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Effects of Round Goby Presence on Invertebrate and Microbial Communities in Decaying Leaf Matter of a Lake Erie Tributary Stream

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Effects of Round Goby Presence on Invertebrate and Microbial Communities in Decaying Leaf Matter of a Lake Erie Tributary Stream

by

Allyse Fischer

An Abstract of a Thesis
in
Biology

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

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Buffalo State
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Department of Biology

ABSTRACT OF THESIS

Effects of Round Goby Presence on the Microbial Communities
in Decaying Leaf Matter of a Lake Erie Tributary Stream

Microbial communities are ubiquitous and carry out valuable functions in the environment. Decomposition of leaf material by microbial communities is important to return nutrients back to both terrestrial and aquatic organisms. Perturbations to the environment like the arrival of invasive species can have an impact on the structure and functions of the microbial community. The round goby (*Neogobius melanostomus*) is a Ponto-Caspian fish introduced into the Great Lakes which has since secondarily invaded tributary streams and rivers. Studies have shown they alter invertebrate communities, and these alterations have impacted organic matter decomposition. Stream studies suggest leaf litter decomposes less rapidly in the presence of gobies and I sought to determine whether the reduction in decomposition was a function of changes in microbial community richness. Leaf litter packs were used as a substrate for microbial colonization and analyzed for decomposition rates at sites with and without gobies present. Leaf material decayed significantly less rapidly when gobies were present. The invertebrate communities were significantly different between sites. The goby-absent site had a higher abundance of shredders when compared to the goby-present site. There were not significant differences in microbial carbon source consumption richness or average color well development (AWCD) for carbon guilds between goby-present and goby-absent sites. There were significant seasonal differences in ACWD for microbial carbon guild usage for several guilds. These data suggest that the presence of round gobies does not alter carbon usage of microbial communities in stream leaf litter but that microbial communities can vary with season.

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CHAPTER I: Introduction

Microbial organisms are ubiquitous in nature and function as important components to a number of biogeochemical or nutrient cycles and transformations. Nutrient transformations by microbial communities have been studied extensively in terrestrial (forest and agricultural land) and freshwater (lakes, rivers, and stream) environments (Swift *et al.* 1979, Webster and Benfield 1986, Hättenschwiler *et al.* 2005, Allan and Castillo 2007).

Though there are a vast array of microbes and they vary in their specific functions within ecosystems, there are functional and structural similarities between many terrestrial and aquatic communities. Decomposition is a key functional similarity and it refers to the microbe-mediated transformation of organic matter into its constituent elements. Throughout this process microbes absorb nutrients (C, N, P, etc.) required for growth and metabolism and subsequently release useable forms of the same nutrients to the environment via metabolic byproducts or upon cell death for uptake by algae, plant roots, or other microbes. Microbial communities can be described in various ways such as, genetically (Angeloni *et al*. 2006, Lohner *et al.* 2007), by carbon source utilization (Gamo and Shoji 1999, Matsui *et al.* 2001, Lohner *et al.* 2007), or using microscopic techniques (Baldy *et al.* 1995, Hieber and Gessner 2002). Data obtained for any of these methods can be used to generate estimates of microbial community richness or diversity (Garland and Mills 1991).

1.1 Terrestrial Microbial Biogeochemical Cycling

In terrestrial ecosystems, microbe-mediated nutrient cycles have been examined most in forest and agricultural soils. Within these terrestrial ecosystems the decay of plant material serves as a large nutrient reserve for plant growth, and this is largely mediated by the ability of microbial communities to break down litter under a vast array of environmental conditions (see review by Hättenschwiler *et al.* 2005). In particular, plant-microbe interactions play an important role in the acquisition of nutrients to agricultural plants (van der Putten *et al.* 2007).

Research on microbial-rhizosphere interactions related to plant growth (Das and Dkhar 2012) and the transformation of organic nutrients otherwise unavailable to plants (Paterson 2003, Hill *et al.* 2008) shows that the rhizosphere bacteria interact in a positive manner to the addition of nutrients, aiding in supplying these nutrients to the plant and receiving a carbon source in return from the rhizosphere. This suggests an important link exists between organic matter processing and microbial biomass. Different organic and chemical amendments (fertilizers) were found to alter the bacterial population in agricultural soils and bacterial biomass (C) was consistently higher in nutrient-treated plots compared to the control plots (Das and Dkhar 2012, Zhang *et al*. 2012). Isotopically labeled glucose additions to agricultural soil demonstrated the rate of microbial uptake of glucose increased at increasing concentrations, leading to subsequent increases in the respiration of CO_2 (Hill *et al.* 2008). These studies demonstrate that in agricultural soils, rhizosphere microbes are important for the cycling of carbon and carbon can serve as a substrate to stimulate microbial growth. Similar to Das and Dkhar (2012), Li *et al.* (2013) found that microbial biomass C increased significantly, but only to a particular depth. In addition, Li *et al.* (2013) measured microbial biomass N and

found that for both ammonium sulfate and urea additives the measured N biomass decreased. BIOLOG plates were used to assess community level differences under the amended soils. High ammonium concentrations caused the microbial communities to differentially use carbon sources, based on lower average color well development (ACWD) values, as well as decrease the Shannon and Simpson diversity indices at high concentrations (Li *et al.* 2013). Zhang *et al.* (2012) also found that the BIOLOG carbon source profiles were different among treated plots compared to control plots and also varied based on the type of fertilizer treatment but found the amended plots to have higher ACWD values in contrast to Li et *al.* (2013). Addition of organic amendments or chemical constituents that mimic required nutrients for plant growth significantly impacted the structure and function of the microbial community in the soils where they were applied. Fertilizer applied to agricultural land replaces the nutrients which in forest soils are supplied naturally by decaying leaf material.

Microbial organisms in forest soils play a key role in processing leaf litter and regulation of nutrients, though it varies considerably based on the forest type and other environmental factors. In the Hubbard Brook Experimental Forest, a positive correlation between microbial biomass and N cycling (mineralization and nitrification, Bohlen *et al.* 2001) was found. Schädler and Brandl (2005) also found that there was a positive correlation between the initial N content in leaf litter and the breakdown rate observed in a forest. Bohlen *et al.* (2001) attributed increased microbial biomass (C and N) to the elevation and the difference in nutrient qualities of litter fall and variability in tree species at each elevation. When compared to soils beneath oak trees in the Mediterranean, the soils beneath gaps in canopy or under grass-only cover had the lowest microbial C and N

biomass, due to a lack of litter shed, root decay, and exudates (Aponte *et al.* 2010). Exudates are the nutrients and enzymes excreted through the root system of the plants. These data suggest that the microbial fauna is extremely important to the C and N-cycles in a forest.

In the studies above, numerous factors were found to affect the rate of the C and N transformations in soils. Soil moisture, altitude, season (Bohlen *et al.* 2001, Aponte *et al.* 2010) and the presence or exclusion of invertebrates (Schädler and Brandl 2005) all affect C and N transformations in forest soils. Litter amendments to a study area in Costa Rica were shown to affect litter respiration rates as well as decomposition (Leff *et al.* 2012). Leff *et al.* (2012) showed that when litter was increased in a plot, total soil C and N increased, and microbial C biomass was elevated compared to plots where no alterations were made.

In general, microbial communities create a cycle of apparent nutrient depletion as organic matter is transformed and immobilized into microbial biomass. It is then subsequently released to the surrounding environment in a useable form, either via excretion or upon microbial cell death. Biogeochemical processes and nutrient cycling are reliant upon the microbial compartment. These processes occur in a broad range of forest types and agricultural lands. Nutrient cycling by microbes also occurs and is particularly important in aquatic environments such as lakes, rivers and streams. Like terrestrial ecosystems, microbial organisms are also essential to organic matter decomposition and nutrient recycling in aquatic environments (Bärlocher and Kendrick 1975, Heiber and Gessner 2002). Paerl and Pinckney (1996) reviewed the role of

microbes in the transformations of nitrogen, carbon and, sulfur in aquatic ecosystems concluding that microbial communities were essential for nutrient transformations.

1.2 Aquatic Microbial Biogeochemical Cycling

Microbial communities in lakes, stream, and river ecosystems have been studied extensively. In lakes, bacterial communities can be separated into planktonic and sediment communities. Factors such as the depth, oxygen profile, pH and, allochthonous organic inputs are important determinants in microbial community composition throughout a lake (Dodson 2005). Allochthonous inputs are materials originating from an outside source, for example the deciduous leaf material entering a lake or stream.

In the littoral zone of three lakes in Germany allochthonous inputs of pine pollen were shown to be an important source of nutrients to microbial communities in the water column (Rösel *et al.* 2012). Microbial transformation of pollen particulate organic carbon (POC) accounted for >80% of the decrease in POC in experiments within 48-hours as observed by microscopic techniques (Rösel *et al.* 2012). Rösel *et al.* (2012) also found soluble reactive phosphorus and total dissolved nitrogen increased in experimental tanks with pollen relative to control tanks. This suggests that the pulse event of an allochthonous input of pollen can be an important nutrient stimulant to microbial communities within lakes.

A study conducted in Lake Erie revealed that microbial communities within the sediments are affected spatially by the input of dissolved organic matter (DOM) entering from streams (Hoostal and Bouzat 2008). Hoostal and Bouzat (2008) found allochthonous inputs of DOM were dependent on lake basin characteristics. In addition,

microbial communities at sites closest to allochthonous inputs consumed a smaller variety of carbon substrates (glycogen, D-cellobiose, α-D-lactose, N-acetyl-D-glucosamine, β-methyl-D-glucosie, D-xylose and D-mannitol) compared to the range of substrates used by communities at offshore sites in the respective basins (Hoostal and Bouzat 2008). Within a lake, microbial communities can vary largely based on availability of allochthonous resources supplied to the water column.

In the sediments, similar to terrestrial soils, C and N cycles are important. Also important is the phosphorus (P) cycle as it is often a limiting nutrient in aquatic environments. The flux of P is sensitive to the oxygen conditions of the lake. In aerobic conditions P complexes with Fe^{3+}/Fe^{2+}, thus removing P from the water and creating a pool of sediment bound P unavailable to water column organisms. Much like microorganisms are important to the C and N cycle, they are also key players in the P cycle. When compared to sterile sediments, Huang *et al.* (2011) found that microbial activities were responsible for 50% of the P uptake in inoculated sediments under aerobic conditions. Under anoxic conditions, the amount of P released from sediments was greater than from sterile controls (Huang *et al.* 2011). The oxygen profile plays an important role in microbial community structure and function in lakes where water movement is limited. The availability of oxygen in rivers and streams is less of a limiting issue overall but does become important when studying the micro-environments of biofilms and leaf litter.

Similar to lakes, different bacterial communities are present in rivers and streams. They can be classified as planktonic bacteria, free-floating in the water, sediment bacteria, epilithic bacteria attached to rock, and epiphytic bacteria on leaf litter or

macrophytes. The microbial consortia of each of these communities are especially important in cycling of nutrients for stream ecosystem, similar to their counterparts in terrestrial and other aquatic environments.

Breakdown of allocthonous leaf litter is a major contributor to stream nutrient cycles. The bulk of carbon entering stream systems is the result of terrestrial primary production which ultimately becomes detritus. Autochthonous carbon is estimated at less than 1% of the energy supply in small woodland streams (Cummings 1974) with the bulk of stream energy being derived from allochthonous leaf litter (Minshall 1967, Wallace *et al.* 1997). Detritus goes through several phases of decomposition in streams commonly known as leaching, conditioning, and fragmentation (Gessner 1999). Conditioning is the period of time, within about 24 hours, in which microorganisms colonize the leaf material and modify the leaf matrix (Gessner 1999). Leaf material serves as both a platform for microbial growth as well as a carbon substrate for production of additional biomass, initiating the microbial loop (Meyer 1994). Ardón and Pringle (2008) found positive correlations between leaf break down rate and the maximum bacterial biomass in streams. Increased microbial biomass and nitrogen content of the leaf litter promoted shredder fragmentation and breakdown of leaf litter (Inkley *et al.* 2008). Without microbes, the recycling of nutrients from leaf litter to the invertebrate compartment or further up the tropic food chain would be stalled.

Microbial communities in streams play a key role in nitrogen transformations and retention. A consortium of *Nitrosomonas* and *Nitrobacter* microbes are capable of nitrification in the presence of decomposing material and nitrogen fixation by heterotrophic bacteria is also aided by organic matter (Paerl and Pinckney 1996). The

biofilms that develop on detritus and the compacting of leaf material can provide microniches in which anoxia promotes denitrification, while lotic conditions otherwise provide oxygen-rich conditions for nitrification. Microzones created by bacterial biofilms on organic matter have been shown to create an environment where N_2-fixing bacteria can thrive in an otherwise oxic environment (Paerl and Carlton 1988). The relationship between substrate N and microbes plays an important role in the leaf break down rate. A positive correlation was found between detritus %N and the leaf breakdown rate for 48 species of deciduous leaves (Ostrofsky 1997). Mulholland *et al.* (2000) using a ^{15}N tracer study, showed that by day 42 65% of the ^{15}N resided in microbes on decomposing leaves.

Detritus also is a rich source of carbon compounds that are assimilated by microbes for growth, as well as serving as a substrate for attachment. McArthur and Richardson (2002) found that within 1-hour of leaf submergence, bacteria reached a peak in [^{3}H] leucine incorporated, a measure of bacterial growth. Direct counts revealed biofilm communities on leaves increase in number over the course of several days before maintaining a relatively steady abundance (McNamara and Leff 2004a). Other studies showed bacterial abundance on leaf material increased for the first 4-8 weeks before maintaining a constant abundance when enumerated by direct count methods (Baldy *et al.* 1995, Hieber and Gessner 2002). Duarte *et al.* (2010) found that species succession occurs with time on decaying leaf material in streams; microbial biomass increased to a peak around 41 days and subsequently declined. Bacterial biomass was positively correlated to leaf decomposition. Enumeration reveled that there was an order of magnitude difference in the number of bacteria in a stream containing leaf litter

compared to a stream where litter was excluded for a period of time (Hall and Meyer 1998). This suggests course particulate organic matter promotes the assimilation of organic carbon to bacterial biomasss when compared to dissolved organic carbon in stream water alone.

Duarte *et al.* (2010) using denaturing gradient gel electrophoresis (DGGE), showed microbial community diversity changed through time. In studying the diversity of the bacterial components of the leaf litter, succession of species present detected by DGGE was apparent (Duarte *et al.* 2010). This could be, in part, due to the chemical changes the leaf material and the aggregation of epilithic bacteria on the leaf material creating niches more suitable to certain strains of bacteria. Weyers and Suberkropp (1996) also found increasing microbial density and biomass in two study streams during the period of leaf breakdown

Terrestrial derived dissolved organic matter (DOM) is also an important source of nutrients to microbes. Within three hours of DOM addition to experimental tanks, Kreutzweiser and Capell (2003) documented an increase in microbial density leading to increasing respiration on leaf disks. Kreutzweiser and Capell (2003) found that the microbial community experienced a shift in metabolic activity with the addition of DOM. Patterns in population size were observed for different bacterial species based on organic or phenolic content (McNamara and Leff 2004b). Hall and Meyer (1998), in comparing a stream with leaf litter and a litter-excluded stream, found that litter exclusion resulted in less bacterial biomass but ^{13}C uptake was equivalent in both streams. Analysis of the partial carbon budget showed that microbial respiration accounted for up about 18% of leaf mass carbon loss when treated to prevent fungal growth and 26% when fungi and

bacteria worked in conjunction (Suberkropp 2003). These data reflect the importance of detrital particulate carbon for bacterial growth.

Bacteria associated with decaying organic matter are essential to nutrient cycling in streams, aiding in decomposition and the transfer of nutrients from detritus to higher trophic levels. Laboratory studies using *Gammarus* reveal that fungal and microbial conditioned leaves resulted in higher survival rates and were the preferred food choice over sterile leaves (Bärlocher and Kendrick 1975, Kostalos and Seymour 1976). Minshall (1967) studied the diet of a number of invertebrates of various feeding groups from a creek in Kentucky and found that allochthonous leaf detritus was at least 50% of the diet of herbivores and omnivores. In the herbivores the detritus was noted to be only partially digested suggesting the consumers may be using the attached bacteria as an energy source rather than the leaf material (Minshall 1967). Hall and Meyer (1998) showed that invertebrates of various functional feeding groups acquired a significant portion of labeled carbon from microbial sources in a North Carolina stream. This carbon was transferred to higher trophic levels, including predators, demonstrating a transfer of energy originating from a microbial source (Hall and Meyer 1998). Thus, there are important microbe-mediated links between detritus and higher consumers and disruptions to the ecosystem can break or alter these important relationships.

1.3 Disruptions to Natural Nutrient Cycles

Stream ecosystem disruptions such as eutrophication (Gulis and Suberkropp 2003b), pollutants (Dean-Ross and Mills 1989, Hoostal *et al.* 2008) and the introduction of new species (Hahn 2003, Angeloni *et al.* 2006, Lohner *et al.* 2007, Piscart *et al*. 2011,

Winters *et al.* 2011) can have an effect on the microbial community with the potential to alter the structure and function of the natural communities present. Gulis and Suberkropp (2003a, 2003b) found that nutrient enrichments of a stream increased the leaf decomposition rates, the nitrogen content of the leaves after submersion, and bacterial abundance and bacterial carbon associated with the leaf material. The different bacteria communities (platonic, sediment and epilithic) in the Maumee River varied in the biomass present and heavy metal concentrations reduced the capability of the community to use various carbon sources (Dean-Ross and Mills 1989). Hoostal *et al.* (2008) demonstrated that microbial communities in sediments across a heavy metal contamination gradient were adapted to the levels of heavy metal present in their local environment. The extracellular enzymatic activity of the communities was largely based on their exposure to the heavy metal contaminants. Overall enzymatic activity was greater and hydrolytic enzyme activity was decreased in polluted sediments compared to unpolluted sediments of Lake Erie (Hoostal *et al.* 2008). Additionally, indirect measures of microbial biomass suggested a greater biomass in polluted sediments (Hoostal *et al.* 2008). These studies are examples of how perturbations can impact microbial communities due to altered environmental abiotic conditions and similarly changes to the biotic compartment of an ecosystem can inflict changes to the microbial community.

