{-1}) inhibited the aspartate aminotransferase and alamine transferase activities in *Achlya proliferoides* (El-Hissy et al. 1995). Concentrations of 1 mg l^{-1} and 30 mg l^{-1} affected the DNA of *Saprolegnia ferax*, *Achlya proliferoides*, and *Dictyuchus sterilis*. DNA was decreased in *Saprolegnia ferax* and increased in *Achlya proliferoides* and *Dictyuchus sterilis*.

We have investigated whether fungicide concentrations typical of suburban streams affect rates of fungal biomass accumulation and leaf decay rates of tulip-poplar leaves (*Liriodendron tulipifera* L.) in streams. Tulip-poplar is a common riparian tree species in the southeastern U.S., and its leaves have a medium-fast decay rate once they enter streams (Webster and Benfield 1986). Bacterial abundance was also measured to determine if there was an effect of the fungicides on bacteria. Bacterial biomass and productivity have been shown to be lower than fungal biomass and productivity on leaves

in streams and play a smaller role in the breakdown of leaves (Baldy et al. 1995), (Findlay and Arsuffi 1989), (Weyers and Suberkropp 1996).

The concentrations of fungicides used in this experiment correspond to measured concentrations in suburban streams in Peachtree City, Georgia (Table 3.1). The lower concentrations investigated correspond to concentrations measured in first-, second- and third-order suburban streams and a section of stream downstream of a golf course; the higher concentrations correspond to levels measured in a tile drain coming directly from a golf course green (Table 3.1). Golf courses, which are common in the suburban landscape, are very highly managed areas and use high loads of pesticides and fertilizers to maintain growth of turf grasses.

Methods

Water for the experiment was collected from a small stream in a forested watershed in which no pesticides were detected and nutrients and conductivity were very low. Water was filtered through 1μm glass fiber filters. Nutrients were added as KNO_3 and K_2HPO_4 so that concentrations in the flasks were 1.4 mg N l^{-1} and 0.2 mg P l^{-1} and pH averaged 7.5 (Table 3.3). These concentrations were chosen to be within the range measured in suburban streams in Georgia.

Senescent leaves were collected from tulip-poplar trees, or freshly fallen leaves were picked off the ground in November 2001. Leaves were kept in the dark at 22°C in the laboratory for two to four weeks. A cork borer was used to cut 18-mm diameter leaf disks, which were then pre-leached for three days in aerated, deionized water.

Eighty leached leaf disks were added to each of 28 Erlenmeyer flasks containing 400 ml of nutrient-enriched water, along with the fungal inoculum described below and allowed to colonize at 17°C for three days before any fungicides were added. Flasks were continuously aerated and covered with cotton wrapped in cheesecloth to minimize evaporation (mean evaporative water loss = 6%) while allowing sufficient air exchange. Fungicides were added on "day 0" and 1 ml ethanol was added to control flasks since fungicides were initially dissolved in ethanol. A blank control containing no ethanol or fungicides was used to determine the effect of ethanol. The experiment ran for 28 days at 17°C with a daily cycle of 12 hours (h) of light (non-photodegradative to fungicides) and 12 h of darkness to mimic natural conditions. Water was changed every five days to minimize the buildup of dissolved organic carbon and to replenish nutrients, but approximately 15% of existing water was left in the flasks. Every time water was changed, nutrients, fungicides and ethanol were added to the appropriate flasks. Flasks were agitated twice a day to remove leaf disks from the sides and bottom of flasks.

Three days before adding fungicides, and again on day 18, a fungal inoculum was added to each flask. To prepare the inoculum, senescent, deciduous leaves (tulip-poplar, oak, maple) were assembled in fine mesh bags and allowed to colonize for several weeks in the stream from which water was collected. After the colonization period, leaves were rinsed, and fifteen 10-mm diameter leaf disks were placed in each of fifteen aerated sporulation chambers (see Suberkropp 1991 for description) with 40 ml of filtered, nutrient-enriched stream water at 17°C and covered with cotton wrapped in cheesecloth. After 24 and 48 h, aliquots of water were filtered onto nitrocellulose filters (5 μm pore size) and stained with 0.1% trypan blue in lactic acid. Fungal spores were counted in 25

microscopic fields at 100x magnification on duplicate filters to approximate the number of spores in a known volume of water, from which sporulation rates were calculated (modified from Suberkropp 1991). Water from all sporulation chambers was composited after 48 h into a stock inoculum and placed on a stir plate to homogenize. Ten ml containing approximately 4,000 spores were added to each flask for the first inoculation, and 12 ml containing approximately 3,500 spores were added on day 18.

Chlorothalonil and flutolanil were obtained from ChemServ in West Chester, Pennsylvania (98% purity); 4OH-chlorothalonil was a gift from Syngenta Crop Protection in Greensboro, North Carolina (99% purity). Each fungicide was weighed and dissolved in ethanol and stored in the dark at -16°C. Four fungicide concentrations with two replicate flasks per concentration were used in the experiment (Table 3.4).

A composite leaf disk sample for establishing initial conditions was obtained by combining two leaf disks from each flask on day 0. From this composite sample, three sets of six disks were used for determination of ash free dry mass (AFDM); three sets of six disks were preserved for ergosterol analysis and three individual disks were preserved for counting bacteria. To determine sporulation rates, three disks from each replicate flask were transferred directly from flasks to sporulation chambers and followed sporulation procedures discussed earlier.

