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IN-STREAM LEAF DECOMPOSITION AS AN INDICATOR OF MARCELLUS SHALE IMPAIRMENT ACROSS A LAND USE GRADIENT

by

Jordan A. Barton

A Thesis

Presented to the Faculty of
Bucknell University
In Partial Fulfillment of the Requirements for the Degree of
Master of Science in Biology

Approved: Matthew E. McTammany
Adviser

Matthew Heintzelman
Department Chairperson

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Date

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Abstract

Rapid development of hydrofracking, particularly in the Marcellus Shale region, has greatly outpaced ecological research assessing potential impacts on aquatic ecosystems. Increased sedimentation and contamination of streams from unconventional natural gas (UNG) activity could affect stream biota, resulting in altered rates of in-stream leaf decomposition. We deployed leaf packs in seven sites representing a range of UNG activity among different land uses including forest, agriculture, and development. In addition, physical and chemical variables were measured. Summer breakdown rates for all sites, mesh sizes, and leaf species were higher in the presence of UNG activity. Fall breakdown rates demonstrated no consistent trend among land uses or UNG activity. Summer deployment had more storm events than fall, promoting more runoff into streams as well as more sediment release. This suggests that higher physical breakdown rates in UNG sites could have been caused by more disturbed land, modifying stream hydrology. However, fall measurements, under more consistent flow regimes, indicate sites with flashier hydrology are prone to faster breakdown rates due to mechanical fragmentation rather than biological decomposition. Leaf breakdown rates were not a consistent indicator of UNG impairment among our sites due to factors affecting breakdown rates caused by land uses other than UNG and physical breakdown attributed to hydrologic disturbances.

Introduction

Unconventional natural gas extraction

The world has been actively searching for additional sources of energy, preferentially ones with lower carbon dioxide emissions, due to increased understanding of human-caused climate change and rapid depletion of oil reserves. Natural gas has been called a “bridge fuel” to renewable energy sources because its combustion releases fewer contaminants when compared with that of coal or petroleum (Entrekin et al. 2011, Kargbo et al. 2010). However, the process of acquiring natural gas emits 30% more methane and has a larger greenhouse gas footprint compared to the other fossil fuels (Entrekin et al. 2011, Howarth et al. 2011). An unconventional method used to access natural gas in deep shale beds, hydraulic fracturing (“hydrofracking”), utilizes high-pressure injection of fracturing fluids, consisting of large volumes of water and numerous chemical additives, to create fractures in the shale, while added propping agents, such as sand, keep the fractures open allowing the gas to flow (Entrekin et al. 2011, Vidic et al. 2013). Unconventional natural gas (hereafter referred to as UNG) recovery requires construction of extensive infrastructure, such as roads, pipelines, compressor stations, and drilling pads, which, along with drilling, gas extraction, and transport, can have significant environmental effects, including sedimentation and contamination in streams (Entrekin et al. 2011). The U.S. has many abundant shale gas resources, but the most expansive shale gas play, and the main focus of this study, is the Marcellus Shale.

The Marcellus Shale is a Devonian age sedimentary rock formation spanning 240,000 km^2 at a depth of 1200-2500 m, underlying six states in the upper Mid-Atlantic U.S. (Entrekin et al. 2011, Soeder and Kappel 2009). Most UNG activity in the Marcellus Shale has been in Pennsylvania's northern tier where it has grown from 8 wells in 2005 to around 7,234 as of November 2013 (Brantley et al. 2014). The formation underlies important aquatic ecosystems, such as the Chesapeake Bay watershed and the Delaware River basin, and contains one of the country's most diverse regions of amphibians and freshwater fish (Entrekin et al. 2011, Souther et al. 2014). Proximity of UNG activity to sensitive biota and ecosystems causes ecological concern. Nearly 4,000 Marcellus Shale natural gas well sites in Pennsylvania are located within 300 m of streams, with more than 750 located within 100 m of stream channels (Entrekin et al. 2011, Souther et al. 2014). With well sites being so close to freshwater resources, the risk to aquatic ecosystems is exacerbated (Souther et al. 2014).