1.4 Invasive Species Alter Microbial Communities

With the age of globalization, the introduction of new species to environments has dramatically increased. The introduction of a non-native or invasive species can have lasting impacts on the ecosystem, impacting specific trophic levels or having a concomitant effect on multiple trophic levels. As described, disruptions or perturbations

within an ecological community have been shown to alter the microbial fauna of the habitat. This phenomenon has been well documented with invasive plant species (Hahn 2003, Angeloni *et al.* 2006), dreissenid mussels (Lohner *et al.* 2007, Winters *et al.* 2011) and invertebrates (Piscart *et al.* 2011, Hunting *et al.* 2012) in aquatic environments.

Hahn (2003) demonstrated that an invasive plant (Asian Eelgrass, *Zostera japonica*) did not alter the sediment microbial community numerically, but that the community composition as inferred from BIOLOG carbon source utilization profiles was different at sites where invasive eelgrass was present. This suggests that the microbial assemblage in the sediments surrounding the invasive eelgrass differs from the assemblage near the native eelgrass (*Zostera marina L.*) and this could contribute to a functional change in the decay of organic matter at the location.

Another invasive plant, *Typha angustifolia*, a cattail found in Great Lake wetlands, has impacted microbial communities and nutrient cycling. *Typha angustifolia* has decreased plant diversity when compared to native zones. The sediments associated with the invasive cattail had a significant increase in soluble nutrients; ammonium, nitrate and phosphate in sediments were increased 14, 10 and 10-fold respectively when they were compared to sediments near native cattail species (*Typha latifolia*, Angeloni *et al*. 2006). Terminal restriction fragments also showed that the sediments near *Typha angustifolia* had significantly higher bacterial species richness, which was expected due to the altered soil characteristics induced by the invasive typha (Angeloni *et al*. 2006).

Similarly, invasive dreissenid mussels (*Dreissena polymorpha* and *D. bugensis rostriformis*) also have been documented to alter benthic bacterial populations.

Molecular techniques were used to demonstrate that different bacteria strains dominated different cavities of the zebra mussels (*Dreissena polymorpha*) (Winters *et al.* 2011). Increased levels of Cyanobacteria and Bacillariophyta were associated with gut samples (Winters *et al.* 2011), indicating that mussels sequester specific microbes from their environment or their diet, thereby altering the microbial community compositions surrounding their clusters. Likewise, microbial community structure in sediments collected from within a cluster of dreissenid mussels or druse was compared to the community from sediments outside the druse cluster (Lohner *et al.* 2007). Community-level, carbon source profiling showed increased microbial diversity within *Dreissena* clusters. Additionally, DGGE, a molecular fingerprinting method, showed significant differences in microbial community composition between *Dreissena* present and absent communities (Lohner *et al.* 2007). The communities present within druses were consistently able to use more carbon sources than the communities outside of druses, suggesting that in addition to altered diversity, the function of the microbial communities was different as well.

Another mollusc, *Crassosirea gigas*, is an invasive species to a number of areas around the world and has been shown to alter the structure, function, and diversity of microbial communities thereby affecting the carbon and nitrogen cycle in the affected areas (Green *et al.* 2012). The presence of molluscs significantly affected the ammonium concentration when compared to the absence of molluscs (Green *et al.* 2012). Additionally, Green *et al.* (2012) found that at medium and high mollusc density more CO_2 and CH_4 was respired from the sediments. Lastly, microbial activity and diversity

was greater at high mussel density compared to lower mussel density in the oxic sediments (Green *et al.* 2012).

The invertebrate community has effects on detritus processing and have been shown to systematically alter microbial communities. In streams these alterations can impact detritus processing. Piscart *et al.* (2011) studied the interaction between a native amphipod and non-native or introduced species and found that for two streams the potential leaf breakdown rate was significantly decreased based on the shredding capabilities of the species present. The presence of invertebrates not only has an effect on detritus processing but can drastically impact the bacterial community composition and function. Hunting *et al.* (2012), in a microcosm experiment, showed that the specific feeding activities of an isopod and amphipod (biodiffuser, grazing upper layer of detritus), midge larva (ventilated tubes, diffusers) and, two oligocheates (upward conveyers) increased bacterial activity within detritus by affecting the oxygen profile. The magnitude of detritus processing varied depending on the invertebrate treatment. The isopod and amphipod processed 60% more detritus and had up to 500% more bacterial activity than their experimental counterparts (Hunting *et al.* 2012). In addition, these two invertebrates were responsible for an oxygen rich profile at the sediment-water interface (Hunting *et al.* 2012). The bioturbation due to feeding activity of different invertebrate species therefore can impact microbial communities by introducing oxygen and nutrients to microhabitats. Morrison and White (1980) also studied the interactions between a mixed community of gammaridean amphipods on the microbial interactions and leaf break down rates, finding that the presence of grazing amphipods affects the microbial community on the leaves over time. The grazing pressure exerted by the

amphipods stimulated increased biomass and metabolic activity as well as altered the community composition when compared to ungrazed controls (Morrison and White 1980).

These observed differences between plants, invertebrates and other microbes substantiate bacterial community shifts in the presence of a non-native or introduced species and illustrate that microbial communities are relatively sensitive to changes in the environment. With changes in the assemblage of microbes, changes in nutrient cycling and organic matter breakdown are likely to result. Alterations to abundance or diversity of the ichthyofauna also can directly alter microbial communities or alter the invertebrate fauna thereby impacting the microbial community structure and function as outlined above.

In an extreme instance, it has been shown that fish can drastically impact microbial communities. Aquaculture practices shifted the most abundant bacteria in the sediments from gram positive species (in control areas) to gram negative species in the experimental areas (Danovaro *et al.* 2010). Danovaro *et al.* (2010) also found that there was a 3-10 fold increase in bacterial and microbial biomass respectively and this difference evaporated when the aquaculture cages were removed from an area, returning back to conditions similar to the control. Although this is an extreme example it may still be applicable to natural settings where a fish species is introduced, bringing with them a different suite of excreted nutrients. Nishimura *et al.* (2011) looked at the trophic cascade effect of fish additions in a mesocosm experiment and found, unsurprisingly, that there are tropic cascade effects in the presence of fish. In the presence of fish there was a 6.2 fold increase in the abundance of filamentous bacteria when compared to plots

without fish. There were no significant differences in abundance of the single celled morphotypes (rods, cocci) and this was potentially controlled by both top-down and bottom-up forces (Nishimura *et al.* 2011). Though the effects of the fish addition did not directly affect the microbial population, it did have a significant effect on the abundance of organisms and subsequently their interaction with microbial populations. The disruptions to species abundance and diversity at various tropic levels caused by species introduction to a habitat can therefore be expected to directly or indirectly affect the microbial population abundance, structure, and function when compared to pre-introduction conditions. In the work described here, I document the possible effects the invasive round gobies could have on leaf litter microbial communities in streams.

1.5 Round Goby Invasion

The round goby (*Neogobius melanostomus*) was introduced to the Great Lakes via the ballast water of transoceanic ships traveling from brackish ports in the Black and Caspian Seas. Jude *et al.* (1992) first documented round gobies in the St. Clair River in June of 1990. Since their introduction, gobies have spread to all five of the Laurentian Great Lakes (Phillips *et al.* 2003, Krakowiak and Pennuto 2008, USGS 2012), and their population sizes have expanded rapidly. Clapp *et al.* (2001) used trawl samples to show round goby catch per unit effort increased from 0 to 20-70 fish over the course of three years near Grand Haven, Michigan. Furthermore, goby expansion was confirmed at four separate new locations within Lake Michigan from 1997-1999 (Clapp *et al.* 2001), and into tributary rivers and streams (Krakowiak and Pennuto 2008).

Round gobies have a characteristic fused pelvic fin which allows them to attach to substrate, facilitating their migration up streams against the flow of water (Jude *et al.* 1992). The swimming performance of the round gobies was evaluated by Tierney *et al.* (2011), who found that most gobies used a "burst and hold" method and can sustain swimming speeds equal to 37.7cm/s. In addition, regardless of flow speed, all gobies attempted to brake against backward movement (Tierney *et al.* 2011). The ability of round gobies to sustain relatively high swimming speeds, burst and hold to substrate while they energetically recover, and brake using their pelvic fins against strong currents are all attributes allowing them to execute a secondary invasion of the tributaries surrounding the Great Lakes.

In 2003, Phillips *et al.* documented the presence of the round goby in four of six streams sampled in Pennsylvania. The abundance of gobies reached levels of 30% of all fish collected per unit effort. Tributaries to the northern portion of Lake Michigan showed similar findings; six of the sampled streams had round gobies present in abundances $\leq$25% and their range extended up to the first impassable barrier (Poos *et al.* 2010). Karakowiak and Pennuto (2008) documented goby presence in four of eight Lake Erie streams, representing no less than 50% of the total fish collected. Thus, tributary streams are not immune to round goby invasion.

The advancement of round gobies up tributaries of the Great Lakes has had demonstrable effects on native benthic invertebrate and fish communities. Diet overlaps of native benthic fish and the round goby are in part responsible for the observed effects. Small to medium sized round gobies in the nearshore zone feed primarily on benthos and undergo a diet shift as they become larger to a diet primarily composed of mollusks such

as dreissenid mussels (French and Jude 2001). Most tributary streams lack dreissenid mussels, forcing round gobies to consume primarily other invertebrate prey such as chironomid midges and caddisflies even as they mature to larger size classes (Phillips *et al.* 2003, Pennuto *et al.* 2010). The overlap in diet and competitive nature of round gobies are suspected, in part, to be causing a decline in native fish species such as rainbow darters (French and Jude 2001, Krakowiak and Pennuto 2008), mottled sculpin (Jude *et al.* 1995) and logperch (Jude *et al.* 1995, French and Jude 2001, Balshine *et al.* 2005). Krakowiak and Pennuto (2008) showed that macroinvertebrate taxa richness and diversity were altered in streams with round gobies present relative to streams without gobies. Alterations in the native fish and macroinvertebrate community as the result of invasive species might also have profound effects on the microbiota structure of an ecosystem.

1.6 BIOLOG, Carbon Sources Assay

BIOLOG assay plates were developed as an efficient method for identifying bacterial isolates in a clinical setting based on their sole-carbon source utilization "fingerprints". Plates are designed with 96 wells; one control and 95 wells containing different carbon sources or chemical assays. A tetrazolium dye activated by microbial respiration is used as an indicator of carbon use.

Since its inception, the use of carbon source utilization has become a popular method for community level profiling of microbes in various ecological settings. Garland and Mills (1991) were able to use BIOLOG technology to differentiate samples from an assortment of aquatic environments and terrestrial microbial communities. An

ecological community-level assessment of microbes is a popular practice to study the effects of an element on a community rather than an individual. It has been used in various methods to study pollution remediation ability of a community (Powell *et al.* 2003), adaptations to heavy metal stress (Hoostal *et al.* 2008), and microbial resistance (Dean-Ross and Mills 1989). Matsui *et al.* (2001) used BIOLOG to show that a bacterial community changed functionally due to changes in phytoplankton and zooplankton populations. Bacterial communities in the presence and absence of non-native *Dreissena* mussels were shown by Lohner *et al.* (2007) to exhibit variations in carbon utilization profiles as well as banding patterns in DGGE. This suggests that changes or disruptions in the natural biota can have profound effects on the microbial structure of aquatic environments and furthermore can affect organic matter decomposition. The sensitivity of the BIOLOG microplates to detect even dilute strains of bacteria was demonstrated by Gamo and Shoji (1999). This suggests even minor changes in community structure should be detectable.

1.7 Goals of the Current Research

Previous research has shown the direct effects of the round goby on invertebrate abundance and richness in streams and on the rate of leaf litter decomposition, but no studies have investigated the possible indirect effects these fish might have on the microbial community in streams. Since round gobies alter invertebrate community structure and abundance, and since invertebrate shredding and grazing of leaf litter both affect and are affected by microbial communities, the indirect effects of round gobies on microbial communities should be assessed. This study will investigate microbial community diversity in leaf litter from a Lake Erie tributary stream at sites with and

without round gobies, using the round goby as a model to predict if the addition of a benthic fish impacts microbial carbon utilization. I expect to find in this research that the round goby impacts the microbial community. Based on previous research from this lab I hypothesize that leaf litter breakdown will be fastest where the round gobies are absent compared to sites where gobies are present. I also expect that the invertebrate community will vary between the goby-present and goby-absent sites. I anticipate differences in the BIOLOG carbon source utilization patterns between the goby-present site and the goby-absent site and these differences to be attributed to the goby presence. In addition, I expect that the expected observed differences to hold the same trend in both a summer season and fall season though the values are likely to be different between seasons.

CHAPTER 2: Methods

2.1 Sample Locations

Round goby impacts on bacterial communities associated with leaf litter decomposition were assessed at two sites in Ellicott Creek. The sites were above and below Glenn Falls dam in Willilamsville, New York and represent locations without and with round gobies, respectively. The goby-present site was located near the University of Buffalo in Amherst, NY (UB, 43.006983°N, -78.776137°W) and the goby-absent site was located near the Buffalo Niagara Airport in Cheektowaga, NY (AP, 42.939364°N, -78.711637°W) (Figure. 1). Stream dimensions were measured at the onset of the study and water quality measurements were recorded at each of the sample sites at each collection time. Water temperature (◦C), dissolved oxygen (mg/L), percent saturation (%), turbidity (NTU), pH and, conductivity (ms/cm) were measured using a Hydrolab QUANTA multiprobe instrument. Stream velocity (cm/s) and depth (cm) were measured once at the initiation of the study in May. Measurements were collected at 1-m intervals across the width of the stream (Swoffer Model 2100 flow meter). The stream discharge (Q; m^3/s) at both locations was then derived from stream depth and velocity measurements. Sites were matched as closely as possible in terms of water depth, discharge, substrate composition and canopy cover.

2.2 Collection and Processing of Litter Packs

In the fall of 2011, red maple (*Acer rubrum*) leaves were collected soon after natural abscission. All leaves were sealed in plastic bags and then frozen until further processing. In late April 2012 the leaves were dried at 60□C for at least 48-hours or until

a constant weight was obtained. Once dried, precisely 10 grams of dried leaf material was weighed using a top-loading balance. Leaves were then rewetted to prevent breakage while handling and placed into nylon bags having a mesh size of 0.5 cm.

On May 25, 2012, twenty-one leaf litter packs were secured at each of the sample sites (UB and AP) and arranged in a 3x7 array. In the field a brick was added in each leaf pack to weight the bag at the bottom of the stream bed. At this time an additional pack was prepared, secured in the stream, immediately removed and the leaf material was processed as described below to determine a correction factor for loss of material due to handling and transport. Three replicate bags were then retrieved from each location at 14-days, 30-days and 60-days, using a random number table to select bags. Due to vandalism at the AP site some data were reported only until the 30-day collection. To extend the study a second series of leaf packs was placed as described above at each location on September 7, 2012 and replicate collections made at 14-days, 30-days and 60-days.

Upon collection, individually each litter pack was gently lifted into a fine mesh D-net below the water line with care to not lose any loose organic matter. The brick was removed from the leaf pack in the field and all invertebrates were removed from the brick and the whole sample was then placed in a new plastic bag and returned to the lab in a cooler for further processing.

Within 24-hours all organic matter was removed from bags and enough stream water to cover the leaf material was placed in a sterilized flask and agitated by hand for five minutes with sterile glass beads to dislodge bacteria. The resulting liquid was

transferred into sterile 50-mL polystyrene tubes and then slow-centrifuged to remove large particles and debris. The supernatant containing the suspended bacteria was decanted into sterile, 15-mL polystyrene centrifuge tubes. Bacteria were then pelleted from the remaining liquid portion by centrifugation at 5000-8000 RPM for 15-minutes and all pellets for each sample were homogenized and re-centrifuged following the same parameters as above. The homogenized pellet was re-suspended in 4 milliliters of physiological saline buffer. This solution was then added dropwise to BIOLOG inoculating fluid (BIOLOG IF-A, BIOLOG Cat. #72401) until the appropriate turbidity was reached, T= 90%, using a calibrated Biolog Turbidmeter for BIOLOG analysis.

The leaf material from the original agitated flask was dried at 60°C for one week at which time the sample was reweighed to determine percent change in leaf dry mass since colonization. A subsample of dried material was taken for elemental determination of carbon and nitrogen. For every sample three replicates of between 4-6 mg were weighed to the nearest 0.001mg and placed in inert tin capsules, which were first tarred to zero, using a Mettler Toledo microbalance. The tins were then closed up and loaded into an auto sampler carousel for analysis of percent carbon, nitrogen, hydrogen and sulfur on the Flash 2000 Organic Element Analyzer (Thermo Scientific). A calibration plot was constructed with weights between the high and low values of the samples using a BBOT standard (Thermo Scientific, C:72.52%, N: 6.51%, H:6.09%, S:7.44%). The method for this analysis ran with the following parameters: cycle run time 720s, combustion furnace 950°C, reduction furnace 1060°C, oven 65°C, carrier gas 140mL/min, oxygen 205mL/min and, reference gas 100mL/min.

The remaining dried leaf material was then ashed in a muffle furnace at 550°C for 2-hours. Percent organic matter (%OM) and ash free dry mass (AFDM) were calculated to determine the leaf decay coefficient (*k*). Decomposition rate, *k*, of the leaf litter was analyzed by linear regression and a t-test was used to determine statistical differences in decay rates.

2.3 Benthic Invertebrate Processing

As leaf material was being removed and placed in a sterile flask, all invertebrates were removed from the leaves and placed in 70% ethanol. Invertebrates were also picked from the remainder of the sample. This either occurred while the invertebrates were live or the whole sample less the leaf material was preserved and invertebrates were picked at a later date. Once all invertebrates had been preserved they were counted and identified to at least family level and genera where possible using keys of Peckarsky *et al.* (1990) and Merritt *et al.* (2008). Species richness, diversity, and functional group abundance were calculated and analyzed using a students t-test for statistical differences between sites. Functional feeding group percent compositions were compared using a G-test.