On days 7, 14, 21, and 28, six disks per flask were sampled for AFDM. In twenty flasks, leaves were allowed to decompose for an additional 10 days and disks for AFDM were removed on day 38. On days 14 and 28, six disks were sampled for ergosterol extraction; one disk for bacteria was sampled on day 28 and three disks for sporulation were sampled on day 14 from each flask. To determine AFDM, leaves were dried at

60°C for 24 h to obtain dry weight, and were then ashed at 500°C for 3.5 h and weighed again. Leaf disks for ergosterol (a sterol found in the membranes of living aquatic hyphomycete fungi) were stored in 5 ml of methanol at -16°C in the dark and then extracted following methods described in Weyers and Suberkropp (1996) with final concentrations determined by high performance liquid chromatography (HPLC) (University of Georgia Pesticide and Hazardous Waste Laboratory). Leaf disks for bacteria were stored in 10 ml of 5% formalin at 8°C, and bacteria were stained with acridine orange and counted using methods modified from Hobbie et al. (1977). The volume of sample filtered was such that at least 30 bacteria cells could be seen in each field of view and ten fields were counted per filter at 1000x magnification (Kirchman 1993). Leaf disks for sporulation were sampled and counted as they were on day 0.

Water samples were taken on six days throughout the experiment to measure concentrations of fungicides over time. These samples were taken 1, 2, 3, 4, and 5 days after a water change. Twenty ml from each replicate flask were composited and frozen at -16°C in the dark until later analysis. Samples were analyzed by K. Armburst (University of Georgia-Griffin) by injecting 1-ml aliquots into a Waters 2690 Alliance HPLC and separated on an Alltech Associates C-8 column using an acetonitrile : water gradient. Analytes were detected by ultraviolet absorbance at 220 nm using a Waters Model 996 photodiode array detector. All reagents were obtained from Fisher Scientific. The limit of quantification was 0.1 ng l^{-1}.

Leaf decay rates were calculated based on the standard exponential decay regression model (Petersen and Cummins 1974). The slope of the regression (k) of time versus the LN(% AFDM remaining) is the leaf decay rate (day^{-1}). Leaf AFDM for both

replicates was used to calculate one decay rate for each treatment. Leaf breakdown rates were compared using overlapping 95% confidence intervals. Rate of fungal biomass accumulation was determined by regressing time versus the LN(ergosterol mass) for each concentration. Linear regression analyses were performed on log-transformed fungicide concentrations versus ergosterol mass to determine if there was a significant relationship. Analysis of variance (ANOVA) was used to test for differences in bacterial abundance among treatments using JMP® statistical software (SAS 2002).

Results

Flutolanil and 4OH-chlorothalonil did not degrade over time in this experiment, but 4OH-chlorothalonil was detected in those flasks that originally had only chlorothalonil (Table 3.5). Ergosterol mass at the beginning of the experiment was 0.36 µg cm^{-2} leaf area and on day 28 ranged from 0.48 to 1.12 µg cm^{-2} leaf area (Table 3.4). Neither rate of fungal biomass accumulation nor fungal biomass on day 14 or 28 was related to flutolanil or chlorothalonil concentrations (Table 3.4). However, three measures of fungal growth (rate of biomass accumulation, day 28 biomass, and change in biomass from day 14 to 28) declined with increasing 4OH-chlorothalonil concentration (Figure 3.1 and 3.2).

Sporulation rates averaged 145 spores mg^{-1} d^{-1} on day 0 in the controls and ranged from 0 to 357 spores mg^{-1} d^{-1} on day 14 in the controls and treatments (Table 3.6). Chlorothalonil and flutolanil did not appear to alter sporulation rates but no spores were observed at the highest 4OH-chlorothalonil concentration (Table 3.6); therefore, 4OH-chlorothalonil may affect sporulation rates.

Significant leaf decay only occurred in the control and in one of the flutolanil treatments; leaf decay rates were not significantly different from zero in all other fungicide treatments (Table 3.7). Leaf decay rate in the flask without ethanol or fungicides was not significantly different from the control (overlapping 95% CI). (Table 3.7), so the lack of leaf decay in the fungicide treatments can not be attributed to the presence of ethanol.

Mean bacterial abundance in controls and treatments on day 28 ranged from $1.12x10^7$ to $5.94x10^7$ cells cm^{-2} leaf area (Table 3.8). Bacterial abundance in the intermediate and high concentrations of each fungicide on day 28 was not significantly different from abundances in control flasks (ANOVA, $p>0.05$); hence, bacteria do not appear to be affected by these concentrations of fungicides. No correlation was detected between bacterial abundance (number of cells g^{-1} AFDM) and fungal biomass ($\mu g\ g^{-1}$ AFDM) on day 28 ($r=0.3$, $p=0.2$).

Discussion

Temperature, pH, aeration, and duration of this experiment were similar to conditions in other microcosm experiments (Table 3.3). We added lower concentrations of nitrogen and phosphorus than in other microcosms and much lower than those used in culture media so that concentrations were similar to those found naturally in suburban streams in Peachtree City, GA (mean concentrations of NO_3-N <0.2 mg l^{-1} and soluble reactive phosphorus <0.01 mg l^{-1}; L. Shuman pers. comm., University of Georgia-Griffin).