Development of UNG has greatly outpaced ecological research trying to assess potential impacts of natural gas drilling on the environment. UNG development has progressed so quickly that sampling and monitoring of headwater streams has not been sufficient to document impacts over long or short periods of time (Brantley et al. 2014). UNG requires a trade-off between energy development and ecosystem services, which are natural functions and processes of ecosystems that maintain human health and overall well-being (Smith et al. 2012). The processes and functions of stream ecosystems are largely dependent on the flora and fauna residing within them. Therefore, if the natural biota of a stream is impaired, then the stream functions will be altered as well. Potential threats to biota from UNG activities include: surface and groundwater contamination; diminished stream flow; stream sedimentation; habitat loss and fragmentation; localized air, noise, and light pollution; climate change; and cumulative impacts (Souther et al. 2014). Fragmentation of forest land will increase the risk of pollution in headwater streams (Drohan et al. 2012). Of these potential impacts, the primary concerns for aquatic ecosystems are water contamination and sedimentation, which can result from UNG activities. Both sedimentation and contamination impacts can be compounded by diminished stream flow, which could result from water withdrawals for UNG drilling. Each active UNG well consumes between 2-7 million gallons of source water for drilling and the production of the fracking fluids (Entrekin et al. 2011, Souther et al. 2014). Taking water from a small stream concentrates contaminants in the stream water (Burton et al. 2014, Entrekin et al. 2011), allows suspended sediment to settle, and contributes to loss of habitat (Brittingham et al. 2014). Water withdrawal directly impacts small streams, but the collective changes associated with UNG activities and land use have more widespread and severe impacts on streams and associated aquatic biota (Shank and Stauffer 2015).

Functional assessment of UNG impacts on streams

Previous studies have relied on ecosystem structure rather than function to distinguish the health and overall quality of a particular habitat. The structural integrity of a stream is explained by Gessner and Chauvet (2002), to be “the qualitative and quantitative composition of biological communities and their resources.” UNG activity may directly and indirectly influence stream structure by killing the biota or by affecting consumer foraging and consumption rates (Evans-White and Lamberti 2009). These impacts may cause feedbacks altering ecosystem processes and functions, such as leaf decomposition. Monitoring ecosystem functions is an important tool for assessing the health of aquatic ecosystems (Young and Collier 2009). Ecosystem-level processes are suitable indicators of stream health because they provide an integrated response to watershed disturbances like sedimentation (Bunn et al. 1999). Studying changes in leaf breakdown might be particularly useful in detecting changes to the behavior and physiology of biota, rather than just their abundances (Entrekin et al. 2011, Evans-White and Lamberti 2009, Young and Collier 2009).

Two of the most prominent and widely applied measurements of stream function are studies of primary production and organic matter breakdown. These measurements are well-suited for detecting large-scale alterations and will most likely show changes due to impairments caused by UNG (Entrekin et al. 2011). Our study will focus on leaf decomposition, in part because the Marcellus Shale exists in a primarily forested region. Headwater streams in forested regions receive a majority of their organic matter supply for fueling the food web from leaf detritus (Fisher and Likens 1973, Gulis and Suberkropp 2003, Sponseller and Benfield 2001).

The majority of impact from UNG in the Marcellus Shale will be on small headwater streams, which has potential implications both locally and downstream along the river continuum. Functional processes of headwater streams influence river networks through the downstream exportation of CPOM, sediments, nutrients, and FPOM generated due to breakdown (Bott et al. 2012, Gomi et al. 2002, Vannote et al. 1980). Thus, disturbed headwaters may strongly modify the food web and community structure of the watershed through the alteration of these drifted materials and loss of downstream connectivity (Gomi et al. 2002, Meyer and Wallace 2001, Meyer et al. 2007, Wallace et al. 1991). In particular, if impairments from UNG activity reduce leaf decomposition in headwater streams, then the supply of organic material to downstream food webs will be reduced as well (Meyer and Wallace 2001, Wallace et al. 1982). Though the combined effects of water withdrawal, sedimentation, and contamination will be compounded in low volume streams, effects from UNG activity extend beyond the local scale and into watershed networks. This study will focus on low-order streams where impairment to stream function and structure is most likely to be detected from UNG (Entrekin et al. 2011).