2.4 Microbial Assessment of Community Structure

The prepared inoculating fluid (IF) was used for community analysis using the Biolog system. The prepared IF solution was used to inoculate one 96 well GEN III Biolog microplate plate per leaf pack. A multichannel Biolog pipette was used to aliquot 100µl of IF mixture per well. An immediate reading using a Thermo Electron Corp., Multiskan Accent Type 354 instrument and Accent Software V1.3.1 was taken after the

GEN III plates had been inoculated and then every 12 hours for four days. All plates were incubated at about 21□C.

A method of average well color development (AWCD) was used to analyze the metabolic activity of the community. To employ this method, the absorbance value of the control well (C) was subtracted from each of the other 95 wells (R) and the average absorbance value of the whole plate was then taken, [$ACWD = (\sum(R - C))/95$] (Garland 1996, Garland and Mills 1991, Lohner *et al.* 2007). This method resulted in a single ACWD value for each plate which included all carbon sources and chemical assays. The same calculations were done for only carbon sources and additionally per guild within the carbon sources. A diversity index was calculated using the number of substrates consumed by the bacterial community producing absorbance values of greater than 0.25 for all carbon sources, less chemical assay wells, as an accepted value in the literature. It was also used to calculate the diversity of specific substrate guilds for each plate (amides/amines, amino acids, carbohydrates, carboxylic acids, polymers and other sources; Lohner *et al.* 2007). A percentage was calculated to determine the number of positive wells (>0.25) that occurred per guild.

The ACWD per guild was compared between sites, between seasons, and between days using ANOVA. The G-test was used to compare the substrate diversity for guilds that contained at least five wells. Any guilds with < 5 wells on a plate were condensed into "Other Carbon".

All statistics were preformed with an $\alpha = 0.05$ to determine statistical significance and were done using Statistix (version 9).

CHAPTER 3: Results

3.1 Water Chemistry

The goby-present and goby-absent locations were about 14.5-kilometers apart with a USGS gauge station (04218518) located between them on Ellicott Creek at Sheridan Drive (Figure1). The stream discharge measured by the gauge station for both seasons was variable, on average 1.27 m^3/s in May and 0.85 m^3/s in November, but followed an expected trend (Figure. 2). There was a large storm event at the end of May shortly after the leaf packs were placed, as might be expected towards the end of the rainy season in western New York. The channel width at the downstream goby-present (UB) site was about 10 meters wider than the upstream goby-absent site (AP). The center of the stream at UB resembled that of AP in terms of substrate heterogeneity and velocity and that is where the leaf packs were placed. The mean depth at UB was roughly 10-centimeters less than AP (Table 1).

Water chemistry varied slightly between locations on each sampling date (Table 1). With the exception of the final fall sampling (14-November), the UB site was warmer than the upstream, AP site. The range of difference in temperature between locations was 0.60-2.06◦C and 0.90-3.72◦C in the spring and fall respectively. Dissolved oxygen followed the same trend as temperature where measures of oxygen were higher at the UB location when compared to the AP location. Turbidity also followed this common trend between sites with the exception of two dates in the fall sampling period, 20-September and 14-November, where the AP site was slightly more turbid than the UB site. For all sampling periods conductivity was consistently higher at the AP site when compared to

Figure 1. Map of Ellicott Creek drainage and study location with goby-present (black circle, UB) and goby-absent (black triangle, AP) sampling locations. The goby-present site is separated from the goby-absent site by Glenn Falls, located in Williamsville, NY which prevents migration of the goby further upstream.

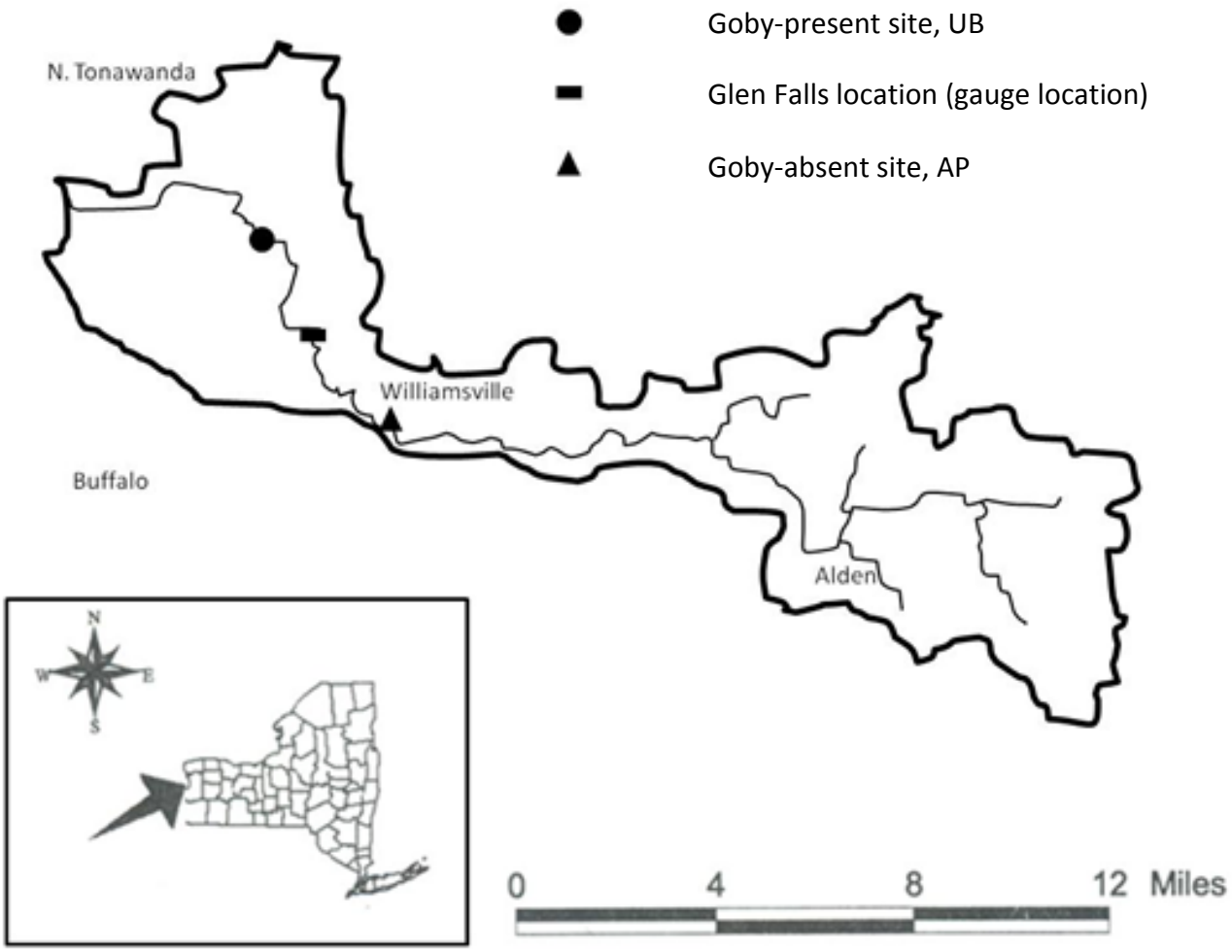

Goby-present site, UB
Glen Falls location (gauge location)
Goby-absent site, AP
N. Tonawanda
Williamsville
Buffalo
Alden
N
W
E
S
0
4
8
12 Miles

Figure 2. Ellicott Creek stream discharge (m^3/s) for 2012. Available stream discharge data recorded by the gauge station between the two study sites for the study period May 2012-September 2012. The data are not available after September due to state budget restrictions.

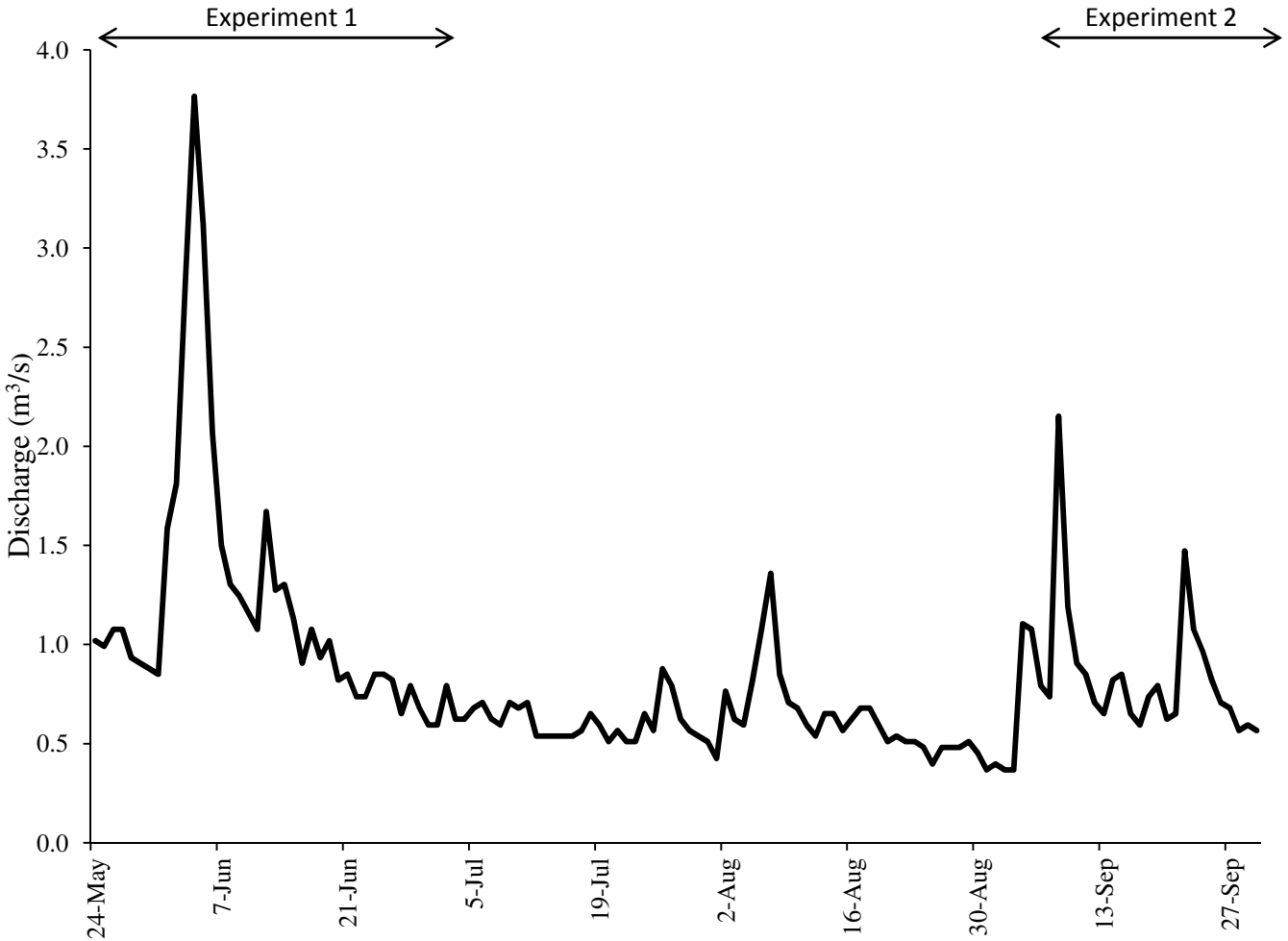

Experiment 1
Experiment 2
Discharge (m3/s)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
24-May
7-Jun
21-Jun
5-Jul
19-Jul
2-Aug
16-Aug
30-Aug
13-Sep
27-Sep

Table 1. Water chemistry and physical conditions for the downstream, goby-present (UB) site and the upstream goby-absent (AP) site at each sampling date. Discharge for the stream was determined from USGS gauging station located midway between sampling locations.

Date	Site	Temp (◦C)	D.O. (mg/L)	Turb (NTU)	Cond (mS/cm)	pH	Discharge (m3/s)
25-May	AP	22.10	8.54	7.0	1.70	7.84	0.99
25-May	UB	22.70	8.90	7.2	1.72	n/a	
14-Jun	AP	18.90	8.35	6.4	1.74	8.20	1.3
14-Jun	UB	19.80	8.68	10.9	1.74	8.40	
28-Jun	AP	19.31	6.57	6.4	2.04	7.97	0.79
28-Jun	UB	21.37	8.16	12.5	1.93	8.14	
25-Jul	AP	22.13	5.83	9.1	2.04	7.63	0.57
25-Jul	UB	23.78	7.62	13.7	1.93	7.98	
7-Sep	AP	22.09	7.75	13.8	2.02	7.19	0.73
7-Sep	UB	22.99	9.59	21.2	1.76	7.52	
20-Sep	AP	13.26	8.83	12.2	2.10	6.97	0.62
20-Sep	UB	16.98	8.88	9.9	1.97	7.50	
12-Oct	AP	10.28	10.43	8.0	2.07	7.50	n/a
12-Oct	UB	11.47	13.69	8.1	2.02	8.33	
12-Nov	AP	10.55	10.33	21.6	1.69	7.70	n/a
14-Nov	UB	7.13	12.85	18.1	1.66	8.22	

the UB site. The contrast was observed when comparing the upstream and downstream location for pH, the UB site always had a slightly elevated pH.

Within the spring sampling period sampling date had a significant effect on conductivity and temperature (Table 2a), and a location effect was observed for temperature (Table 2a) and very marginal effect on pH (Table 2a). Similarly in fall, sampling date also had a significant effect on conductivity (Table 2b) and temperature (Table 2b). Date also was a factor for a significant difference in pH (Table 2b) for the fall sampling period and like spring was also significantly different for locations (Table 2b). Seasonal differences were observed for four of the five water chemistry measures at $p < 0.05$, with the exception of conductivity.

3.2 Leaf Breakdown

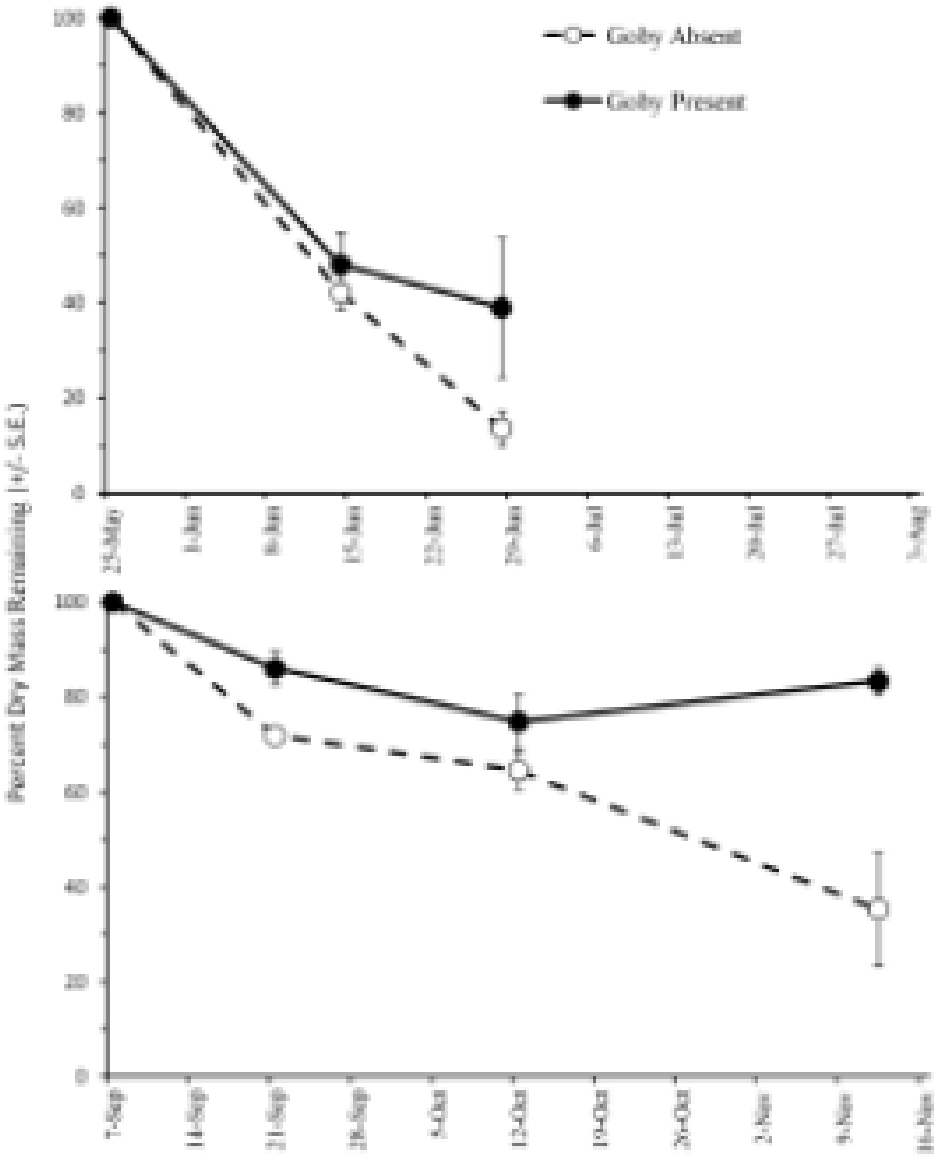
In the spring sampling period at least 50% of the initial leaf mass had been lost at both locations during the first two weeks of incubation. Leaf breakdown rate or the decay rate (k) up to day 30 in the spring sampling period was 0.058 day^{-1} at AP and 0.028 day^{-1} at UB. The decay rate (Figure 3), k, was roughly 2 times quicker at the AP site without round gobies than the UB site where round gobies were present. There was not a significant difference in the breakdown rates between sites in the spring ($F_{1, 2} = 5.97$, $P = 0.134$) or fall ($F_{1, 2} = 0.51$, $P = 0.550$) through day 30, but there was a significant site difference in the fall through day 60 ($F_{1,4} = 14.68$, $P = 0.019$). The number of days the leaf packs were in the stream was a significant predictor of the leaf mass remaining through day 30 for both sampling periods ($P \leq 0.02$) and through day 60 was a less significant predictor of leaf mass remaining ($P = 0.058$).