The fungal inoculum (# spores mm^{-2} leaf area) was composed of multiple species (e.g. *Tetrachaetum elegans* Ingold, *Lunulospora curvula*) and was an order of magnitude less than what has been used in other studies. This may have contributed to the lower sporulation rates and lower ergosterol mass observed in control flasks (Table 3.3). Sporulation rates measured in other laboratory studies reported in Table 3.3 are much higher than what is seen on tulip-poplar leaves in hardwater and softwater streams in Alabama; these higher rates have been attributed to increased nutrients, especially nitrogen, in laboratory microcosms (Suberkropp 1991). Day 14 sporulation rates in this study are similar to rates seen in softwater streams at day 15 (Weyers and Suberkropp 1996). While sporulation is stimulated by turbulence (Webster 1975), (Webster and Towfik 1972), violent agitation can inhibit the activity of some fungi (Newell 1993), (Bergbauer and Newell 1992), and high aeration rates might have played a role in limiting sporulation in this experiment. Low calcium concentration in the flasks may also explain low sporulation rates (Table 3.3), although conflicting findings on the impact of calcium on sporulation have been reported. Suberkropp (1998) found no effect of calcium on sporulation rates, whereas Sridhar and Barlocher (1997) found that sporulation was stimulated when calcium was added.

Overall, ergosterol in this experiment was lower than in other laboratory experiments that used pure cultures of aquatic fungal species (Table 3.3) (Suberkropp et al. 1993). Since the inoculum was composed of several fungal species, we might be seeing a compensatory response whereby one species is resistant to fungicides while another species is severely affected and is not able to accumulate biomass as a result of fungicide exposure. However, the significant rates of ergosterol mass accumulation in

six of the treatments (Table 3.4) show that fungi were able to grow. In addition, rates of ergosterol mass accumulation were higher in the laboratory experiment than in the field experiment (Chapter 2).

Leaf decay in the controls was slower than those in other microcosms with higher concentrations of nutrients (Suberkropp 1991). Leaf decay rates of the controls and the flask without ethanol were not significantly different; hence, the presence of ethanol did not affect leaf decay (Table 3.7). However, even low concentrations of the three fungicides slowed leaf decay, so that rates measured were not significantly different from zero. Leaf decay rates in the controls (d^{-1}) were faster than those measured in the field experiment (dd^{-1}) (Chapter 2).

Bacterial abundance in the controls was slightly higher than the number of bacteria cells found on decaying tulip-poplar leaves in two hardwater and softwater streams in Alabama (Weyers and Suberkropp 1996). Chlorothalonil is a bactericide as well as a fungicide (Tomlin 2000), but bacterial abundance in the medium and high fungicide treatments was not significantly different from the controls (ANOVA, $p>0.05$).

Chlorothalonil concentrations tested were similar to ranges seen in three river basins in Georgia and Florida during a tropical storm and slightly lower than mean concentrations in Peachtree City streams (Table 3.1) (USGS 1994). The LC_{50} of all three chemicals for fish and *Daphnia* are greater than the concentrations tested in this study (Table 3.1 and 3.2) (USEPA 1999a, USEPA 1999b). Chlorothalonil levels tested were slightly less than the level at which the hatching success and survivability of fathead minnows are affected (Table 3.2) (USEPA 1999a, USEPA 1999b); leaf decay rates were reduced at those concentrations but no effect was seen on fungi.

Flutolanil is a systemic fungicide targeting certain terrestrial fungal species. It causes hyphae to collapse and prevents fungal growth, but it does not appear to inhibit hyphal growth of aquatic hyphomycetes at concentrations similar to those measured downstream of a golf course (Table 3.1). Even though no effects were found as a result of short-term exposure to flutolanil, chronic effects could be occurring in streams. Researchers have found that chronic, low concentrations of insecticides affect emergence of aquatic insect larvae at concentrations four orders of magnitude lower than the LC_{50} for aquatic insects (Schulz and Liess 1995). In addition, toxic concentrations found in a natural setting are sometimes lower than the toxic concentrations estimated in laboratory studies (Dewey 1986). Further research is needed to examine the chronic effects of fungicides on aquatic fungi at these low concentrations in natural settings.

The degradation product tested in this study, 4OH-chlorothalonil, was the only chemical that reduced the growth of aquatic hyphomycetes, and its impact increased at higher concentrations (Figures 3.1 and 3.2). A literature review and comparison of the ecotoxicity of 78 pesticide transformation products to their respective parent compounds found numerous cases where transformation products were more toxic than their parent compounds (Belfroid et al. 1998), as was found in this study.

Conclusions

Leaf decay was affected by chlorothalonil and 4OH-chlorothalonil and perhaps by flutolanil. The observed lack of leaf decay in the 4OH-chlorothalonil and chlorothalonil treatments could be a consequence of fungal suppression. The evidence to support this includes: bacteria, which play a role in leaf decay, were not affected by fungicides; there were no spores in the highest 4OH-chlorothalonil treatment; and three measures of fungal

growth decreased with increasing 4OH-chlorothalonil concentration. As noted by Gilliom (2001), there is a need for additional research on the potential effects of chronic exposure to low-level concentrations of pesticides to aquatic life. Chronic low-level exposure as well as pulse exposure during storms to higher levels of pesticides could be having an effect on aquatic fungi and subsequently leaf breakdown. Monthly monitoring of fungicides in Peachtree City streams shows that these chemicals are present year round. Useful future research includes performing additional laboratory experiments using higher concentrations of fungicides, while providing higher nutrient concentrations and a greater inoculum of fungal spores to see if there are greater effects than what were observed in this experiment. Decreased fungal activity and little leaf decay as a result of exposure to turf care fungicides has significant implications for stream food webs. Slower leaf breakdown reduces food availability for secondary consumers and could lead to lower consumer productivity in stream food webs.