Studying both stream structure and function in conjunction with one another provides the most insight on overall health and level of impairment of a stream because some forms of stream degradation may impact structure but not function, function but not structure, or both (Young et al. 2008). However, most studies conducted on the effects of UNG have focused on the structural component of aquatic ecosystems (Entrekin et al. 2011); thus an emphasis will be put on functional integrity to assess the impact of natural gas extraction. Therefore if UNG development impacts the biota of small streams, it will also compromise the manner in which leaves are processed, which will potentially affect downstream ecosystems as well. Though this study's primary focus is on leaf breakdown, it is important to note that stream metabolism is also worth assessing because UNG activity will likely induce measurable changes in the factors contributing to metabolism (light, substrate composition, turbidity, nutrients, pH, riparian vegetation, and flow fluctuations) (Young et al. 2008).

Organic matter decomposition

Organic matter breakdown is the decomposition of organic matter into its inorganic components by leaching of soluble compounds, physical fragmentation, microbial conditioning and decay, and invertebrate feeding (Tank et al. 2010). Leaves are most commonly used in decomposition experiments, and rich literature exists for comparing breakdown rates of different leaf species in streams suffering from various types of human impairment. Leaf breakdown effectively links riparian vegetation and activities of both microbial and invertebrate communities of streams (Young et al. 2008). Processing and decomposition of leaves entering the aquatic food web begins with retention of leaves in the stream by some obstacle (rocks or debris). Initiated by contact with the water, soluble materials begin to leach out from the leaves (Benfield and Webster 1985). Leaves are then colonized by microbes, primarily bacteria and fungi, initializing decomposition and attracting detritivorous invertebrates (shredders) to feed on the microbially conditioned, protein-rich leaf mass (Benfield and Webster 1985, Reice 1974, Sponseller and Benfield 2001). Thus the two groups of biota influencing breakdown rates are microorganisms and invertebrates, which both have the potential to be negatively impacted by UNG activity.

According to Pozo et al. (2011), detritivore activity has a stronger impact on breakdown rates than microbial activity. Several abiotic factors, including light, substrate composition, turbidity, nutrients, pH, riparian vegetation, and flow fluctuations, may also affect leaf decomposition (Young et al. 2008). Anthropogenic factors stemming from land use that can impose variations on breakdown rates include sedimentation, increased nutrient loads, and chemical contamination (Sponseller and Benfield 2001). These factors and their impacts on stream structure and function will be discussed in the following sections.

Sediment transport and deposition

The influx of sediment from surrounding environments can be potentially detrimental to small headwater streams. Sediment runoff has been detected from well pads, and well-pad density is positively correlated to stream turbidity (Brittingham et al. 2014, Entrekin et al. 2011, Williams et al. 2008). The contribution of UNG development to sediment load varies geographically, depending on local hydrology, geology, industry practices, and existing forms of land use (Souther et al. 2014).