Table 2. Results of 2-way ANOVA without replication examining each sampling period a) Spring and b) Fall for the effects of sampling date and location on water chemistry data. All df for date = 3 and location = 1 and significant differences at p = 0.05 are denoted by asterisk (*). Two of the five measures in the spring suggested lack of homogeneity of variance but the log transformed data did not change resulting conclusions therefore the data presented is based on the raw values.

a) Spring	df	M.S.	F	*P*
Conductivity (µS/cm)				
Date	3	0.045	18.64	0.0192*
Location	1	0.005	2.05	0.2472
Dissolved Oxygen (mg/L)				
Date	3	1.799	5.90	0.0895
Location	1	2.071	6.79	0.0800
Temperature (°C)				
Date	3	5.778	25.68	0.0122*
Location	1	3.393	15.08	0.0303*
Turbidity (NTU)				
Date	3	6.438	2.01	0.2909
Location	1	29.26	9.12	0.5680
pH				
Date	3	0.084	18.03	0.0530
Location	1	0.086	18.58	0.0498*

b) Fall	df	M.S.	F	*P*
Conductivity (µS/cm)				
Date	3	0.060	10.95	0.0400*
Location	1	0.028	5.07	0.1098
Dissolved Oxygen (mg/L)				
Date	3	6.338	6.72	0.0760
Location	1	7.354	7.80	0.8120
Temperature (°C)				
Date	3	73.40	16.73	0.0224*
Location	1	0.714	0.16	0.7137
Turbidity (NTU)				
Date	3	60.35	5.06	0.1080
Location	1	0.361	0.03	0.8729
pH				
Date	3	0.281	13.15	0.0312*
Location	1	0.611	28.60	0.0128*

Figure 3. Mean (+/- SE) % dry mass remaining for replicate leaf pack bags collected at 2-weeks, 1-month and 2-month for the a) spring and b) fall sampling periods. The spring 2-month collection data is absent due to vandalism of samples in the field.


Goby Absent
Goby Present
Percent Dry Mass Remaining (+/- S.E.)
100
80
60
40
20
0
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
7-Sep
14-Sep
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct
2-Nov
9-Nov
16-Nov

The observed leaf mass loss after two weeks of incubation in the stream during the fall was about half of the observed values at the two week sampling in the spring; about 20% for both sites in fall, when compared to the spring which was at least 50% for both sites. As was true for the spring, the leaves decomposed faster at the AP site without round gobies in the fall compared to the UB site with round gobies present. The leaf breakdown rates for AP and UB in the fall were 0.008 day^{-1} and 0.012 day^{-1} respectively through day 30 and 0.015 day^{-1} and 0.003 day^{-1} respectively through day 60 (Figure 3b).

In the spring there was more leaf material remaining at the goby-present site when compared to the goby-absent site but the difference was not significant ($t = 1.15$, $df = 4$, $P = 0.204$) at day 30. The same also was found for September ($t = 1.42$, $df = 4$, $P = 0.227$). The difference between goby-present and goby-absent leaf mass remaining is more dramatic at 30 days in May than September (Figure 4). The difference in leaf mass loss at the 60-day observation in September was more variable but also was not found to be significantly different ($t = 3.03$, $df = 3$, $P = 0.094$).

3.3 Leaf litter chemistry

The initial litter chemistry in both seasons contained roughly 45-50% DM carbon and 0.60-1.00% DM nitrogen. The leaf chemistry had no date, site or interaction effects during the spring for carbon or nitrogen (Table 3). In the fall only date, or number of days in the stream, had a significant effect on carbon concentration (Table 3). In both seasons the C:N ratio was significantly impacted by date and in the spring site differences also impacted the ratio. Bonferroni pairwise comparisons showed that carbon decreased over time and was higher at the goby-absent site in both seasons. The nitrogen concentration in the leaf material in the spring

Figure 4. Percent (+ S.E.) dry leaf mass remaining at 30 day endpoint for spring (May-June) and fall (September-November) trails. The students t-test assuming equal variances for the spring and unequal variances for the fall showed no significant differences between sites for leaf mass remaining on day 30 for either season.

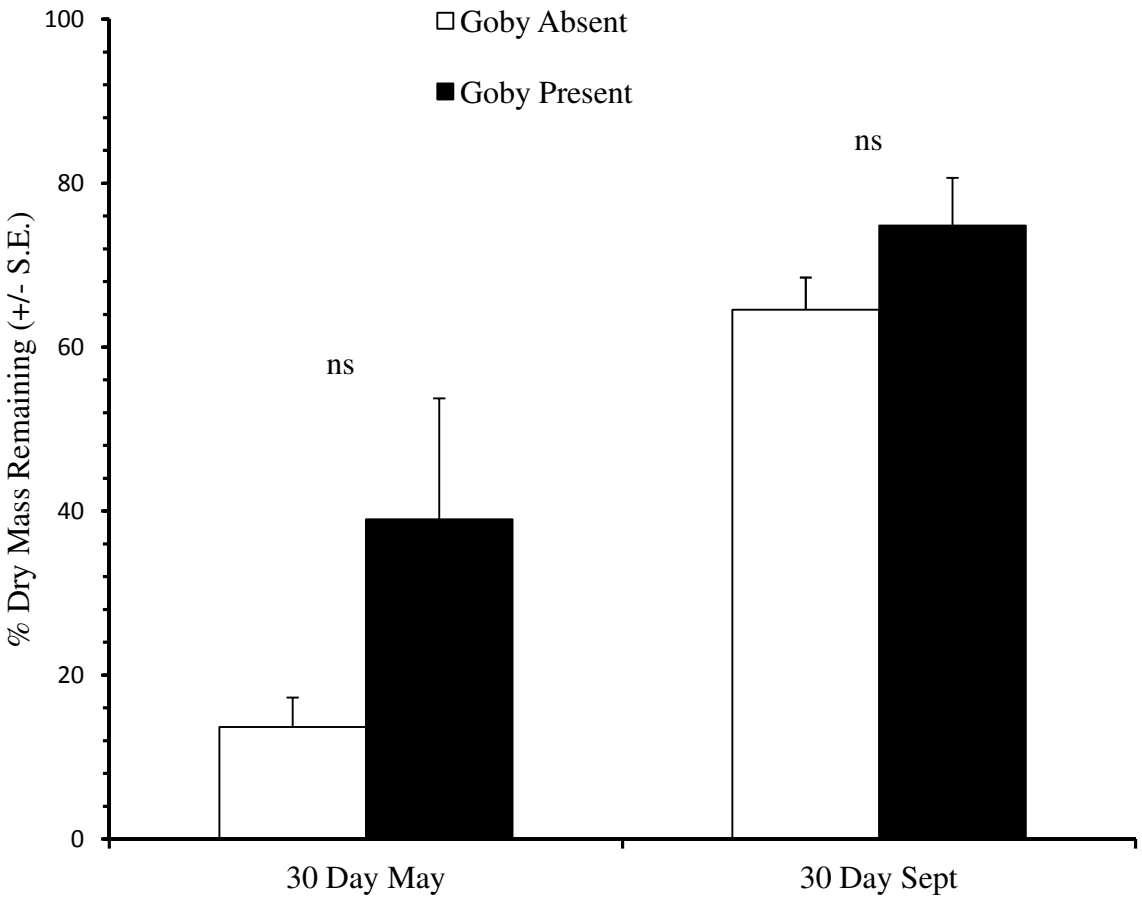

Goby Absent
Goby Present
% Dry Mass Remaining (+/- S.E.)
100
80
60
40
20
0
ns
ns
30 Day May
30 Day Sept

Table 3. Results of 2-way ANOVA examining the leaf chemistry of dried leaf material recovered at the sampling dates throughout each sampling period a) Spring and b) Fall for the main effects of sampling date and location. Significant differences at P = 0.05 are denoted by asterisk (*).

A) Spring	df	M.S.	F	P
%C				
Date	2	93.98	1.45	0.298
Site	1	93.78	1.44	0.269
Date*Site	2	11.71	0.18	0.839
%N				
Date	2	0.17	1.76	0.240
Site	1	0.23	2.37	0.168
Date*Site	2	0.13	1.34	0.322
C:N				
Date	2	837.73	19.79	0.001*
Site	1	704.91	16.66	0.005*
Date*Site	2	27.22	0.64	0.554
B) Fall	df	M.S.	F	P
%C				
Date	3	154.73	6.34	0.009*
Site	1	28.09	1.15	0.306
Date*Site	3	39.71	1.63	0.240
%N				
Date	3	0.14	2.72	0.095
Site	1	0.01	0.18	0.676
Date*Site	3	0.02	0.41	0.747
C:N				
Date	3	1626.58	30.47	0.000*
Site	1	41.18	0.77	0.399
Date*Site	3	101.02	1.89	0.189

peaked at the two-week collection date, but over the course of time generally increased from the beginning. During the spring the average nitrogen content of leaf material was higher at the goby present site. In the fall the nitrogen content increased consistently with time, but did not peak at the two-week mark as it did in the spring, yet the general trend was the same. The goby-absent site on average had a higher average nitrogen content in the leaf material during the fall which was different than the spring results. The C:N ratio for both seasons decreased over time and was consistently higher in the goby-absent site during both seasons.

The carbon concentrations decreased over time for both sites in both seasons. In both spring and fall, after about two weeks the goby-absent site had roughly 5% more carbon in both seasons than the goby-present site. This difference became more dramatic at one month with about 10% more carbon measured at the goby-absent site than the goby-present site (Figure. 5A, 5B).

The nitrogen concentrations of leaf litter generally increased with time in both seasons, but showed a large spike after 2 weeks in the spring (Figure. 5C, 5D). The nitrogen content in the spring at two weeks at the goby-present site was roughly double that of the goby-absent site.

The carbon-to-nitrogen (C:N) ratio followed a similar pattern to the mean % organic matter remaining; as the amount of organic matter remaining declined, the molar ratio of C:N also declined (Figure 6).

3.4 Invertebrates

There were a total of 41 invertebrate taxa identified across all sampling periods (Appendix A). Of these, four (Psephenidae: Psephenus; Siphonuridae: Siphlonurus; Asellidae: Lirceus; Ashenidae: Boyeria) were found only at the goby-absent site, one (Tipulidae: Tipula) was found

Figure 5. Mean percent carbon (A and B) and percent nitrogen (C and D) of dry leaf material at each collection time. Each data point represents the average of triplicate samples taken from each leaf pack (+/- S.E.).

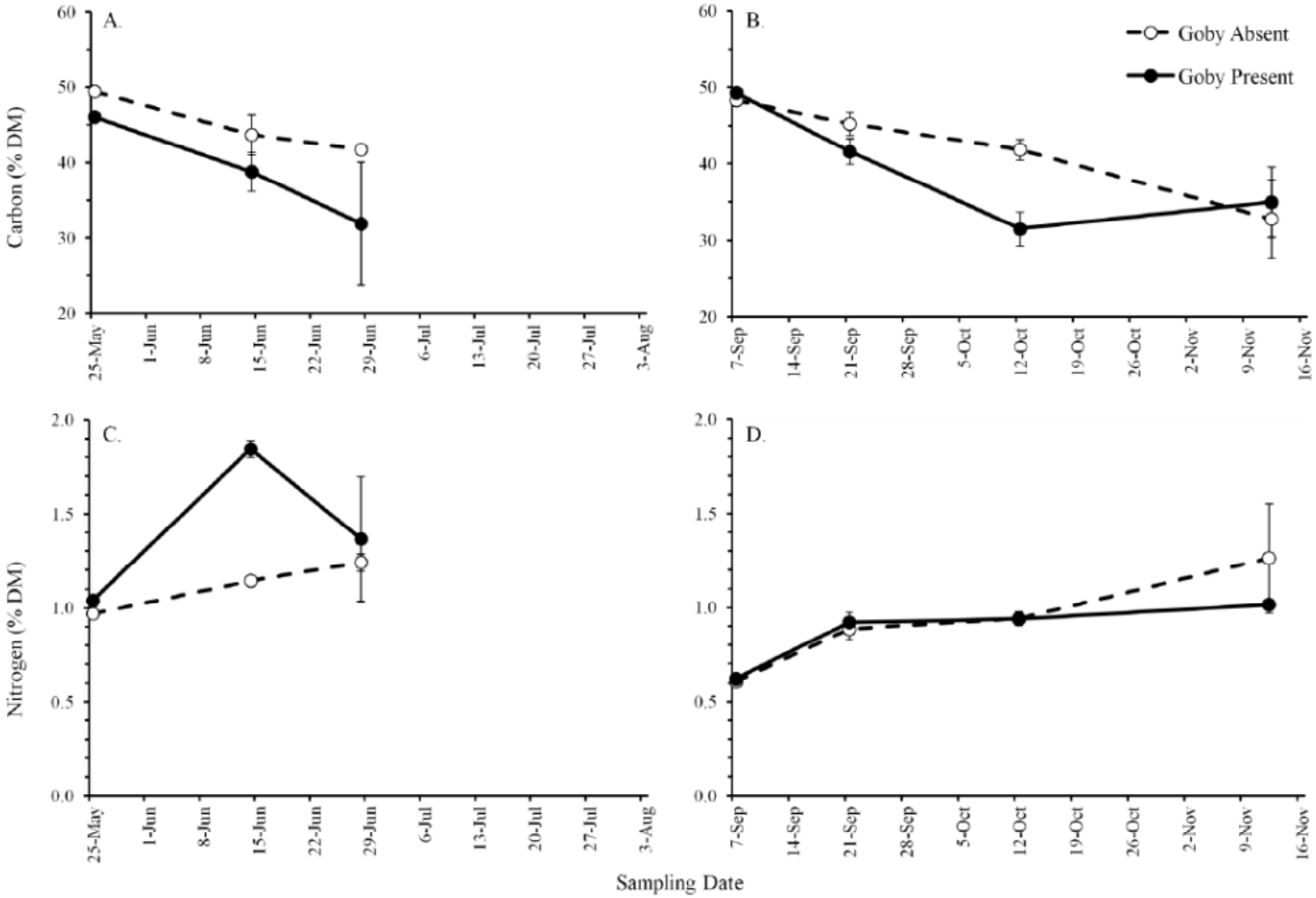
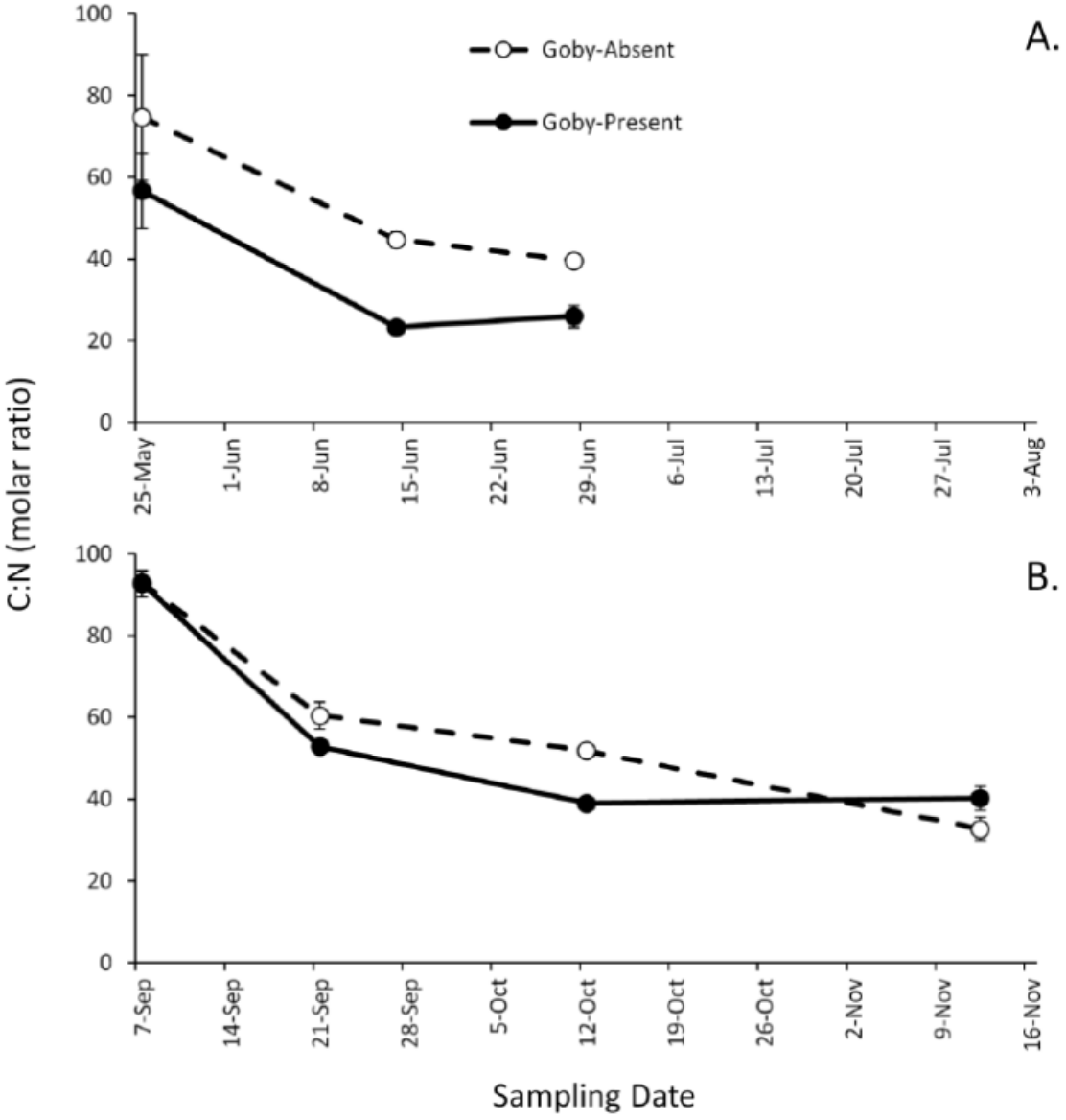

A.
B.
C.
D.
Goby Absent
Goby Present
Carbon (% DM)
Nitrogen (% DM)
Sampling Date
60
50
40
30
20
2.0
1.5
1.0
0.5
0.0
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
7-Sep
14-Sep
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct
2-Nov
9-Nov
16-Nov

Figure 6. C:N molar ratio of dry leaf material at each collection time. Each data point represents the average of triplicate samples taken from each leaf pack (+/- S.E.).