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Table 3.1. A comparison of mean fungicide concentrations (ranges) used in this study to those concentrations found in suburban streams in Peachtree City, (PTC), Georgia, downstream of a golf course, and a tile drain of a green from the same golf course.

Fungicide (common trade names)	**PTC streams** [a] ($\mu g\ l^{-1}$)	**Detection frequency in PTC streams** [a] (%)	**Golf Course tile drainage from greens** ($\mu g\ l^{-1}$)	**Downstream of Golf Course** ($\mu g\ l^{-1}$)	**Ocmulgee, Flint, and Apalachicola R. basins** [d] ($\mu g\ l^{-1}$)	**This study** ($\mu g\ l^{-1}$)
4OH-chlorothalonil (degradation product of chlorothalonil)	0.0035 (0 – 0.0260)	99%	2,890	0.0930 (0.00001 – 0.3934) [b]		1.0 – 450
Chlorothalonil (Bravo®, Daconil®, Fungiless®)	0.0006 (0 – 0.0134)	94%	120	0.0088 (0.00001 – 0.0378) [b]	0.04 – 0.05	0.03 – 2.0
Flutolanil (Moncut®, Prostar®)	no data	no data	150	0.0086 (0.0021 – 0.0144) [c]		0.02 – 11.0

[a] based on 14 monthly samples from July 2000 to September 2001; concentrations that were detected below the limit of quantification, 0.1 ng l^{-1}, were included in calculations of means as 0.05 ng l^{-1} (APHA 1998)

[b] based on 6 samples from August 2000 to September 2001

[c] based on 3 samples from June 2001 to September 2001

[d] river and tributary samples in the Ocmulgee, Flint, and Apalachicola River basins during flooding caused by tropical storm Alberto in Georgia and Florida (USGS 1994).

Table 3.2. Toxicity of the fungicides 4OH-chlorothalonil, chlorothalonil, and flutolanil to fish, *Daphnia*, and algae, and their environmental fate in freshwater systems. Toxicity values were taken from USEPA (1999a), USEPA (1999b), Tomlin (2000), and Guillebeau (2001). Adsorption coefficients (k_{oc}) as reported in various studies with a median (range) reported for chlorothalonil by Caux et al. 1996, van der Pas et al. 1999, Tomlin 2000, Sigler et al. 2000, Suzuki et al. 1998, Wauchope et al. 1992.

Fungicide	**Fish Toxicity ($\mu g\ l^{-1}$)**	**Fish Species**	***Daphnia* Toxicity ($\mu g\ l^{-1}$)**	**Algae Toxicity ($\mu g\ l^{-1}$)**	**k_{oc}**	**Environmental Fate**
4OH-chlorothalonil	15,000 and 45,000 [a]	bluegill sunfish	26,000 [a]		465 and 430 (low-humic sandy soils)	moderately mobile in soils
Chlorothalonil	47 [a] 60 [a] 43 [a] 3 – 6.5 [b]	rainbow trout bluegill sunfish channel catfish fathead minnow (hatching success and survivability affected)	70 [d] 39 - 79 [b] (reproduc-tion affected)	210 [f] 100 [g] (*Selanastrum capricornatum*)	1600 (790-14,000) (sandy and silty soils)	low mobility to immobile in soils in aquatic systems: DT_{50} (aerobic) <8 hr [h] DT_{50} (anaerobic) <10 d [h]
Flutolanil	5,400 [a] 5,400 [a] 4,650 [a] 2,800 [c]	rainbow trout bluegill sunfish striped mullet carp	50,000 [e]		420 (turf grass soil)	moderately mobile in soils

[a] 96-h LC_{50}
[b] NOEL and LOEL
[c] 72-h LC_{50}
[d] 48-h LC_{50}
[e] 6-h LC_{50}
[f] 120-h EC_{50}
[g] 120-h NOEC
[h] DT_{50} = degradation time for 50%

Table 3.3. A comparison of conditions and results of this study to other laboratory studies using aquatic hyphomycetes.

Conditions	**This Study**	**Other Studies**
Temperature	17°C	15°C [ah]
Water and Nutrients	1.4 mg N l^{-1}, 0.2 mg P l^{-1}, 0.15 mg Mg^{2+} l^{-1}, 0.30 mg Ca^{2+} l^{-1} filtered stream water	13.9 mg N l^{-1}, 4.4 mg P l^{-1} 2 mg Mg^{2+}, 27 mg Ca^{2+} l^{-1} [a] distilled water [a]
pH	7.5	7 [a]
Leaf disks	*Liriodendron tulipifera* L.	*Liriodendron tulipifera* L. [abg]
Aeration	Yes	Yes [b]
Frequency of water change (d)	5	2 [a]
Duration (d)	28	26-35 [ab]
Inoculum (# spores mm^{-2} leaf area)	0.37	1.96 [ah]
Pre-leaching of leaves	Yes	Yes [b]
Results		
Sporulation rates (# spores $mg^{-1}d^{-1}$)	69 [c] (control)	4,800 (*Anguillospora filiformis*) [d] 20,000 (*Lunulospora curvula*) [d] 15,000 (*Flagellospora curvula* Ingold) [e] <50 (softwater stream) [g] 800 (hardwater stream) [g]
Ergosterol concentration (mg g^{-1} leaf AFDM)	0.2 at day 28 (control)	0.8 at day 28 (*Anguillospora filiformis*) [f] 1.2 at day 26 (*Flagellospora curvula* Ingold) [f]
Leaf breakdown rates (d^{-1})	-0.0050 (control)	-0.0234 (*Anguillospora filiformis*) [b] -0.0208 (*Lunulospora curvula*) [b]