UNG activities in the Marcellus Shale region have the potential to greatly increase inputs of sediments to streams due to the extent of land disturbance for each well pad (1.5-3.5 ha) and proximity of well pads to streams (Brantley et al. 2014, Drohan et al. 2012, Entrekin et al. 2011, Olmstead et al. 2013, Trexler et al. 2014). In addition to well pads, land is also cleared and manipulated to construct roads and pipelines, which frequently include stream crossings (Weltman-Fahs and Taylor 2013). Many of these roads are unpaved, elevating runoff rates and increasing the risk of sedimentation to receiving water bodies (Brittingham et al. 2014). Newly exposed land, high volumes of truck traffic, and lack of controls for erosion have led to many Notices of Violations (NOV's) regarding sedimentation, though violations have decreased with time (Brantley et al. 2014). Although most sediment reaches streams with runoff during storms, it can also be delivered to streams in landslides, failure of water containment structures, and broken pipelines. Larry's Creek, near Salladasburg, PA, suffered an input of sediment from a fractured pipeline that caused turbidity of the stream to increase for over two months, even in the absence of precipitation (Brantley et al. 2014).

Sedimentation influences structure of aquatic ecosystems via habitat manipulation, which in turn may result in changes in stream function, such as reduced leaf decomposition due to decreased shredder and microbial biomass and burial of the leaf litter. Sedimentation can cause physical abrasion of leaf material, reducing the amount of available food at a local scale and abrading sensitive microbial biofilms on the leaf surface. Burial and abrasion of leaf material by sediments may lead to loss of macroinvertebrate diversity (Wood and Armitage 1999), density, and biomass (Waters 1995) due to the reduction of accessible leaf material for consumption. Leaf burial temporarily removes energy from the local aquatic food web and decreases exposed surface area available for microbial activity, minimizes physical abrasion, creates anaerobic conditions, and prevents feeding by detritivores, all of which will slow decomposition rates (Herbst 1980, Sponseller and Benfield 2001, Webster and Waide 1982). On the other hand, leaf burial may act as a homeostatic mechanism trapping leaves and allowing them to persist longer in a local stream to become a richer food source (Herbst 1980). A study done by Cornut et al. (2010) showed that if leaf litter was buried in sediment out of reach of shredders, microbial decomposers become more abundant but do not fully compensate for the lost role of shredders in leaf decomposition.

Waters (1995) also notes that suspended sediments may result in increased invertebrate drifting due to abrasion or other physical actions. Increased drifting would cause losses of invertebrate taxa and density, which could lead to reduced leaf breakdown. The most profound consequence of sediment deposition is the modification of the streambed (Waters 1995) and stream substrate conditions (Rabení et al. 2005). Deposited sediment may alter the benthic habitat by burying coarse substrate and by filling interstitial spaces of rocks commonly used by invertebrates as refuge from current and predators. Decomposition rates will slow if detritivore habitat becomes unsuitable to sustain populations that feed on leaf litter. There is also evidence of sediments adsorbing nutrients and other chemicals that could accumulate over time and potentially contaminate the stream (Burton et al. 2014).

Contamination

In addition to sedimentation, another concern of UNG activity is the release or runoff of chemicals into streams. Equipment failure, illegal disposal or spills of fracking fluids or flow-back water, chemical migration in groundwater, and wastewater escape are all potential sources of contamination from UNG extraction and are in need of research (Souther et al. 2014). The use of fracking fluids is of primary concern because the Safe Drinking Water Act excludes regulation of UNG activities, allowing for the formulas of fracking fluids to be kept confidential (Kargbo et al. 2010, Vidic et al. 2013) and making it difficult to predict and research potential impacts on the environment. Contaminants most likely to increase in streams due to UNG extraction would be Na, Ca, Cl, Sr, Ba, and Br (Brantley et al. 2014). UNG wastewater used to be released directly into surface waters, but this practice was replaced with more environmentally friendly techniques, such as ion-exchange treatment and other wastewater treatment plant methods (Brittingham et al. 2014). Recently, studies have determined that retrieved water from UNG drilling has significantly high concentrations of total dissolved solids (TDS), specifically chlorides and bromides, and is inadequately treated at treatment plants in Pennsylvania (Brittingham et al. 2014, Ferrar et al. 2013, Olmstead et al. 2013). If these wastewaters are released into streams, the streams may demonstrate elevated concentrations of the elements listed above, which could be detrimental to aquatic ecosystem structure and function.