A.
B.
Goby-Absent
Goby-Present
C:N (molar ratio)
Sampling Date
100
80
60
40
20
0
25-May
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
7-Sep
14-Sep
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct
2-Nov
9-Nov
16-Nov

at only the goby-present site and the remainder were found at both locations. Some taxa showed variations with season, occurring only during the spring or the fall at either location or only at one location in spring or fall.

The dominant taxa differed in the spring between sites. At the goby-absent site (AP) shredder species dominated, accounting for about 60% of the community abundance, while at the goby-present site (UB) the shredders represented about 1% (Figure 7). Collector-filters represented just over 50% of the total population at the goby-present site (UB) in the spring (Figure 8). In the fall both locations were dominated by collector-filters largely compromised of Chironomidae. The second most dominant species at each location in the fall differed. At the goby-absent site (AP) the remaining invertebrates were comprised of predators and then shredders, together composing about 25% of the remaining population. At the goby-present site (UB) the collector-filters dominated most of the remainder of the community leaving less than 2% to the remaining three functional feeding groups (predator, scraper and shredder). When looking at taxonomic groupings, the Gammeridae and Elmidae were more abundant throughout both seasons at the goby-absent site. The Chironomidae were more abundant at the goby-present site and there was a large flux of them during the fall sampling. Hydropsychidae also were consistently throughout both seasons more abundant at the goby-present site. Due to the large number of Chironomidae, this was less apparent in the fall. The category of 'other', which includes a number of other invertebrate species, was variable between sites and seasons (Figure 9).

The absolute abundance of invertebrates was less in the spring when compared to the fall, again largely due to the number of Chironomidae found at both locations

Figure 7. Percent of total invertebrate abundance by functional feeding groups for average invertebrate abundance in three leaf packs at each location. For grouping purposes Chironomidae were split between two categories collector-gather and collector-filter.

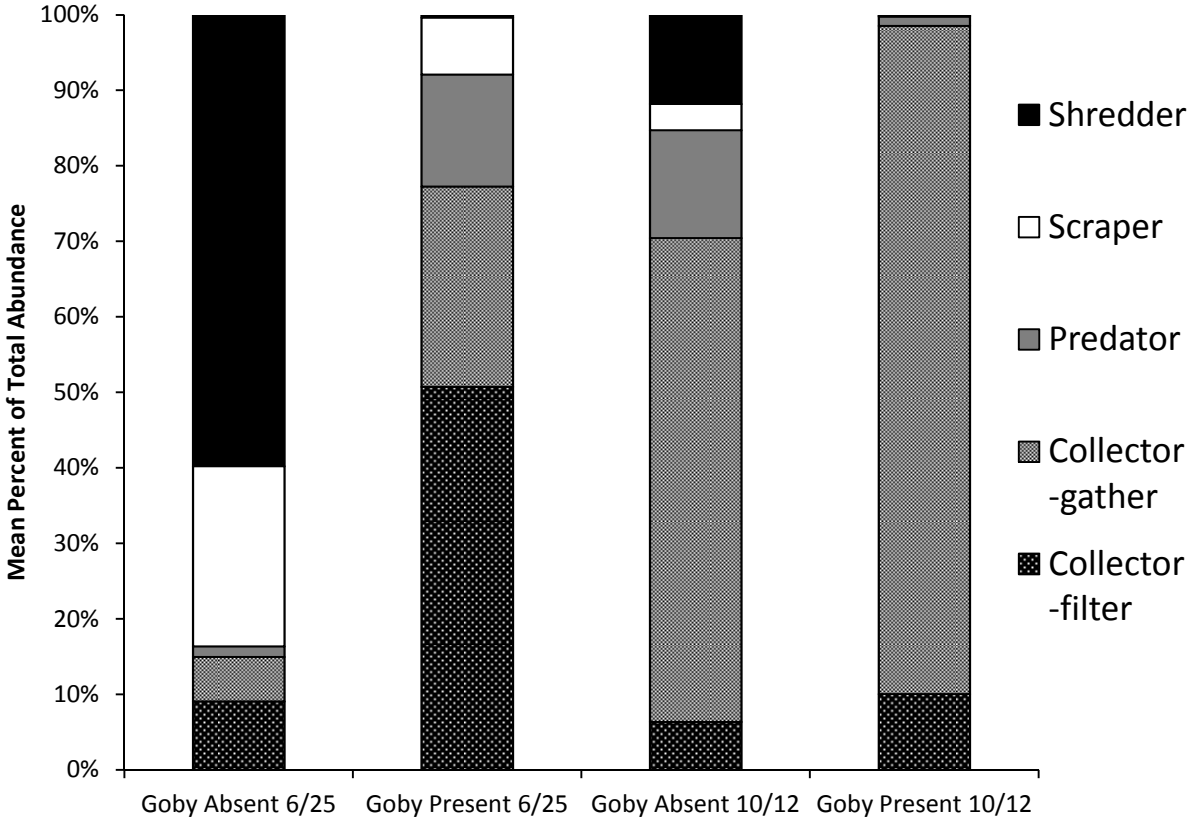

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Mean Percent of Total Abundance
Goby Absent 6/25
Goby Present 6/25
Goby Absent 10/12
Goby Present 10/12
Shredder
Scraper
Predator
Collector -gather
Collector -filter

Figure 8. Mean relative abundance (+/- S.E.) of invertebrate functional feeding groups for leaf packs retrieved from each location at day 30, for a) spring and b) fall.

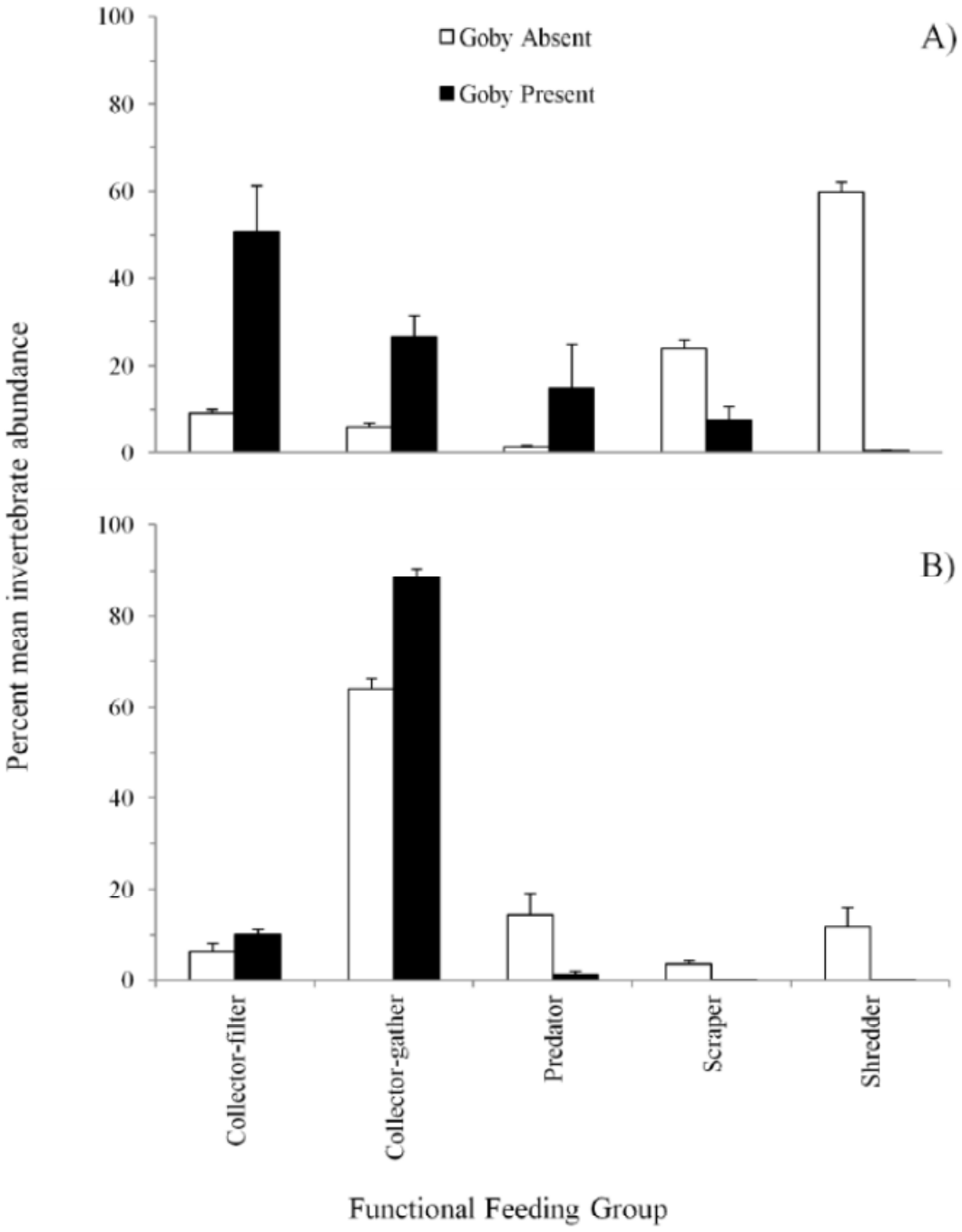
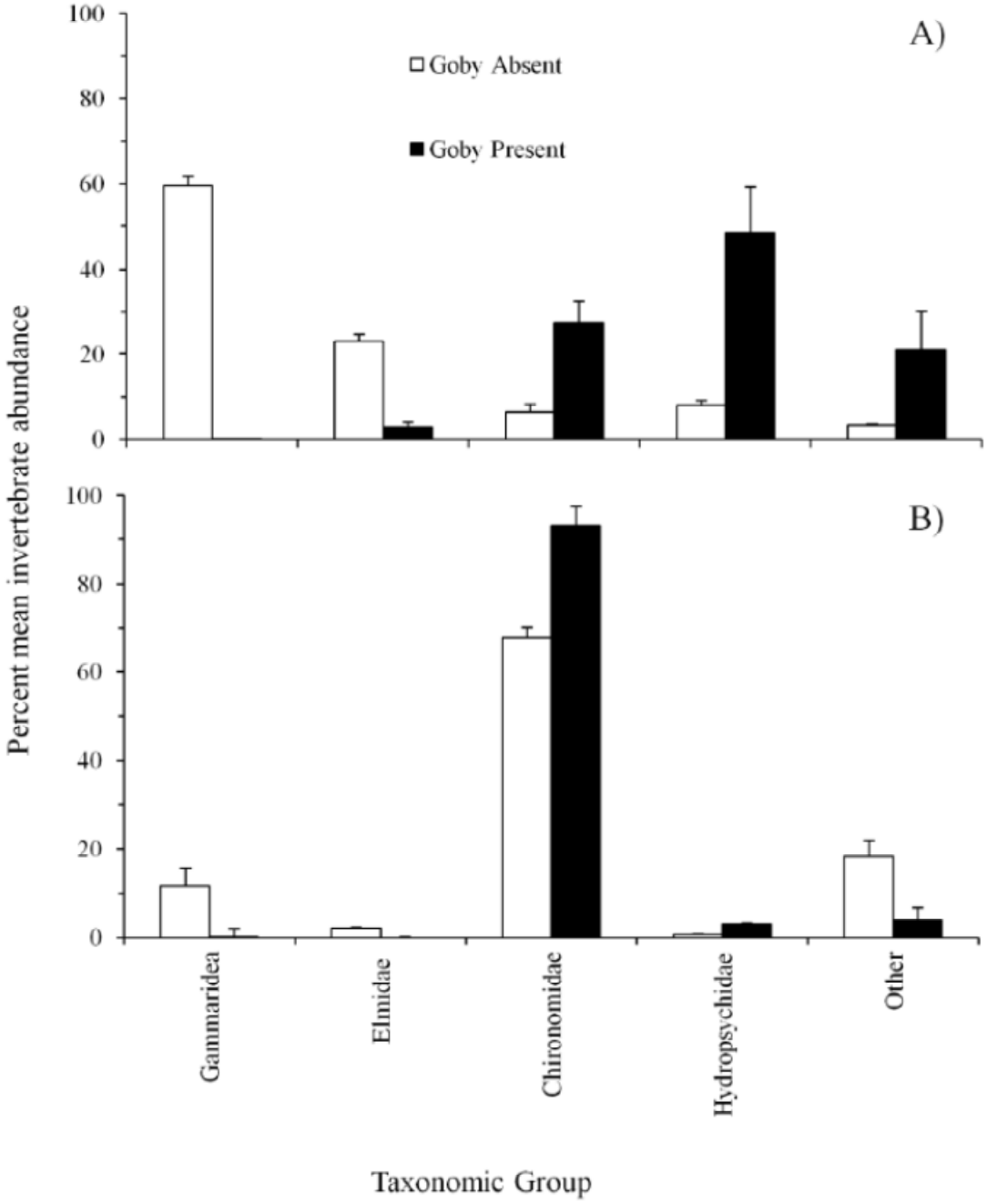

Goby Absent
Goby Present
A)
B)
100
80
60
40
20
0
Percent mean invertebrate abundance
Collector-filter
Collector-gather
Predator
Scraper
Shredder
Functional Feeding Group

Figure 9. Percent of total invertebrate abundance by major taxonomic groups present for average invertebrate abundance in three leaf packs at each location.


A)
Goby Absent
Goby Present
100
80
60
40
20
0
B)
100
80
60
40
20
0
Percent mean invertebrate abundance
Gammaridea
Elmidae
Chironomidae
Hydropsychidae
Other
Taxonomic Group

in the fall season. In the spring the invertebrate communities were highly significantly different between the goby-absent and goby-present locations (Figure 8; G = 711.61, df = 4, P << 0.0001) in terms of functional feeding groups. With the large collector-gather numbers in the fall, the difference also was found to be highly significantly different between locations (Figure 8; G= 486.55, df = 4, P << 0.0001). When the functional feeding group for collector-gathers was removed from the fall calculations, the remaining functional feeding groups were still significantly different between locations (G = 305.50, df = 3, P << 0.0001). The same significant differences were found between the taxonomic groupings for both spring (Figure 9, G = 160.03, df = 4, P<<0.0001) and fall (Figure 9, G = 197.74, df = 1, P<<0.0001). In the fall all groups were collapsed to other except Diptera to satisfy the sample size assumptions of the G-test.

3.5 BIOLOG

The goby-absent and goby-present site data were lumped together for the following results because there were no significant location differences in the tested parameters. Visual observations of the Biolog plates for both sites suggested that by 12-hours post incubation, all of the carbon sources (excluding the three columns of chemical assays) were varying shades of purple, indicating use of substrate by bacteria. This included control well A1, containing no substrate. At the 48-hour mark and all time-points thereafter the number of visually purple wells decreased and created a common pattern among all samples (Appendix B).

Average well color development (AWCD) for all sample collections at all locations followed a sigmoidal curve with time in agreement with Garland and Mills

(1991, Figure 10). In the spring the goby-absent site consistently developed higher ACWD values with time compared to the goby-present site. The trend in the fall remained the same but with more variation. At the two week collection in the fall the ACWD varied between sites, from 0-24 hours the goby-absent site was higher than the goby-present, the converse was true from 36-48 and then the goby-absent site was equal to or greater than the goby-present site. At the fall one- and two-month collections the goby-present site ACWD was higher only at the 12-hour reading and then the goby-absent site remained consistently higher than the goby-present site, similar to the spring (Figure 11, Appendix C).

No significant effects were found for location (goby presence or absence) for ACWD for all carbon sources or when the carbon sources were analyzed respective to guild (Table 4). With respect to season the overall carbon ACWD was significantly different in the spring compared to fall. When separated by specific guild, five guilds (carboxylic acids, amino acids, polymers, phosphorylated chemicals, and other) were found to be significantly different between seasons. For all guilds the ACWD was always higher in the fall (Figure 11).

Carbon consumption richness also was not different between locations in either season based on a threshold value of 0.25 at the 48-hour endpoint. In the spring the bacteria used on average, 30 wells at the goby-present site and 34 wells at the goby-absent site at day 30. The consumption richness increased for both locations. On day 30 in the fall 37 wells and 46 wells were used, on average , for the goby-present and goby-absent locations, respectively. In the spring at both locations, less than 50% of all the

Figure 10. Progression of average well color development for all carbon sources during incubation at the 30-day sampling. Each point represents the average of all carbon sources for three BIOLOG plates.