[a] Suberkropp 1998
[b] Suberkropp 1991
[c] day 14
[d] day 15; Suberkropp 1991
[e] day 15; Suberkropp et al. 1993
[f] Suberkropp et al. 1993
[g] day 15 Weyers and Suberkropp 1996
[h] Chauvet and Suberkropp 1998

Table 3.4. Mean fungicide concentrations (mean ± 1 SE; n=6) in stream-simulating microcosms, rate of ergosterol mass accumulation (μg d^{-1}) to day 28, and day 14 and 28 ergosterol mass per amount of leaf area for control, 4OH-chlorothalonil (H), chlorothalonil (C), and flutolanil (F); <log = below limit of quantification.

Fungicide	Fungicide Concentration (μg l^{-1})	Rate of ergosterol mass accumulation (μg d^{-1})	r^2	Day 14 ergosterol mass (μg cm^{-2} leaf area)	Day 28 ergosterol mass (μg cm^{-2} leaf area)
Control	<loq	0.021	0.89	0.47	0.53
H	1.3 ± 0.1	0.048*	1	0.60	1.12
H	9.5 ± 0.6	0.031*	1	0.46	0.69
H	73.2 ± 3.7	0.027	0.94	0.41	0.63
H	450.1 ± 36.1	0.035	0.78	0.61	0.77
C	0.03†	0.036*	0.99	0.53	0.80
C	1.4 ± 0.0	0.018	0.77	0.29	0.48
C	1.8 ± 0.1	0.025	0.64	0.49	0.85
C	2.0 ± 0.2	0.045*	0.99	0.65	0.59
F	0.02†	0.031*	0.99	0.49	0.69
F	1.0 ± 0.1	0.030	0.96	0.52	0.68
F	2.1 ± 0.1	0.025	0.84	0.54	0.58
F	10.5 ± 0.5	0.032*	1	0.43	0.71

* denotes significance at p = 0.10

† concentrations not detected, and so were calculated based on measured concentrations in stock solutions and amount of stock solution added

Table 3.5. Mean concentrations (n=3) of chlorothalonil (C) and 4OH-chlorothalonil (H) (± 1 SE) after 1, 2, 3, 4, and 5 days in flasks where only chlorothalonil was added.

Days after water change	C ($\mu g\ l^{-1}$)	H ($\mu g\ l^{-1}$)
1	1.6 ± 0.2	1.5 ± 1.2
2	2.0 ± 0.2*	0.9 ± 0.3*
3	1.5 ± 0.1	0.9 ± 0.6
4	1.7 ± 0.1	0.9 ± 0.6
5	1.7 ± 0.2	0.9 ± 0.6

* mean based on 4 samples

Figure 3.1 Regression of log (4OH-chlorothalonil concentration) vs. day 28 ergosterol mass (■), ($y = -0.13 + 0.99$, $r^2=0.46$, $p=0.32$) and difference in ergosterol mass between days 28 and 14 (△), ($y = -0.13x + 0.46$, $r^2=0.79$, $p=0.11$).

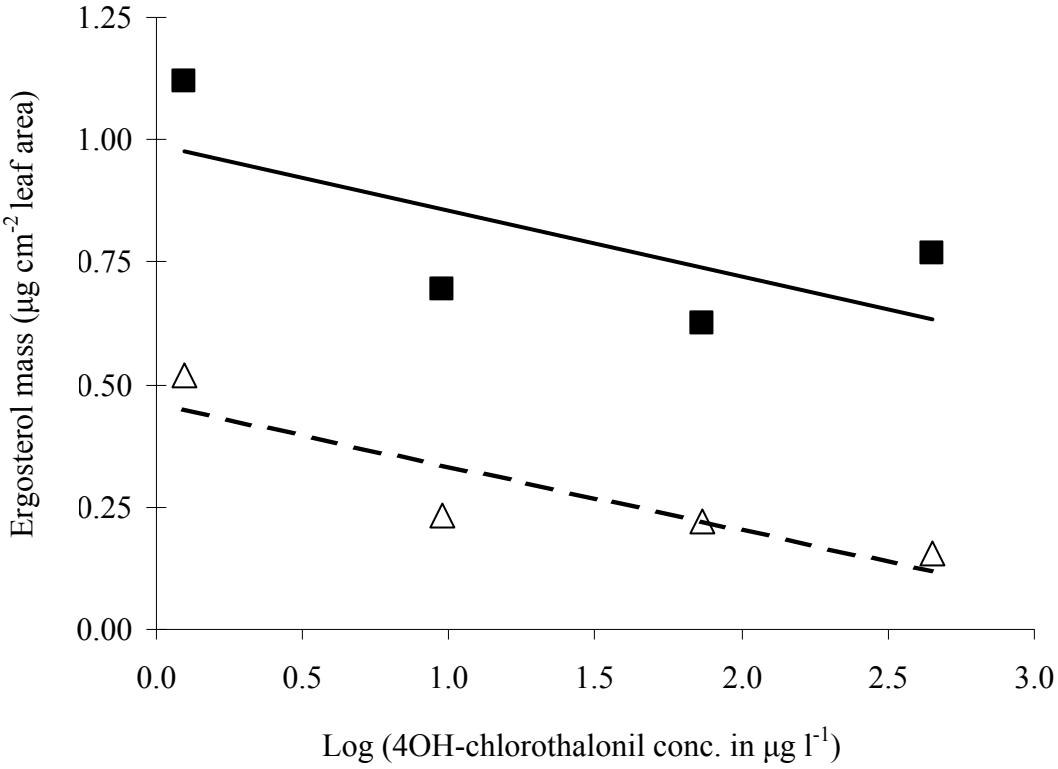
1.25
1.00
0.75
0.50
0.25
0.00
Ergosterol mass (µg cm-2 leaf area)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Log (4OH-chlorothalonil conc. in µg l-1)