Evans-White and Lamberti (2008) explain that even at sub-lethal levels, contaminants can indirectly affect ecological processes (e.g., leaf decomposition) by directly affecting primary consumers. Contamination may also affect microbial populations because many freshwater microbes are sensitive to low pH, dissolved metals, salinity, and deposition of metal oxides (Niyogi et al. 2001). Contamination of streams by UNG activity might stimulate or inhibit ecosystem functions, like leaf decomposition, depending on specific effects of contamination on stream biota (Entrekin et al. 2011). Carlisle and Clements (2005) concluded that leaf decomposition was extremely sensitive to contaminant-induced changes due to metal toxicity reducing biomass and feeding efficiency of shredders. Therefore contamination from UNG extraction is likely to negatively impact the two most important biotic factors contributing to leaf decomposition. This reinforces the use of leaf decomposition rates as an appropriate indicator of UNG impact on stream ecosystems at a local scale.

Another impact on leaf breakdown from UNG activity could result from hydrological alterations caused from the clearing of land for well pads, pipelines, and roads. This newly exposed land could increase runoff to streams due to the higher amounts of impervious surfaces. The higher runoff could cause flashier hydrology within UNG watersheds. Faster more turbulent flows along with increased sediment inputs have the ability to enhance physical breakdown of leaf material, which may overshadow biological influences on leaf decomposition.

Land use

In addition to UNG influence, many streams are also impacted by preexisting land uses, such as agriculture and development, each of which has unique impacts on leaf decomposition. It is important to consider influences of land use on streams when trying to elucidate UNG influences because UNG occurs in watersheds with co-occurring land uses whose effects may be similar to those attributed to UNG activity. Watersheds with a particular land use do not necessarily mean streams within it are going to be impacted in the same way or to the same degree. The extent of land use impacts on stream ecosystems may depend on spatial distribution of development in the watershed and spatial scale at which this distribution is evaluated (Sponseller and Benfield 2001). The level of impact also depends on how dense the land use impairment is within the watershed, as well as the specific location of the impairment in regards to the stream and hydrologic flow paths.

Streams in forested watersheds are usually cooler due to shade from canopy cover. It is this canopy cover and dense riparian vegetation that supplies these forested streams with one of their most important food sources, leaves (Webster and Waide 1982). Due to the regular and high availability of leaves as a food source, leaf breakdown is a very important process in these streams and is dominated by leaf shredding macroinvertebrates. Forested streams are typically least impacted by anthropogenic disturbances and are often used as reference sites to detect impairment by other land uses.

Agricultural watersheds contain much more cleared land than forested landscapes and have less dense riparian vegetation, which results in more light reaching streams and correspondingly warmer water. Runoff from agricultural land use has high nutrient concentrations from fertilizers, which can increase leaf breakdown rates (Hagen et al. 2006) and microbial activity (McTammany et al. 2008). High sedimentation, soil erosion, and bank instability are also associated with agricultural land use, and these too may alter breakdown rates from increased physical breakdown (Allan et al. 1997, Hagen et al. 2006). Agriculture can have positive effects on shredders due to increased light, elevated water temperature, high nutrients, and adequate food supplies associated with agriculture (Hagen et al. 2006, Paul et al. 2006). However, extensive agriculture may reduce shredder populations due to high rates of sedimentation and reduction in riparian vegetation quantity and diversity. This would imply that physical breakdown and microorganisms are major influences on leaf decomposition in sites with extensive agriculture. Agriculture has both positive and negative effects on leaf breakdown; therefore, the rates must be interpreted in context of other structural and functional variables associated with stream categories (Hagen et al. 2006).

Urbanization in watersheds increases impervious land cover and storm water drainage efficiency, which leads to more frequent and flashy water flows, potentially increasing physical fragmentation of leaf litter and invertebrate drift (Paul et al. 2006, Schueler 1994). Impervious surface runoff is also associated with non-point source pollution, which could increase conductivity and pollutant concentrations causing lower numbers of macroinvertebrates in urban streams (Paul et al. 2006). Due to negative influence on macroinvertebrates, biological leaf decomposition would be slowed in developed land uses allowing physical fragmentation induced by storm runoff to be the driving factor on leaf breakdown.