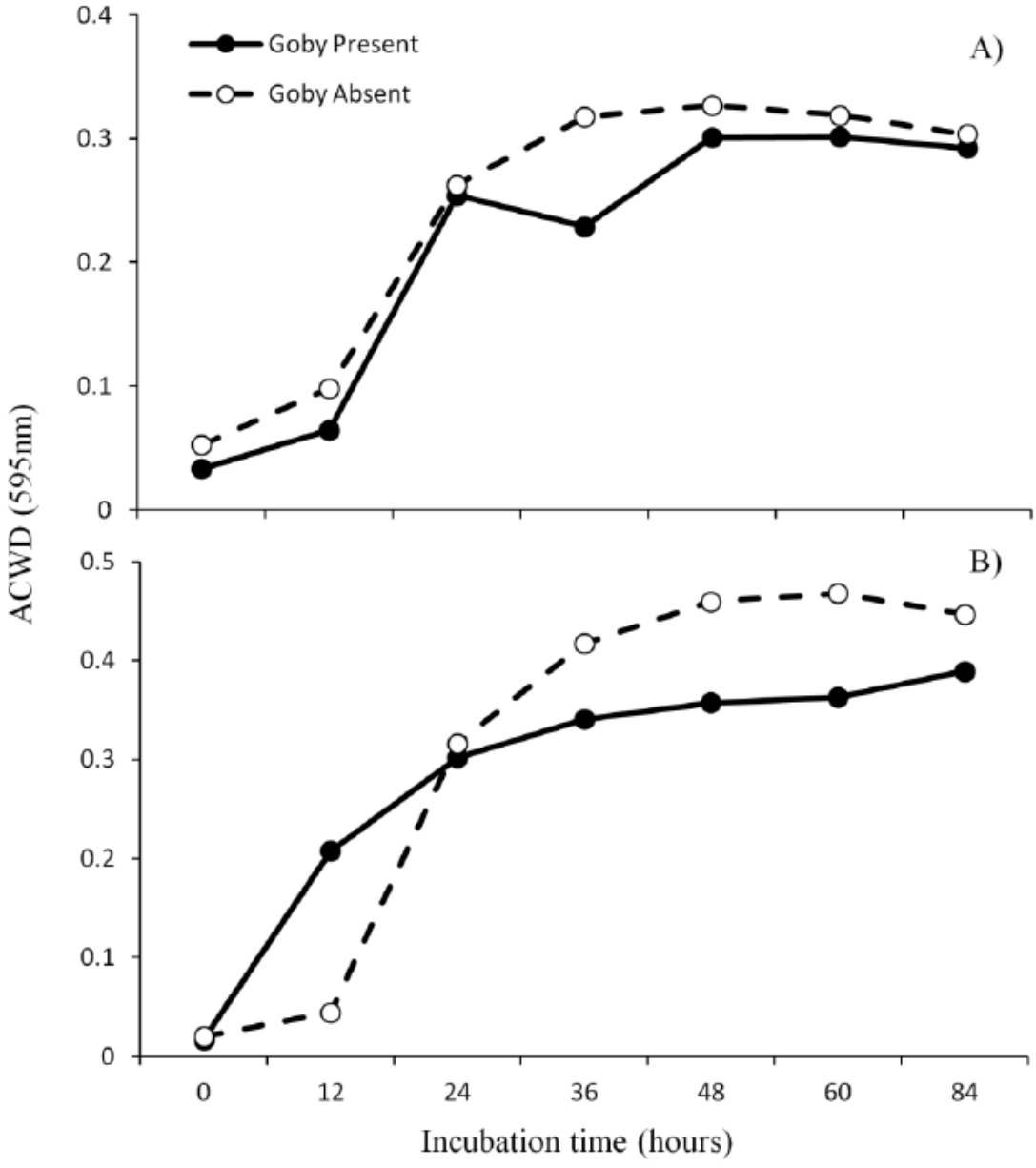
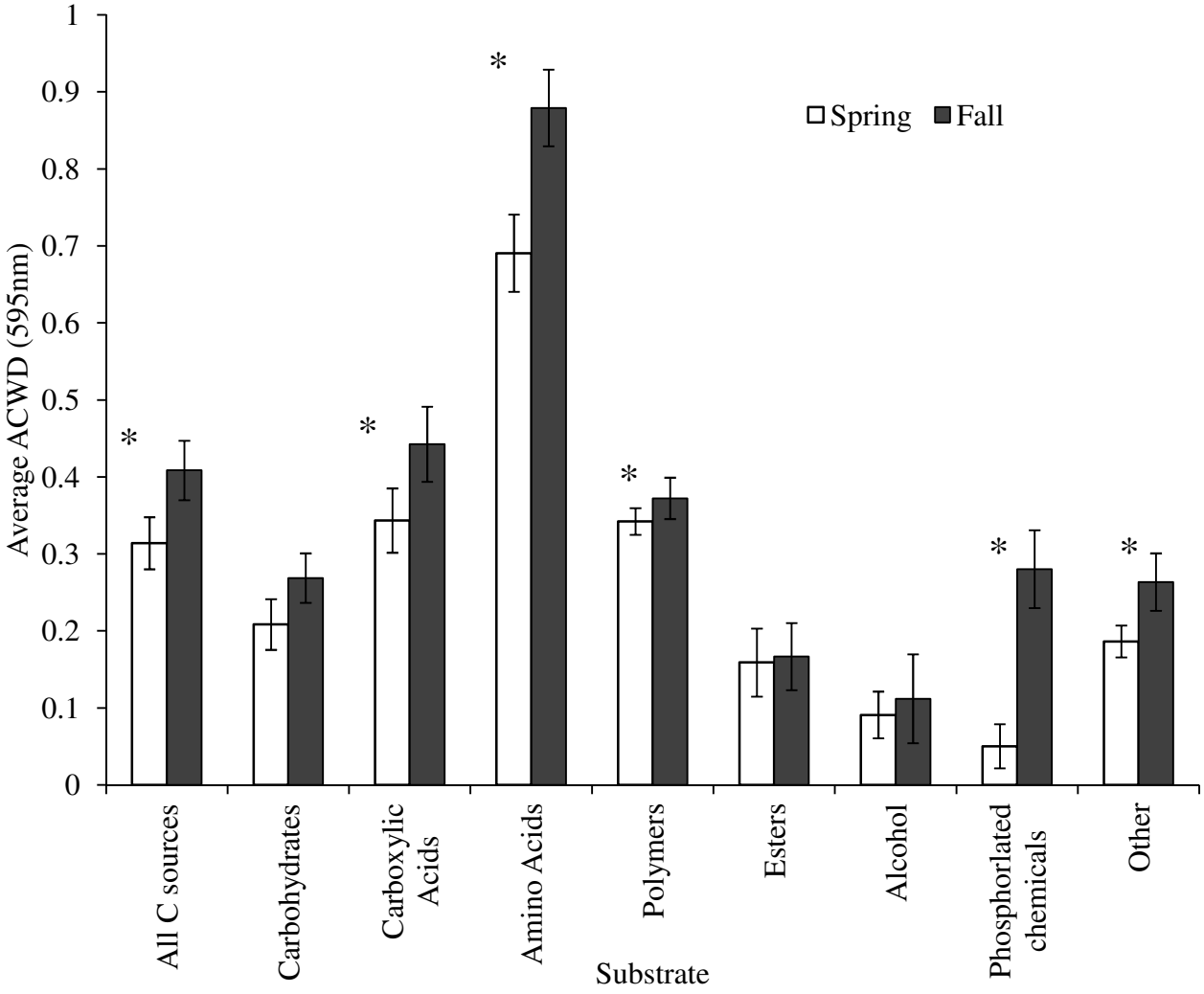

Goby Present
Goby Absent
A)
B)
ACWD (595nm)
0.4
0.3
0.2
0.1
0
0.5
0.4
0.3
0.2
0.1
0
0
12
24
36
48
60
84
Incubation time (hours)

Table 4. ANOVA results for location and season main effects on microbial usage of all carbon sources based on average well color development and then by guild. Bold values represent significant differences at P<0.05 and all d.f.=1.

	MS	F	P		MS	F	P
All Carbon				Polymers			
Location	0.0087	0.58	0.4518	*Location*	0.0001	0.01	0.9408
Season	0.1539	10.38	**0.0034**	*Season*	0.0751	4.5	**0.0435**
Location x Season	0.0002	0.02	0.9024	*Location x Season*	0.0167	0.12	0.7352
Carbohydrates				Esters			
Location	0.0016	0.12	0.7292	*Location*	0	0	0.9671
Season	0.0541	4.11	0.053	*Season*	0.0728	2.82	0.1052
Location x Season	0.0006	0.04	0.8349	*Location x Season*	0.2851	1.1	0.3031
Carboxylic Acids				Alcohols			
Location	0.0227	1.33	0.2587	*Location*	0.0218	1.34	0.2575
Season	0.1974	11.58	**0.0022**	*Season*	0.0085	0.52	0.4766
Location x Season	0.0046	0.27	0.6076	*Location x Season*	0.0008	0.05	0.8237
Amino Acids				Other			
Location	0.0247	1.3	0.2638	*Location*	0.0112	0.65	0.4277
Season	0.4196	22.2	**0.0001**	*Season*	0.2544	14.77	**0.0007**
Location x Season	0.0039	0.19	0.6628	*Location x Season*	0.0048	0.28	0.5992
Phos. Chemicals							
Location	0	0	0.9677				
Season	0.693	17.52	**0.003**				
Location x Season	0.0092	0.23	0.633				

Figure 11. Average well color development (+/- S.E.) at 595nm for guilds by season. Asterisk (*) indicates significant differences based on two way ANOVA results testing season and location main effects on ACWD of guild usage. All d.f. for both location and season were 1 and significance level of $P < 0.05$. No significant location x season interactions were recorded.


Average ACWD (595nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Spring
Fall
*
*
*
*
*
*
All C sources
Carbohydrates
Carboxylic Acids
Amino Acids
Polymers
Esters
Alcohol
Phosphorlated chemicals
Other
Substrate

carbon sources were used at either location compared to in the fall when over 50% of carbon sources were utilized at both locations. The goby-absent site consistently utilized more of the available carbon sources compared to the goby-present location. For both seasons there was a positive correlation between the number of positive wells and time. The differences observed for richness across the incubation time scale were not significant at 30 days when the regression was compared by location in the spring ($F_{1,10}$ = 0.00, P = 0.946).

When the locations were compared at day 30 in the fall, there also was no significant difference in the regression of positive wells ($F_{1,10}$ = 0.74, P = 0.409). The rate of increase of positive wells in the spring between the goby-absent and goby-present locations were nearly identical with slopes of 0.384 and 0.372 respectively.

CHAPTER 4: Discussion

4.1 Stream Chemistry

The stream chemistry parameters measured in Ellicott Creek were slightly variable between the two sites and the variability was expected based on seasonal changes in the stream. The range of difference within a sampling date between the two sites was low, therefore the water chemistry was assumed to be similar on each day sampled. Consistently lower recorded water temperatures at the goby-absent (AP) when compared to the goby-present (UB) site could be attributed to more shading from riparian trees at the goby-absent site. In addition, the largest range between sites for water temperature occurred on the 20 September sampling date and this could be due to a small storm event that occurred around this date (Figure 1).

4.2 Leaf Breakdown

Leaf break down rates are dependent on a number of factors such as leaf species (Webster and Benfield 1986), invertebrate communities (Covich *et al.* 1999, Jonsson *et al.* 2001), microbial communities (Morrison and White 1980, Ardón and Pringle 2008), and stream chemistry (Jonsson *et al.* 2001, Findlay 2010). In this study stream, as previously mentioned, chemistry did not differ between sites, with the exception of seasonal temperature changes and pH. Generally leaf material will breakdown faster in warmer waters due to increased microbial and invertebrate shredding. Faster leaf breakdown was observed in the summer experiment when compared to the fall when water temperatures were lower. Leaf species was controlled by using the same leaf species for all leaf packs. Thus, invertebrate and microbial community differences were

the variables potentially impacting leaf decay rate. As is well established, leaf material is an important source of nutrients to the food webs present in aquatic environments (Cummins 1974, Meyer 1994, Hauer and Lamberti 2007).

Webster and Benfield (1986) published estimates with variation due to the aforementioned effects on leaf breakdown rate for a number of different types of leaf and plant species in all types of aquatic systems. The Aceraceae (maples), which would contain the red maple used in this study, has an estimated breakdown rate of -0.0035 to -0.0050 day^{-1} (Webster and Benfield 1986). A larger range of rates were observed ranging from -0.0019 day^{-1} to -0.0089 day^{-1} in other studies (Ostrofsky 1997, Gulis and Suberkropp 2003b). In addition, the rate for red maple in Ellicott Creek was calculated in 2009 to be -0.0539 day^{-1} at the goby-absent site and -0.0375 day^{-1} at the goby-present site (Janik and Pennuto unpublished data). The observed values in 2009 are a magnitude higher than the estimated values of others (Webster and Benfield 1986, Ostrofsky 1997, Gulis and Suberkropp 2003b). The rates observed by Janik and Pennuto (unpublished data) however, are closer to the observed rates in the same sites in the spring while the fall rates more closely reflect the other studies. The values observed for the spring sampling period in this study and the 2009 study are comparable. The goby-absent site has a faster decay rate than the goby-present site, ranging from 1.44 times faster (Janik and Pennuto unpublished data) to 2.04 times faster (this study). During the fall sampling period it is clear again that leaves at the goby-absent site are decaying faster, compared to the goby-present site, and the decay rates are comparable to the estimated values of Webster and Benfield (1986) and Gulis and Suberkropp (2003b).

Spring decay rates were faster than fall decay rates by 3.87 times at the goby-absent and 10.88 times at the goby-present, likely due to temperature differences between the seasons. This is not surprising as it is established that decay rates are faster in water with warmer temperatures (Webster and Benfield 1986). The dry mass remaining at 30 day at the goby-absent site for the spring sampling in the present study is close to the observed values for summer input maple leaves in a northern Michigan stream at 14 days (Maloney and Lamberti 1995). The difference between day 14 in Maloney and Laberti (1995) study and day 30 in the present study could be due to the differences between streams. It is valuable information that even when stream differences are considered the undisturbed goby-absent site breaks down leaf material at a rate closer to the study by Maloney and Lamberti (1995) than the goby-present site in both the fall and the spring. The fall values have a higher percentage remaining likely due to colder temperatures during this sampling period compared to the spring values. Kobayashi and Kagaya (2005) demonstrated the reverse, with higher leaf breakdown rates occurring in the winter through early spring for riffle habitats. The differences seen between studies could be due to macroinvertebrate assemblages during the specific seasons and specific characteristics of the streams but it is important to note that seasonal differences are important in stream habitats, for leaf litter breakdown.

Leaf decay occurs in three generalized steps: leaching, conditioning, and fragmentation (Gessner 1999). The leaf break down rate can be impacted by a number of factors mentioned above, but initial litter chemistry can also impact the rate of decay and nutrient availability can affect microbial colonization. Some leaf constituents such as phenolic content prevent microbial growth and colonization and the release of organic

matter whilst others promote rapid decay (Gullis and Suberkropp 2003b, McNamara and Leff 2004b, Talbot and Treseder 2012). In this study, the red maple leaf C:N ratio, percent carbon and percent nitrogen were in line with other studies in other east coast streams. Red maple leaf material was between 45-50% carbon and 0.60-1% nitrogen while reported literature values range from 48.2-52.9% and 0.56-1.2% respectively after a period of decay (Delaney *et al.* 1996, Ostrofsky 1997). The initial C:N ratio also coincided with the reported values found by Delaney *et al.* (1996). The C:N ratio observed after two months of decay in this study, roughly 26-40, were in agreement with those reported for a number different climate types; most closely the temperate and boreal regions (Manzoni *et al.* 2010).

Gulis and Suberkropp (2003b) found that there was an increase in N concentration of leaf litter over the duration of leaf submergence. Nitrogen concentration increased marginally throughout this study with the exception of one peak and subsequent decline during the spring at the goby-present location, ultimately resulting though in an overall increase. It may be that the difference lies in the assimilation efficiency and nitrogen requirements of the decomposers and invertebrate communities working on the leaf material at the time. A study conducted by Hall and Meyer (1998) shows that the microbial compartment is capable of assimilating a significant portion of nutrients including N from leaf litter. Increases in N concentration of leaf material over the duration of leaf submergence were on par with those found in another study done comparing unaltered streams and nutrient-enriched streams (Gulis and Suberkropp 2003b). Leaf litter break down has been correlated to increased bacterial biomass and negatively correlated with carbon (Ardón and Pringle 2008). This study also showed that

leaf litter breakdown was correlated with percent carbon remaining in the leaf litter, as leaf decay progressed the amount of carbon remaining in leaf material also declined. Gulis and Suberkropp (2003a) observed increasing bacterial carbon content over the course of the study on decomposing leaf material. This would explain a decrease in carbon content of leaf material as we observed in this study. A decrease in leaf C:N ratio was observed during both study periods and was initially rapid then became more gradual. As the C:N ratio decreased, so did the leaf mass remaining throughout the study. The declining C:N ratio can be correlated to an increase in bacterial growth (McArthur and Richardson 2002), but was not measured in this study.

4.3 Invertebrate compartment

Invertebrates are important to leaf litter break down in terms of fragmentation and consumption. Alterations in invertebrate community structure such as those seen following to the introduction of the round goby can affect litter processing in streams (Karakowiak and Pennuto 2008). Depending on which invertebrates are lost, leaf litter breakdown might decrease, resulting in a reduction on the flux of nutrients to stream food webs (Covich *et al.* 1999, Allen and Castillo 2007). Exclusion of invertebrates from leaf litter packs has been shown to drastically reduce leaf break down rates (Schädler and Brandl 2005). Shredders are an important feeding group increasing leaf litter breakdown rates when they are present compared to when they are not present (Swan and Palmer 2006, Tiegs *et al.* 2008). As a shredder taxon, Gammeridae are clearly an important component to leaf litter break down. Gammurus species, when compared to other invertebrate species, have demonstrated up to 60% higher detritus processing rates as well as up to 500% higher bacterial activity than other shredder species (Tiegs *et al.*

2008, Hunting *et al.* 2012). Thus it is reasonable to suggest, throughout this study a major difference that likely contributed to the differences in leaf break down rates was the presence or relative absence of shredder species between the two sites.

The invertebrate results were as expected for Ellicott Creek based on past preliminary research conducted by the lab. At the upstream, goby-absent site there was a much higher abundance of shredders consisting largely of Gammeridae. Though some shredders were found at the downstream, goby-present site the number was very greatly reduced and at times zero. In streams, such as this one, goby diets consist primarily of invertebrates. A decrease in amphipod abundance has been associated with invasion and increase in round goby presence (review by Kornis *et al.* 2012). Hunting *et al.* (2012) estimated that up to half of the bacterial community in a sample was introduced by the invertebrates present. Furthermore, the structure of the invertebrate community affected the structure and function of the bacterial community. Differences in the carbon source utilization, and probably the structure and functionality of the bacterial community were not observed in this study. This could be because the differences were minute and not detectable or the gobies present in the downstream site reintroduce, through excrement, the missing components of the bacterial community that are void due to the absence of certain invertebrate species. Alternatively, the procedures for isolating the microbial community for inoculation the BIOLOG plate was such that only the most common, culturable taxa survived, leading to the finding of no significant differences between goby-present and goby-absent sites.