Figure 3.2. Regression of log (4OH-chlorothalonil concentration) vs. rate of ergosterol mass accumulation (μg d^{-1}), ($y = -0.005x + 0.035$, $r^2=0.41$, $p=0.36$).

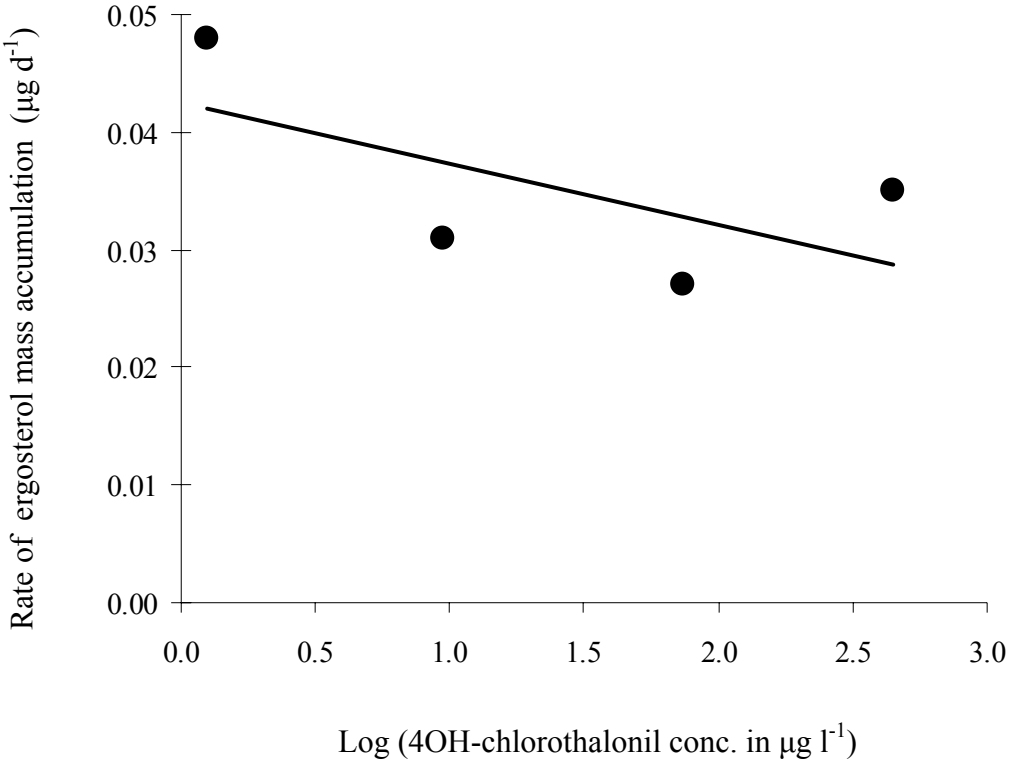
Rate of ergosterol mass accumulation (µg d-1)
0.05
0.04
0.03
0.02
0.01
0.00
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Log (4OH-chlorothalonil conc. in µg l-1)

Table 3.6. Sporulation rates (mean of 2 filters ± 1 SE) at day 0 and 14 for the controls, the lowest and highest concentrations of chlorothalonil (C), and flutolanil (F), and all four concentrations of 4OH-chlorothalonil (H) at day 14; <loq = below limit of quantification.

Day	Fungicide	Mean fungicide conc. ($\mu g\ l^{-1}$)	Sporulation rates (# spores $mg^{-1}\ d^{-1}$)
0	Control	<loq	145 ± 72
14	Control	<loq	69 ± 49
14	H	1.3	58 ± 136
14	H	9.5	74 ± 52
14	H	73.2	143 ± 93
14	H	450.1	0
14	C	0.03	194 ± 137
14	C	1.8	192 ± 136
14	C	2.0	75 ± 53
14	F	0.02	357 ± 252
14	F	10.52	227 ± 161

Table 3.7. Leaf decay rates (± 1 SE) to day 38 for tulip-poplar leaf disks in flasks with different concentrations of 3 fungicides: 4OH-chlorothalonil (H), chlorothalonil (C), and flutolanil (F); (<loq) refers to below limit of quantification and (–) refers to leaf decay rates that were not signigicantly different from zero.