Continued human development may reduce retention and processing of organic matter in headwater streams draining developing watersheds (Sponseller and Benfield 2001).

Study objective

In stream ecosystems, abiotic structure influences biotic structure, and biotic structure influences stream function. UNG activity in the Marcellus Shale region may be affecting aquatic ecosystems by increasing inputs of sediments and chemical pollutants to streams. This in turn may lead to changes in biotic communities in impacted streams, causing an alteration in stream processes and functions. This study aimed to determine the impact of UNG activity in the Marcellus Shale on leaf decomposition in low order streams. We measured leaf breakdown rates in streams with varying degrees of UNG presence and a gradient of different land uses, including agriculture and human development. Differences in breakdown rates were compared with abundance of macroinvertebrates and shredders, as well as other physical and chemical characteristics, in part by comparing breakdown rates of leaves from coarse mesh bags with fine mesh bags, which exclude macroconsumers. This study is part of a project being led by scientists from the Susquehanna River Basin Commission (SRBC) to explore sources of sediment to streams from natural gas drilling development and to recommend practices to minimize sediment inputs to headwater streams. We sought to determine if leaf decomposition could act as a potential indicator of UNG impacts on streams across a gradient of land use.

Hypotheses

We hypothesized that 1) leaves will decompose more slowly in streams with more UNG presence due to increased sedimentation and contamination, and 2) alterations in leaf breakdown rates due to UNG presence will be larger for coarse mesh bags than for fine mesh bags due to effects of UNG activity on the shredder community rather than by its effects on the microbial community.

Methods

Study sites

The SRBC specifically selected 15 sites for its assessment of UNG activity and sedimentation based on high quality long-term data on turbidity from remote monitoring stations along with regularly surveyed benthic macroinvertebrate diversity and brook trout abundance. Of these 15 sites, 7 were selected for use in this study - Grays Run, Loyalsock Creek, Apalachin Creek, Bowman Creek, Wappasening Creek, Blockhouse Creek, and Nanticoke Creek. These sites represent a gradient of dominant land cover types, including forested, agricultural, and developed, and presence or absence of UNG development. The sites are all in relatively small watersheds with drainage areas ranging from 16 sq. mi. to 54 sq. mi., making them ideal to examine effects of UNG activity.

Sites were put into groups based on land use categories and presence/absence of UNG. The two forested sites were chosen based upon having the highest % stable vegetation and lowest % Agriculture (% Ag) and % Developed (% Dev). The agriculture sites were determined due to % Ag being higher than forested sites and % Dev being less than Dev/Ag sites. Dev/Ag sites were determined based on % Dev being the highest compared to other sites. % Ag was actually higher in Dev/Ag sites than in the Ag sites, making Dev/Ag sites the most heavily influenced by existing land uses (Table 1). In addition to land cover information provided by SRBC in Table 1, several other characteristics of each watershed basin were quantified using USGS StreamStats (USGS 2016). These traits include drainage area, total length of streams, stream density, mean basin slope, and mean annual precipitation (Table 2).

Grays Run represented our forested site with UNG presence (FrstY). Grays Run starts in northern Lycoming County and flows south through Loyalsock State Forest until its confluence with Lycoming Creek near the town of Gray, PA (SRBC 2016). Grays Run was our most forested watershed (Table 1) and was densely forested along our study reach. Of the nine well pads within the watershed, one UNG pad was located within sight of our study reach.

Loyalsock Creek represented our forested site without the presence of UNG (FrstN). Loyalsock Creek watershed spans from western Wyoming County and northeastern Sullivan County to central Lycoming County, where it joins the West Branch Susquehanna River (SRBC 2016). Our study site was in upper Loyalsock Creek near Lopez, PA in Sullivan County. Though the watershed is highly forested (Table 1), upper Loyalsock Creek drains some open-canopied boggy areas and contains some abandoned coal mining operations.