Gammeridae are capable of breaking down large amounts of leaf material per day and a decrease or replacement of native species can alter the leaf break down rates

significantly (Piscart *et al.* 2011). Ruetz *et al.* (2006) also noted that the assemblage of invertebrates colonizing red maple leaf packs were composed of a shredding amphipod species and leaf breakdown rates were highest when shredders were present. The leaf break down rate at the goby-absent site was greater than at the goby-present site and likely was due to the presence of shredder species, namely Gammeridae.

Pennuto *et al.* (2010) and Phillips *et al.* (2003) found that the Chironomidae were consumed most by the round goby in streams. During both the spring and fall sampling periods, our data suggest that within the leaf pack communities there were more Chironomidae at the goby-present location and the numbers in the fall were much larger. The presence or absence of gobies did not seem to affect the Chironomidae. In streams, round gobies do not undergo a drastic otogenic diet shift from invertebrates to Dressiena as they do in the lakes, but they do have an affinity for mollusks such as fingernail clams (French and Jude 2001). Though there was not a trend observed in this study for goby presence and fingernail claims (Sphaeriidae) there was a clear trend apparent with the gastropod Physidae. At the goby-absent site Physidae were present in almost every leaf pack whereas at the goby-present site they were rarely present, if at all. This trend did not hold true for other gastropods collected from leaf packs in this study. The other common gastropod group, Pleroceridae, possess thick shells which are difficult to crush. This shell feature may provide them safe refuge from round goby predation.

4.4 Microbial compartment

Morrison and White (1980) showed altered microbial community function and an increase in microbial biomass when the leaf material was grazed by a Gammarus

amphipod. The presence of amphipods altered the biofilm community growing on the leaves visible by scanning electron microscopy, increased the biomass of bacteria despite amphipod grazing and increased metabolic activity. Leaf decay has been linked to both microbial conditioning and activity of shredders and it seems difficult to tease out the factors responsible for each. The current study did not measure microbial biomass and contrary to Morrison and White (1980) did not find functional community differences in the microbial profile. However, in the presence of Gammaridae, leaf decay rate was faster suggesting there may be bottom up controls impacting the microbial populations and therefore the shredding activities of the amphipods at the goby-absent site. At the goby-present site it is likely that the introduced goby is putting pressure on the amphipod link of this trophic web, decreasing their abundance, and therefore decreasing the microbial growth on leaf material, hence slowing leaf break down rates. Hunting *et al.* (2012) found in mesocosms between 30-60% of the bacteria present was of invertebrate origin. Again this study did not find differences in the function of the microbial communities in the presence or absence of round gobies, it also did not look at the microbial community members as individuals. If molecular analysis had been conducted differences may have detected.

A study by Kostalos and Seymour (1976) revealed that the survivorship of Gammarus may be related to the microbiota present on the leaf material and suggested that leaf material containing fungi > bacteria > sterile leaf material was best in supporting the survivorship of Gammarus. Fungi contribute about 98% of total microbial biomass on decaying leaf litter (Daurte *et al.* 2010). Though the microbes only contribute about 2% of the total biomass because of the rapid turnover, the fungi and bacteria break down

leaf material at about an equal rate. This study did not look at the presence, absence or differences in the fungi present on the leaf material and in addition found that the bacterial communities based on carbon source profiles were the same between locations. Survivorship of Gammarus downstream could be affected by an altered fungal community when round gobies are present in addition to the likelihood they are the main prey item for round gobies.

A control of sterile leaves or a day 0 microbial analysis of leaf material was not conducted. It can be assumed that the leaf material had a microbial community present prior to drying and possibly a different one prior to placement in the stream. The microbial colonization of leaf litter from the stream water is expected to be rapid. A comparison between exposed leaves and dialysis bag-enclosed leaves showed varying results when microbial cells were counted but the numbers for both exposed and dialysis-enclosed leaves were both increasing over time and in general was more rapid on the exposed leaves (McNamara and Leff 2004a). Based on this, I assume that the microbial community present on the leaf material when it enters the stream has a marginal effect on the community that eventually develops on the leaf material.

I expected to find microbial communities that differed between the two sites and potentially between the sampling dates. In previous studies, it was found that fungal and microbial communities changed with time based on denaturing gradient gel electrophoresis and microscopic analysis, respectively (Duarte *et al.* 2010). It is likely the succession of microflora varies based on the leaf constituents available as conditioning progresses. The present study focused on the functionality of the whole community present and found no significant differences on date sampled or stream

locations, only seasonally different guild usage. The microbial communities were not functionally different between the sites in the spring or the fall. The communities appear to be cosmopolitan throughout the stream reach studied, yet variable between seasons. It is likely that the drastic change in temperature in the fall could have affected the structure and function of the microbial community present on the leaf litter (Allan and Castillo 2007). A factor that may have affected our ability to detect differences is the rapid substrate consumption in all wells of the BIOLOG GEN III, including the control well. This was likely due to the method used to concentrate and purify the environmental bacterial sample. The shake-centrifuge method used allowed residual organic matter from the leaf material to be present in the bacterial pellet. It was nearly impossible to separate the organic matter to obtain a pure bacterial sample, without streaking plates and greatly diminishing the diversity of the sample that we used to inoculate the BIOLOG plates. Similar color development in the control wells also was found in other environmental studies (Garland and Mills 1991, Smalla *et al.* 1998). Before analysis, the density value obtained in the control well at each reading was subtracted from every other well to obtain a corrected absorbance reading for each well and time. Once this was done the AWCD curve over the course of incubation followed the same sigmoidal curve as found by Garland and Mills (1991). Garland (1996) also found that the pattern observed on the plates was more defined as incubation time progressed based on scores from coordination plots. A similar trend was observed in this study, though the results were not quantified. The change in patterns over time could be due to enrichment of certain community members or, reduction of diversity leading to functional changes in the microbial community in the various wells (Smalla *et al.* 1998, Matsui *et al.* 2001).

Bacterial response may be tied to the leachate produced by the leaf species being used. In the present study the same leaf species was used for all trials. Microbial populations respond rapidly to and grow rapidly from the DOC leached from the leaf material (McArthur and Richardson 2002, Kreutzweiser and Capell 2003). The aforementioned study showed varying growth rates of bacteria based on the DOC leachate obtained for the different leaf types. It is possible then that the expected differences in microbial communities at the goby-present and goby-absent locations were in part masked because of the limitations imposed by using only one leaf type. It is also possible that the bacterial communities change so rapidly using the DOC leached from the leaves that the collection schedule for samples used in this study was not adequate enough to provide these data. Daily data collections for the first week, and possibly hourly during the first 24 hours may have observed more significant changes to the microbial carbon utilization profiles during the most rapid period of leaf decay but processing time of each sample would not have allowed for this in the current project. By doing so we may have observed a shift in the microbial community over time, which is expected for both locations. If differences were present these would have been more apparent with more data points during analysis.

Well color development is a result of three factors (Gamo and Shoji 1999): density of microbes that can utilize the substrate, growth rate of the utilizers and incubation period. The overall density of microbes in the sample was the only thing accounted for in this study as it is impossible to know and determine the density of all functional utilizers present, or their growth rate. The lack of a difference between goby-present and goby-absent locations could have been due to minute differences were

masked by the residual organic matter. Additionally, a limited number of numerically dominant microbes might overwhelm the input of the minor components of the community (Smalla *et al.* 1998). It is also probable that a majority of the community members living on leaf material have a suite of similar functions and therefore have the ability to metabolize a number of the same carbon sources producing the common pattern observed on all plates in this study. Several studies have documented an increase in microbial diversity or richness over the course of leaf decay (Ardón and Pringle 2008, Duarte *et al.* 2010, Kominoski *et al.* 2011) and I expected communities in this study to change in a similar manner. Since carbon consumption richness did not change as expected it suggests that the microbial community has redundant functionality in terms of the carbon assay used. However, differences in microbial community carbon usage have been detected in larger ecosystems such as Lake Erie using the BIOLOG ECO plates. Though Lake Erie is a lentic environment, due to its shallow depth it undergoes a significant amount of mixing. The results of a study by Hoostal and Bouzat (2008) revealed that there were different community profiles in the different basins of Lake Erie based on the availability of organic matter which was chemically different. Lohner *et al.* (2007) also discovered community differences within Lake Erie related to the presence or absence of Dreisenna. The introduction of the round goby has been shown to affect the breakdown of leaf litter and invertebrates. Literature suggests that the structure of the invertebrate community can impact the microbial profiles (Hieber and Gessner 2002). These studies support the hypothesis that there should be different community profiles in the presence and absence of round gobies, but the data from this study does not support this expectation. The difference of the lentic occasional mixing of Lake Erie and the

constant "mixing" of a lotic system may create an environment where microbial communities are cosmopolitan throughout and the disturbance of an introduced species is not significant enough to disrupt the community at this level.

A number of drawbacks exist in using the BIOLOG plates to understand the microbial community structure and function (Graham and Haynes 2005 and references therein). One of the major issues is how the BIOLOG plates affect the initial community, with high concentrations of the carbon source not likely representing the sample environment and the potential toxicity of some substances to certain bacteria. Yet it has still been established as a screening method for community profiling. Many researchers use BIOLOG in conjunction with other methods, such as molecular analysis, to paint a more clear, complete picture. This study could have benefited from an additional technique to compare the study sites. The carbon use profiles suggest that the presence of the round goby does not alter the community function but it may alter the community structure and this is where the BIOLOG plates are insufficient. Throughout incubation of the BIOLOG plates, changes in the original community have been documented (Matsui *et al.* 2001) putting into question what time point should be used to do analysis as different results may be obtained at each 12-hour reading. This study found no differences between the 48-hour time point used and the end point of the BIOLOG readings.

The method to remove the microbes from the leaf material needs to be refined for future studies. As leaf material decays, removing pure microbe inoculants from the leaf material becomes increasingly difficult since the leaf material becomes more fragile and breaks apart easily. Sonication maybe a less destructive method than agitation to achieve a representative microbial community (Gulis and Suberkropp 2003b). This also has

drawbacks associated with excessive heat and cell rupturing that could potentially alter the community present. Another option would be to streak the environmental sample and take a representative sample of colonies for inoculation. The limitation to this method is a probable loss of the representative community due to the culturability of different microbes.

In addition, this study did not consider the relevance of anerobic bacterial strains. These strains can thrive in microenvironments created by the leaf material as it is packed together and they can be responsible for consumption, conversion and excretion of nutrients derived from the leaf material. The BIOLOG plates are not capable of detecting the carbon usage profiles of anaerobic strains and future studies may want to attempt to detect the presence or absence of anoxic environments in leaf packs using microprobe instrumentation or detection of anaerobic bacteria. This, along with data on the fungal community would provide a better picture of the whole microbial environment.

In conclusion, the leaf decay rate was greatest in leaf packs from the goby-absent site and was likely due to round gobies impacting the invertebrate community. However the data presented do not support the hypothesis that the goby impact filters down to the microbial level as determined by community carbon usage. Therefore it is likely that the leaf decay rate differences are a result of the altered invertebrate community and not the microbial community. Further research is needed to assess the microbial compartment of the stream and determine if the lack of difference was a result of experimental methods used or a true lack of variation among sampling sites.

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Appendix A

				AP 6/14/12			UB 6/14/12		
Order	Family	Genus	Functional Feeding	1	2	3	1	2	3
Amphipoda	Gammaridea		Shredder	311	286	352	0	0	0
Coleoptera	Elmidae (adult)		Scraper; Collectors-gatherer	90	119	180	1	2	3
Coleoptera	Elmidae (larva)		Scraper; Collectors-gatherer	5	14	26	6	1	2
Coleoptera	Psephenidae		Scraper	0	2	0	0	0	0
Coleoptera	Scirtidae	cyphon	Scraper	0	0	0	0	0	0
Diptera	Athericidae	atherix	Predator	0	0	0	0	0	0
Diptera	Chironomidae		Collector-gather, filter	48	81	168	72	35	122
Diptera	Chironomidae (adult)		N/A	0	0	0	0	0	0
Diptera	Chironomidae (paupa)		N/A	1	2	3	1	0	1
Diptera	Empididae	hemerodromia	Predator	1	0	1	7	0	2
Diptera	Muscidae		Predator	0	0	0	0	0	0
Diptera	Simuliidae		Collector-filter	0	0	0	0	0	2
Diptera	Tipulidae	tipulda	Shredder	0	0	0	1	0	0
Diptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Ephemoptera	Baetidae		Collectors-gatherer; Scraper	0	0	0	0	0	0
Ephemoptera	Caenidae	caenis	Collectors-gatherer; Scraper	0	0	0	0	0	0
Ephemoptera	Heptageniidae	stenacon	Collectors-gatherer; Scraper	0	2	3	2	0	3
Ephemoptera	Siphlonuridae	siphlonurus	Collectors-gatherer; Scraper	1	0	0	0	0	0
Gastropoda	Physidae		Scraper	2	3	2	0	0	0
Gastropoda	Pleuroceridae		Scraper	0	1	0	1	0	8
Hempitera	Unidentified (terresterial)		N/A	0	1	1	0	0	1
Isopoda	Asellidae	caecidotera	Shredder	0	0	1	0	0	0
Isopoda	Asellidae	lirceus	Shredder	0	0	0	0	0	0
Unionida	Sphaeriidae		Collector-filter	0	0	0	1	0	0
Gastropoda	Unidentified		N/A	0	0	0	0	0	0
Odonata	Aeshnidae	boyeria	Predator	0	0	0	0	0	0
Odonata	Coenagrienidae	nehalennia	Predator	0	0	0	0	0	0
Oligochaeta	Unidentified		N/A	0	0	0	12	0	0
Platyhelminthes	Planariidae	planaria	Predator	0	0	0	98	6	31
Plecoptera	Taeniopterygidae	taenipteryx	Shredder	0	0	0	0	0	0
Tricoptera	Brachycentridae	Micrasema	Shredder	0	0	0	0	0	0
Tricoptera	Heilopsychidae		Scrapper	0	0	0	0	0	0
Tricoptera	Hydropsychidae		Collector-filter	25	28	68	27	10	35
Tricoptera	Hydroptilidae		Collectors-gatherer; Scraper	0	0	0	2	0	0
Tricoptera	Leptoceridae	nectopsyche	Shredder	0	0	0	0	0	0
Tricoptera	Philopotamidae		Collector-filter	0	0	0	0	0	0

Tricoptera	Rhyacophilidae		Predator	0	0	0	0	0	0
Tricoptera	Sericostomatidae		Shredder	0	0	0	0	0	0
Tricoptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Zygoptera	Unidentified		N/A	0	0	0	0	0	0
	Gobiidae	neogobius		0	0	0	0	0	0
	Hydrachnidiae		Predator	0	0	0	3	0	1
				AP 6/25/12			UB 6/25/12		
Order	Family	Genus	Functional Feeding	1	2	3	1	2	3
Amphipoda	Gammaridea		Shredder	220	209	323	0	1	1
Coleoptera	Elmidae (adult)		Scraper; Collectors-gatherer	67	52	91	3	1	19
Coleoptera	Elmidae (larva)		Scraper; Collectors-gatherer	30	27	20	4	0	25
Coleoptera	Psephenidae		Scraper	3	0	0	0	0	0
Coleoptera	Scirtidae	cyphon	Scraper	0	0	0	0	0	0
Diptera	Athericidae	atherix	Predator	0	0	0	0	0	0
Diptera	Chironomidae		Collector-gather, filter	22	9	57	59	64	245
Diptera	Chironomidae (adult)		N/A	0	0	0	0	0	0
Diptera	Chironomidae (paupa)		N/A	1	0	0	0	1	0
Diptera	Empididae	hemerodromia	Predator	4	2	11	11	2	13
Diptera	Muscidae		Predator	0	0	0	0	0	0
Diptera	Simuliidae		Collector-filter	0	0	0	1	0	0
Diptera	Tipulidae	tipulda	Shredder	0	0	0	1	0	0
Diptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Ephemoptera	Baetidae		Collectors-gatherer; Scraper	0	0	0	0	0	0
Ephemoptera	Caenidae	caenis	Collectors-gatherer; Scraper	0	0	0	1	0	0
Ephemoptera	Heptageniidae	stenacon	Collectors-gatherer; Scraper	0	0	0	9	0	0
Ephemoptera	Siphlonuridae	siphlonurus	Collectors-gatherer; Scraper	0	0	0	0	0	0
Gastropoda	Physidae		Scraper	2	2	0	0	0	1
Gastropoda	Pleuroceridae		Scraper	0	0	2	5	6	32
Hempitera	Unidentified (terresterial)		N/A	0	0	0	0	0	0
Isopoda	Asellidae	caecidotera	Shredder	0	0	0	0	0	0
Isopoda	Asellidae	lirceus	Shredder	0	1	0	0	0	0
Unionida	Sphaeriidae		Collector-filter	0	0	0	0	0	6
Gastropoda	Unidentified		N/A	0	0	0	0	0	0
Odonata	Aeshnidae	boyeria	Predator	1	0	2	0	0	0
Odonata	Coenagrienidae	nehalennia	Predator	0	0	0	0	0	0
Oligochaeta	Unidentified		N/A	0	0	0	0	0	0
Platyhelminthes	Planariidae	planaria	Predator	0	0	0	0	88	0
Plecoptera	Taeniopterygidae	taenipteryx	Shredder	0	0	0	0	0	0
Tricoptera	Brachycentridae	Micrasema	Shredder	0	0	0	0	0	0
Tricoptera	Heilopsychidae		Scrapper	0	0	0	0	0	0

Tricoptera	Hydropsychidae		Collector-filter	33	19	54	61	99	827
Tricoptera	Hydroptilidae		Collectors-gatherer; Scraper	0	0	0	0	0	3
Tricoptera	Leptoceridae	nectopsyche	Shredder	0	0	0	0	0	0
Tricoptera	Philopotamidae		Collector-filter	0	5	4	0	0	0
Tricoptera	Rhyacophilidae		Predator	0	0	0	0	0	1
Tricoptera	Sericostomatidae		Shredder	0	0	0	0	0	0
Tricoptera	Unidentified (paupa)		N/A	0	0	1	1	3	2
Zygoptera	Unidentified		N/A	0	0	1	0	0	0
	Gobiidae	neogobius		0	0	0	0	0	0
	Hydrachnidiae		Predator	0	0	0	2	0	6