Fungicide	Mean fungicide conc. ($\mu g\ l^{-1}$)	Leaf decay rate (d^{-1})	p
No ethanol	<loq	-0.0054 ± 0.0020	0.04
Control	<loq	-0.0050 ± 0.0028*	0.10
H	1.3	–	ns
H	9.5	–	ns
H	73.2	–	ns
H	450.1	–	ns
C	0.03	–	ns
C	1.4	–	ns
C	1.8	–	ns
C	2.0	–	ns
F*	0.02	–	ns
F*	1.0	–	ns
F	2.1	-0.0051 ± 0.0027	0.09
F	10.5	–	ns

ns = no significant decay at p=0.1
* denotes decay to day 28

Table 3.8. Mean bacterial abundance (n=2 ± 1 SE) on day 28 for the control, 4OH-chlorothalonil (H), chlorothalonil (C), and flutolanil (F) on day 28.

Fungicide	Mean fungicide conc. ($\mu g\ l^{-1}$)	Bacterial abundance (10^7) (# cells cm^{-2} leaf area)
Control	0	2.31 ± 1.34
H	1.3	1.12 ± 0.79
H	9.5	5.90 ± 4.17
H	450.1	1.83 ± 1.29
C	1.4	5.94 ± 4.20
C	1.8	2.24 ± 1.58
F	1.0	2.34 ± 1.65
F	10.5	1.74 ± 1.23

CHAPTER 4

GENERAL CONCLUSIONS

I hypothesized that lawn care practices by homeowners and lawn care professionals in neighborhoods that differ in socioeconomic status will have an impact on fungi and leaf breakdown in streams. I tested this hypothesis by measuring pesticide and nutrient concentrations in streams and examining the indirect effects of these products on fungi and a key ecosystem process, leaf breakdown. Making a link between lawn care practices and effects in the stream proved to be difficult because stream velocity was confounding the relationship between nutrient and fungicide concentrations and leaf breakdown (Chapter 2). Stream velocity overshadowed other factors in explaining differences observed in leaf breakdown rates. Although fungicides were repeatedly detected in all streams, the concentrations were very low and were correlated with stream velocity. Nutrient concentrations were also low in all streams.

In Chapter 3, the physical and chemical environment was more controlled in the laboratory experiment, and low fungicide concentrations inhibited leaf decay, but only a fungicide degradation product reduced fungal biomass. Results from the laboratory microcosm shows the potential for direct fungicide effects on fungi and leaf breakdown rates. However, we were not able to detect this same effect in nature where hydrologic changes associated with development produce alterations in the hydraulic environment. These alterations have resultant effects on rates of leaf breakdown, thus obscuring any effects on breakdown rates resulting from the chemical environment.

In suburban watersheds, where numerous physical and chemical disturbances are changing stream hydrology, geomorphology, and ecology, continued stream degradation is likely. It is necessary to isolate the factors affecting ecosystem processes to better determine the key drivers. For example, by choosing streams that have similar hydrologic regimes and are of similar size and channel morphology, we can better isolate differences in ecosystem processes due to differences in the chemical environment. In this study, the reference streams were larger with higher stream flow than the suburban streams. Age of watershed development varied among the suburban streams studied and channel adjustment was most likely at different stages in response to changes in the hydrologic regime. By selecting streams with more similar hydrology and geomorphology, we can better isolate changes due to chemical differences.

Results from this thesis will be compiled with the toxicity analyses based on macroinvertebrates and biomarker analyses in bivalves to generate a more comprehensive ecological assessment of the response of these suburban streams to lawn care practices. Biological, chemical, and physical data will be integrated with findings by social scientists in their assessment of Peachtree City residents and lawn care professionals to look for overall patterns in lawn care practices and their consequences in streams.

Our team of researchers will disseminate information to local watershed groups in Peachtree City and around Atlanta to educate the public and provide advice to regulatory agencies about links between residential use of lawn care products and effects on biota in stream ecosystems. These data are essential for developing community-based environmental monitoring and protection programs.

It is important that we understand why people are choosing to use pesticides and how their use patterns vary in space and time so that we can better explain the resultant higher concentrations of pesticides and nutrients found in urban and suburban streams than in agricultural streams (USGS 1994). Understanding this is essential to public education on making environmentally-conscious decisions about lawn care as well as advising water quality managers on where to focus stream rehabilitation efforts.

This thesis provides a piece of the puzzle in improving the understanding of human impacts on stream ecosystems. The significant population growth around Atlanta and in other areas of the country makes it imperative that aquatic ecologists consider human impacts and their link to the degradation of streams. Gaining a better understanding of the mechanisms by which human impacts affect the biota in streams and the essential ecosystem services they provide will help to focus mitigation and rehabilitation efforts to improve our Nation's waters.

Literature Cited

USGS. 1994. *Water quality of the Apalachicola-Chattahoochee-Flint and Ocmulgee River Basins related to flooding from tropical storm Alberto: Pesticides in urban and agricultural watersheds; and nitrate and pesticides in ground water, Georgia, Alabama, and Florida*. Water-Resources Investigations Report 94-4183. Atlanta, GA.

APPENDIX A

MEDIAN, MINIMUM, AND MAXIMUM CONCENTRATIONS OF NUTRIENTS AND FUNGICIDES IN STREAMS AND PROPERTY VALUE CLASSES

Table A.1. Median, minimum, and maximum concentrations of nitrate-N (NO_3-N), ammonium-N (NH_4-N), and soluble reactive phosphorus (SRP) during the leaf pack experiment (μg l^{-1}); n=17 for IN (CB) and LO (ON) and n=18 for R1 (CA), R2 (KG), H1 (SR), and H2 (SB); nd = not detected and detection limits for NO_3-N, NH_4-N, and SRP were 13, 4, and 6 μg l^{-1}.