Apalachin Creek was one of our agricultural sites with UNG (AgY). Apalachin Creek begins in northwest Susquehanna County, PA and flows north into the Susquehanna River at Apalachin, NY (SRBC 2016). Our study reach was located in Apalachin, NY. UNG drilling is not yet permitted in NY, so all of the

UNG presence is located in the PA section of the watershed and not near our study site.

Bowman Creek was our agricultural site without UNG (AgN). Bowman Creek begins in Ricketts Glen State Park and flows northeast into the Susquehanna River a few miles south of Tunkhannock, PA (SRBC 2016). Our study stretch was located in the town of Noxen, PA and was locally surrounded by fields and cropland.

Wappasening Creek was another site chosen to represent agriculture with UNG (WappAgY). Wappasening Creek flows northwest into the Susquehanna River at Nichols, NY (SRBC 2016). Wappasening Creek was chosen because its watershed contains the most UNG pads (23) of all our sites (Table 1). A large UNG pad was visible from the stream reach used in our study. AgN was used as the non-UNG counterpart to both AgY and WappAgY in data analysis.

Blockhouse Creek represented our Dev/Ag site in the study with UNG presence (DAgY). Blockhouse Creek begins in southern Tioga County and flows south into central Lycoming County where it joins Little Pine Creek north of English Center, PA (SRBC 2016). The study reach was located at the base of a mountain with a steep slope of the mountain leading into the stream.

Nanticoke Creek represents our Dev/Ag site without UNG (DAgN). Nanticoke Creek flows south and joins the Susquehanna River south of West Corners, NY (SRBC 2016). Nanticoke Creek has the highest % Dev of all the sites (Table 1). Our study reach was located near Maine, NY, where the stream flows through a narrow channel downstream from a beaver pond.

Water quality and chemistry

Water quality was measured and samples for water chemistry were collected from all sites on dates corresponding to leaf retrievals. Dissolved oxygen, pH, temperature, depth, turbidity, and conductivity were measured using a calibrated YSI 6920 sonde. Stream discharge was calculated by measuring velocity and depth of subsections of known widths across the stream, multiplying velocity by depth and width of interval subsection, and summing these values across the entire stream. Velocity at 60% depth was measured in each increment with an electronic flow meter (Marsh-McBirney Flo-Mate 2000).

Total suspended solids (TSS) were measured from three 1-L grab samples that were collected from each stream on each field visit and kept on ice until returned to lab for analysis. Water from each sample was vacuum filtered through a pre-weighed glass fiber filter (1-µm pore size). Filters were then dried to constant weight at 55^{o}C and reweighed. TSS was calculated by subtracting initial filter weight from the dry weight of filter plus retained solids and dividing by the volume of water, determined by subtracting the empty bottle weight from the weight of the full bottle.

Water samples for analysis of concentrations of nutrients (dissolved inorganic nitrogen, soluble reactive phosphorus), cations (Ca^{+2}, Mg^{+2}, Na^{+1}, K^{+1},

NH_4^+), and anions (Cl^{-1}, NO_3^{-1}, PO_4^{-3}, SO_4^{-2}) were collected during each field visit by filtering stream water in the field (Pall-Gelman GF/F, 0.7 um pore size) and storing samples on ice until returning to lab for analysis. Ammonium was measured using the OPA fluorescence assay method (Holmes et al. 1999). Phosphorus was measured using the ascorbic acid method (APHA 1998). Cations and anions were measured using ion chromatography in the Environmental Science and Engineering Laboratory at Bucknell University.