				AP 7/25/12			UB 7/25/12		
Order	Family	Genus	Functional Feeding	1	2	3	1	2	3
Amphipoda	Gammaridea		Shredder	23	99	52	5	2	0
Coleoptera	Elmidae (adult)		Scraper; Collectors-gatherer	29	153	43	0	2	8
Coleoptera	Elmidae (larva)		Scraper; Collectors-gatherer	2	45	4	7	10	61
Coleoptera	Psephenidae		Scraper	0	0	0	0	0	0
Coleoptera	Scirtidae	cyphon	Scraper	0	0	0	0	0	0
Diptera	Athericidae	atherix	Predator	0	0	0	0	0	0
Diptera	Chironomidae		Collector-gather, filter	5	29	13	0	20	0
Diptera	Chironomidae (adult)		N/A	0	0	0	0	0	0
Diptera	Chironomidae (paupa)		N/A	1	1	1	0	0	0
Diptera	Empididae	hemerodromia	Predator	0	0	1	0	3	0
Diptera	Muscidae		Predator	0	0	0	0	0	0
Diptera	Simuliidae		Collector-filter	1	2	0	0	0	0
Diptera	Tipulidae	tipulda	Shredder	0	0	0	0	0	1
Diptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Ephemoptera	Baetidae		Collectors-gatherer; Scraper	0	0	0	0	0	0
Ephemoptera	Caenidae	caenis	Collectors-gatherer; Scraper	0	0	0	0	2	0
Ephemoptera	Heptageniidae	stenacon	Collectors-gatherer; Scraper	1	9	0	0	3	0
Ephemoptera	Siphlonuridae	siphlonurus	Collectors-gatherer; Scraper	0	0	0	0	0	0
Gastropoda	Physidae		Scraper	6	2	2	0	0	0
Gastropoda	Pleuroceridae		Scraper	0	3	0	25	10	10
Hempitera	Unidentified (terresterial)		N/A	0	0	0	0	0	0
Isopoda	Asellidae	caecidotera	Shredder	0	2	0	0	0	0
Isopoda	Asellidae	lirceus	Shredder	0	0	1	0	0	0
Unionida	Sphaeriidae		Collector-filter	0	0	0	0	0	0
Gastropoda	Unidentified		N/A	0	0	0	0	0	0
Odonata	Aeshnidae	boyeria	Predator	0	1	0	0	0	0
Odonata	Coenagrienidae	nehalennia	Predator	0	0	0	0	0	0
Oligochaeta	Unidentified		N/A	0	0	0	0	0	0

Platyhelminthes	Planariidae	planaria	Predator	0	14	23	18	59	200
Plecoptera	Taeniopterygidae	taenipteryx	Shredder	0	0	0	0	0	0
Tricoptera	Brachycentridae	Micrasema	Shredder	0	1	0	0	0	0
Tricoptera	Heilopsychidae		Scrapper	5	13	0	0	0	0
Tricoptera	Hydropsychidae		Collector-filter	10	19	13	4	31	22
Tricoptera	Hydroptilidae		Collectors-gatherer; Scraper	0	2	0	0	0	0
Tricoptera	Leptoceridae	nectopsyche	Shredder	0	0	0	0	0	0
Tricoptera	Philopotamidae		Collector-filter	0	11	7	1	0	0
Tricoptera	Rhyacophilidae		Predator	0	0	0	0	0	0
Tricoptera	Sericostomatidae		Shredder	0	0	0	0	0	0
Tricoptera	Unidentified (paupa)		N/A	0	0	1	0	0	1
Zygoptera	Unidentified		N/A	0	0	0	0	0	0
	Gobiidae	neogobius		0	0	0	0	0	0
	Hydrachnidiae		Predator	0	0	0	0	1	1

				AP 9/20/12			UB 9/20/12		
Order	Family	Genus	Functional Feeding	1	2	3	1	2	3
Amphipoda	Gammaridea		Shredder	65	30	54	2	1	0
Coleoptera	Elmidae (adult)		Scraper; Collectors-gatherer	0	2	0	0	0	0
Coleoptera	Elmidae (larva)		Scraper; Collectors-gatherer	0	3	1	0	0	0
Coleoptera	Psephenidae		Scraper	0	0	2	0	0	0
Coleoptera	Scirtidae	cyphon	Scraper	0	0	0	1	0	0
Diptera	Athericidae	atherix	Predator	0	0	0	0	0	0
Diptera	Chironomidae		Collector-gather, filter	50	21	53	91	89	66
Diptera	Chironomidae (adult)		N/A	0	0	0	0	0	0
Diptera	Chironomidae (paupa)		N/A	1	3	1	0	4	2
Diptera	Empididae	hemerodromia	Predator	0	0	0	0	0	0
Diptera	Muscidae		Predator	0	0	0	0	0	0
Diptera	Simuliidae		Collector-filter	4	0	6	1	8	10
Diptera	Tipulidae	tipulda	Shredder	0	0	0	0	0	0
Diptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Ephemoptera	Baetidae		Collectors-gatherer; Scraper	0	0	0	1	2	0
Ephemoptera	Caenidae	caenis	Collectors-gatherer; Scraper	0	0	0	2	4	0
Ephemoptera	Heptageniidae	stenacon	Collectors-gatherer; Scraper	0	0	0	1	0	0
Ephemoptera	Siphlonuridae	siphlonurus	Collectors-gatherer; Scraper	0	0	0	0	0	0
Gastropoda	Physidae		Scraper	0	3	4	0	1	0
Gastropoda	Pleuroceridae		Scraper	0	0	0	4	1	0
Hempitera	Unidentified (terresterial)		N/A	0	0	0	0	0	0
Isopoda	Asellidae	caecidotera	Shredder	0	0	0	0	0	0
Isopoda	Asellidae	lirceus	Shredder	0	0	1	0	0	0
Unionida	Sphaeriidae		Collector-filter	0	0	0	1	0	1

Gastropoda	Unidentified		N/A	0	0	0	0	0	1
Odonata	Aeshnidae	boyeria	Predator	0	0	1	0	0	0
Odonata	Coenagrienidae	nehalennia	Predator	0	0	0	0	0	1
Oligochaeta	Unidentified		N/A	0	2	0	5	0	0
Platyhelminthes	Planariidae	planaria	Predator	13	12	41	8	0	0
Plecoptera	Taeniopterygidae	taenipteryx	Shredder	0	0	0	0	0	0
Tricoptera	Brachycentridae	Micrasema	Shredder	0	0	0	1	0	0
Tricoptera	Heilopsychidae		Scrapper	0	2	1	0	0	0
Tricoptera	Hydropsychidae		Collector-filter	1	0	2	3	4	3
Tricoptera	Hydroptilidae		Collectors-gatherer; Scraper	0	0	0	0	0	0
Tricoptera	Leptoceridae	nectopsyche	Shredder	0	0	0	0	0	0
Tricoptera	Philopotamidae		Collector-filter	0	1	0	0	0	0
Tricoptera	Rhyacophilidae		Predator	0	0	0	0	0	0
Tricoptera	Sericostomatidae		Shredder	0	0	1	0	0	0
Tricoptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Zygoptera	Unidentified		N/A	0	0	0	0	0	0
	Gobiidae	neogobius		0	0	0	1	0	0
	Hydrachnidiae		Predator	0	0	0	1	0	0

				AP 10/12/12			UB 10/12/12		
Order	Family	Genus	Functional Feeding	1	2	3	1	2	3
Amphipoda	Gammaridea		Shredder	38	70	75	0	4	0
Coleoptera	Elmidae (adult)		Scraper; Collectors-gatherer	3	3	3	0	0	0
Coleoptera	Elmidae (larva)		Scraper; Collectors-gatherer	11	13	3	0	0	0
Coleoptera	Psephenidae		Scraper	0	0	0	0	0	0
Coleoptera	Scirtidae	cyphon	Scraper	3	0	0	0	0	0
Diptera	Athericidae	atherix	Predator	0	0	0	0	0	0
Diptera	Chironomidae		Collector-gather, filter	514	430	253	2520	2722	2504
Diptera	Chironomidae (adult)		N/A	0	0	0	0	0	1
Diptera	Chironomidae (paupa)		N/A	1	7	8	11	45	34
Diptera	Empididae	hemerodromia	Predator	13	8	3	18	3	8
Diptera	Muscidae		Predator	0	0	0	0	0	0
Diptera	Simuliidae		Collector-filter	2	7	18	96	39	67
Diptera	Tipulidae	tipulda	Shredder	0	0	0	6	0	0
Diptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Ephemoptera	Baetidae		Collectors-gatherer; Scraper	0	0	0	0	0	0
Ephemoptera	Caenidae	caenis	Collectors-gatherer; Scraper	0	0	1	0	0	1
Ephemoptera	Heptageniidae	stenacon	Collectors-gatherer; Scraper	0	0	0	0	0	4
Ephemoptera	Siphlonuridae	siphlonurus	Collectors-gatherer; Scraper	0	0	0	0	0	0
Gastropoda	Physidae		Scraper	1	9	2	0	0	0
Gastropoda	Pleuroceridae		Scraper	0	0	0	0	0	1

Hempitera	Unidentified (terresterial)		N/A	0	0	0	0	0	1
Isopoda	Asellidae	caecidotera	Shredder	0	0	0	0	0	0
Isopoda	Asellidae	lirceus	Shredder	0	0	0	0	0	0
Unionida	Sphaeriidae		Collector-filter	1	0	0	0	0	0
Gastropoda	Unidentified		N/A	0	0	0	0	0	0
Odonata	Aeshnidae	boyeria	Predator	0	0	0	0	0	0
Odonata	Coenagrienidae	nehalennia	Predator	0	0	0	0	0	0
Oligochaeta	Unidentified		N/A	1	4	0	0	0	0
Platyhelminthes	Planariidae	planaria	Predator	124	122	14	24	0	7
Plecoptera	Taeniopterygidae	taenipteryx	Shredder	0	0	0	0	0	0
Tricoptera	Brachycentridae	Micrasema	Shredder	0	0	0	0	0	0
Tricoptera	Heilopsychidae		Scrapper	4	9	1	6	0	0
Tricoptera	Hydropsychidae		Collector-filter	0	5	4	126	68	53
Tricoptera	Hydroptilidae		Collectors-gatherer; Scraper	0	0	0	0	0	9
Tricoptera	Leptoceridae	nectopsyche	Shredder	0	0	0	0	0	0
Tricoptera	Philopotamidae		Collector-filter	0	1	3	0	0	1
Tricoptera	Rhyacophilidae		Predator	0	0	0	0	0	0
Tricoptera	Sericostomatidae		Shredder	0	0	0	0	0	0
Tricoptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Zygoptera	Unidentified		N/A	0	0	0	0	0	0
	Gobiidae	neogobius		0	0	0	0	0	0
	Hydrachnidiae		Predator	0	0	0	30	1	9

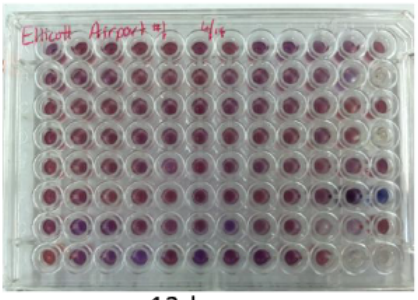
				AP 11/14/12			UB 11/14/12		
Order	Family	Genus	Functional Feeding	1	2	3	1	2	3
Amphipoda	Gammaridea		Shredder	103	161	82	12	9	N/A
Coleoptera	Elmidae (adult)		Scraper; Collectors-gatherer	4	8	5	0	0	N/A
Coleoptera	Elmidae (larva)		Scraper; Collectors-gatherer	6	19	10	24	1	N/A
Coleoptera	Psephenidae		Scraper	0	0	1	0	0	N/A
Coleoptera	Scirtidae	cyphon	Scraper	0	0	0	0	10	N/A
Diptera	Athericidae	atherix	Predator	0	0	1	0	0	N/A
Diptera	Chironomidae		Collector-gather, filter	1503	1668	3070	7416	10434	N/A
Diptera	Chironomidae (adult)		N/A	0	0	0	0	0	0
Diptera	Chironomidae (paupa)		N/A	1	1	2	0	0	2
Diptera	Empididae	hemerodromia	Predator	11	14	18	12	12	N/A
Diptera	Muscidae		Predator	0	0	1	0	0	N/A
Diptera	Simuliidae		Collector-filter	159	145	476	2952	2811	N/A
Diptera	Tipulidae	tipulda	Shredder	0	0	0	0	0	N/A
Diptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Ephemoptera	Baetidae		Collectors-gatherer; Scraper	0	0	0	0	0	N/A
Ephemoptera	Caenidae	caenis	Collectors-gatherer; Scraper	0	0	0	36	18	N/A

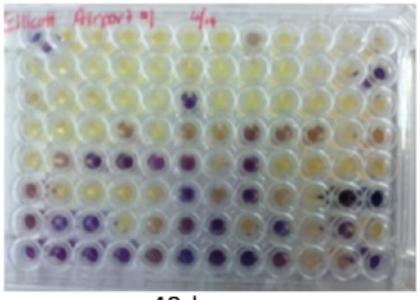
Ephemoptera	Heptageniidae	stenacon	Collectors-gatherer; Scraper	0	0	0	0	0	N/A
Ephemoptera	Siphlonuridae	siphlonurus	Collectors-gatherer; Scraper	0	0	1	0	0	N/A
Gastropoda	Physidae		Scraper	2	8	1	0	0	N/A
Gastropoda	Pleuroceridae		Scraper	0	1	1	12	5	N/A
Hempitera	Unidentified (terresterial)		N/A	0	0	1	0	0	0
Isopoda	Asellidae	caecidotera	Shredder	0	0	0	0	0	N/A
Isopoda	Asellidae	lirceus	Shredder	0	0	0	0	0	N/A
Unionida	Sphaeriidae		Collector-filter	2	19	0	0	6	N/A
Gastropoda	Unidentified		N/A	0	0	0	0	5	N/A
Odonata	Aeshnidae	boyeria	Predator	0	0	0	0	0	N/A
Odonata	Coenagrienidae	nehalennia	Predator	1	1	0	0	0	N/A
Oligochaeta	Unidentified		N/A	0	0	1	0	1	N/A
Platyhelminthes	Planariidae	planaria	Predator	28	41	29	108	7	N/A
Plecoptera	Taeniopterygidae	taenipteryx	Shredder	0	1	1	0	0	N/A
Tricoptera	Brachycentridae	Micrasema	Shredder	0	2	0	0	0	N/A
Tricoptera	Heilopsychidae		Scrapper	2	6	0	12	0	N/A
Tricoptera	Hydropsychidae		Collector-filter	19	12	20	156	178	N/A
Tricoptera	Hydroptilidae		Collectors-gatherer; Scraper	0	0	0	0	15	N/A
Tricoptera	Leptoceridae	nectopsyche	Shredder	0	1	0	0	0	N/A
Tricoptera	Philopotamidae		Collector-filter	2	5	6	0	0	N/A
Tricoptera	Rhyacophilidae		Predator	0	0	0	0	0	N/A
Tricoptera	Sericostomatidae		Shredder	0	0	0	0	0	0
Tricoptera	Unidentified (paupa)		N/A	0	0	0	0	0	0
Zygoptera	Unidentified		N/A	0	0	0	0	0	N/A
	Gobiidae	neogobius		0	0	0	0	0	N/A
	Hydrachnidiae		Predator	1	2	2	0	25	N/A

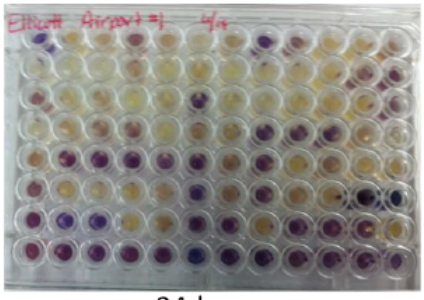
Appendix B

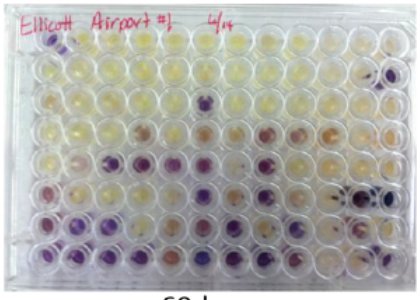
Incubation series of one BIOLOG plate for one bag during the spring collection. As mentioned in the paper at 12-hours almost the entire plate is purple and then a distinct pattern develops and this pattern was consistent for both sites throughout all the collections.

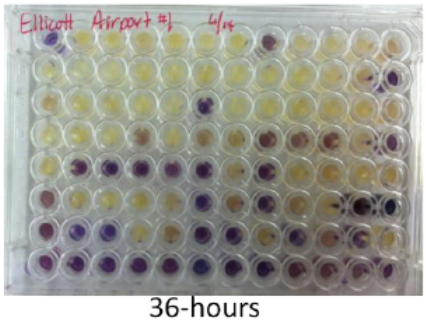
Airport 14 June 12
Incubation Series for one
BIOLOG Plate

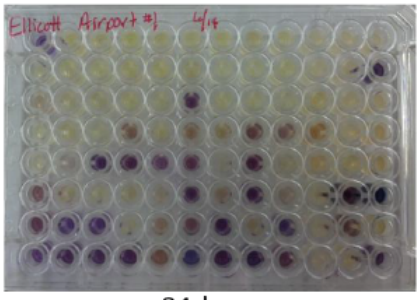

12-hours


48-hours


24-hours


60-hours


36-hours


84-hours

Appendix C

Progression of average well color development for all carbon sources during incubation A) Spring, two-weeks B) Spring, one month C) Fall, two-weeks D) Fall, one month, E) Fall, two months. Each point represents the average of all carbon sources for three BIOLOG plates.