Stream	NO_3-N Median	NO_3-N Min	NO_3-N Max	NH_4-N Median	NH_4-N Min	NH_4-N Max	DIN Median	DIN Min	DIN Max	SRP Median	SRP Min	SRP Max
H1 (SR)	163	49	338	nd	nd	11	169	nd	344	nd	nd	7
H2 (SB)	199	nd	380	nd	nd	33	199	nd	386	nd	nd	7
IN (CB)	115	nd	400	6	nd	100	122	nd	500	nd	nd	8
LO (ON)	264	85	440	nd	nd	49	290	91	489	nd	nd	6
R1 (CA)	220	95	348	nd	nd	237	233	102	354	nd	nd	7
R2 (KG)	188	45	348	6	nd	169	188	051	517	nd	nd	7
H1 and H2	180	nd	380	nd	nd	33	192	nd	386	nd	nd	7
IN and LO	203	nd	440	4	nd	100	206	nd	500	nd	nd	8
R1 and R2	199	45	348	6	nd	237	217	51	517	nd	nd	86

Table A.2. Median, minimum, and maximum fungicide concentrations from baseflow samples (ng l^{-1}) for streams and property value classes during the leaf pack experiment from November 2000 to May 2001 (n=5). Concentrations that were detected below the limit of quantification (loq), 0.1 ng l^{-1}, were included in calculations as 0.05 ng l^{-1}. Chlorothalonil (C), 4-hydroxy-chlorothalonil (H), chlorothalonil and 4-hydroxy-chlorothalonil (H+C).

Stream	C Median	C Min	C Max	H Median	H Min	H Max	H+C Median	H+C Min	H+C Max
H1 (SR)	0.76	0.05	1.44	0.64	0.11	3.93	1.24	0.16	5.21
H2 (SB)	0.27	0.05	1.87	0.36	0.20	12.51	0.50	0.37	14.38
IN (CB)	0.36	0.05	4.39	2.10	0.10	7.61	2.43	0.19	10.59
LO (ON)	0.20	<loq	13.4	0.45	0.17	6.17	0.74	0.17	19.57
R1 (CA)	0.05	<loq	1.27	2.73	0.01	19.09	2.73	0.01	19.59
R2 (KG)	0.10	<loq	1.90	2.98	0.05	25.99	3.95	0.05	26.14
H1 and H2	0.31	0.05	1.87	0.36	0.11	12.51	0.83	0.16	14.38
IN and LO	0.20	<loq	13.4	0.45	0.10	7.61	0.74	0.17	19.57
R1 and R2	0.05	<loq	1.90	2.73	0.01	25.99	2.73	0.01	26.14

APPENDIX B

SEDIMENTS, INSECT AND NON-INSECT TAXA IN LEAF PACKS

Table B.1. Median, minimum, and maximum fine (<250>1 μm) and coarse (>250 μm) sediments per leaf pack (g pack^{-1}) from days 22 to 77 for fine and days 22 to 105 for coarse sediments.

Stream	Median fine sediments (g pack^{-1})	Min	Max	Median coarse sediments (g pack^{-1})	Min	Max
H1 (SR)	0.0022	0.0007	0.0102	2.6	0.3	61.5
H2 (SB)	0.0020	0.0005	0.0130	4.0	0.3	245.4
IN (CB)	0.0037	0.0007	0.0113	36.9	1.9	304.5
LO (ON)	0.0058	0.0006	0.0145	13.1	0.8	226.2
R1 (CA)	0.0017	0.0002	0.0056	68.4	6.2	567.1
R2 (KG)	0.0097	0.0009	0.0272	5.5	0.1	276.1

Table B.2. Insect and non-insect taxa found in leaf packs. An asterisk denotes those insects that were considered to be in the shredder functional feeding group.

Insect taxa

Order	Family	Genus
Diptera	Ceratopogonidae	
	Chironomidae	
	Tipulidae	*Tipula* spp.*
Plecoptera	Chloroperlidae	
	Leuctridae	*Leuctra* spp.*
	Perlodidae	*Cultus* Ricker
Ephemeroptera	Baetidae	
	Caenidae	*Caenis* spp.
	Ephemerellidae	*Ephemerella* spp.
		Serratella spp.
	Ephemeridae	*Hexagenia* spp.
	Heptageniidae	
	Leptophlebiidae	*Leptophlebia* spp.
		Paraleptophlebia spp.*
	Metretopodidae	*Siphloplectron* spp.
	Siphlonuridae	
Trichoptera	Brachycentridae	*Brachycentrus* spp.
	Hydropsychidae	
	Limnephilidae	*Pycnopsyche* spp.*
Coleoptera	Dytiscidae	*Hydroporus* spp.
	Elmidae	*Ancyronyx* spp.
Odonata	Calopterygidae	*Calopteryx* spp.
	Coenagrionidae	
Megaloptera	Sialidae	*Sialis* spp.
Collembola (Class)		

Non-insect taxa

Phylum	Class	Order
Nematoda		
Tardigrada		
Arthropoda	Malacostraca	Amphipoda
	Maxillopoda	Copepod
		Ostracod
	Arachnida	Acari
Mollusca	Bivalvia	Veneroida
	Gastropoda	
Annelida	Oligochaeta	