Leaf breakdown

Oak (*Quercus* spp.*)* leaves and maple (*Acer* spp.*)* leaves were used to measure leaf breakdown because oak and maple trees were common in the study areas and because these leaves have different expected decomposition rates, with maple leaves being more quickly decomposed than more recalcitrant oak leaves (Bott et al. 2012, Webster and Benfield 1986). Leaves from each tree species were collected, air dried, and placed into both coarse mesh bags (6 mm mesh, 10 g initial dried leaf material) and fine mesh bags (0.5 mm mesh, 5 g initial dried leaf material).

Deployment 1, the summer deployment, was conducted from May 28, 2015 to September 18, 2015. For this deployment, we placed 15 leaf packs of each species in both coarse and fine mesh bags in each stream (105 coarse mesh maple = CM, 105 coarse mesh oak = CO, 105 fine mesh maple = FM, and 105 fine mesh oak = FO bags total). Leaf bags were deployed in all streams over a two day period starting on May 28, 2015. Three leaf bags of each type (3 CM, 3 CO, 3 FM, 3 FO) were secured to 6-foot long pieces of rebar using 26-gauge wire. We secured 5 rebar stakes into the substrate of each stream to keep the leaf bags in a fixed location. After deployment in May, 12 bags (3 of each type) were removed from each stream after 14, 28, 42, 55, 72, and 83 days of incubation or until all bags were collected, destroyed, or lost. Collected litter bags were stored in individual Ziploc bags and transported back to the lab on ice and refrigerated until processed. To account for loss of leaf material in handling, fashioning, and deploying the leaf packs, an extra set of 20 leaf packs (5 CM, 5 CO, 5 FM, 5 FO) went through the entire deployment process but was not left in the streams and served as the initial (day 0) amount of leaf material (Benfield 2006).

Due to high precipitation, consequential high water flows, and bag attachment malfunction, a number of leaf bags were lost or destroyed during deployment 1. In FrstY, coarse mesh bags were collected through day 55, but fine mesh bags were only collected until day 41. FrstN coarse bags were collected through day 83, and fine mesh only lasted until day 55. AgY coarse bags were collected through Day 41 and fine bags through day 27. For AgN, WappAgY, and DAgY, both coarse and fine bags were collected through day 27. DAgN got washed out by day 14, when only 4 coarse bags and 2 fine bags were collected. We redeployed bags in DAgN on July 8, 2015, which were collected on 14, 41, and 72 days after redeployment.

Deployment 2, the fall deployment, was conducted from October 16, 2015 through January 15, 2016. Like the first deployment, leaf bags were attached to rebar stakes, only this time bags were directly attached to rebar and then secured with zip ties to minimize loss of bags due to wire corrosion. Due to time constraints and availability of leaves, deployment 2 utilized only maple leaves. Each stream contained only 2 pieces of rebar, and each rebar had 15 bags tethered to it in bundles of alternating sets of three (2 coarse, 1 fine and 2 fine, 1 coarse). The bags were deployed on October, 16, 2015, and one bundle of leaf bags from each piece of rebar was collected to complete a set of 3 coarse and 3 fine bags after 14, 28, 49, 78, and 92 days of incubation. No bags were lost in deployment 2, and all sites except AgN and DAgY had complete pickups until day 92. Bags in AgN and DAgY had very little leaf material remaining on day 78, so we collected all remaining bags on day 78 for these sites. Data from bags that were obviously damaged or had no leaf mass remaining were not included in calculations of breakdown rates.

Leaves were processed in the laboratory by emptying bags into a 250-μm sieve and gently rinsing the leaves to remove silt, debris, and macroinvertebrates. Leaves were then placed into small paper bags, dried to a constant mass at 55°C, and weighed to determine dry mass (DM). Dry material was then ground in a Wiley Mill, and three 0.25 gram subsamples were weighed and combusted at 550^{o}C. Ashed samples were rehydrated, dried overnight, and reweighed to determine ash free dry mass (AFDM). Percent AFDM from these subsamples was used to convert leaf dry mass to AFDM remaining in each leaf bag. The slope of an exponential decay function fit to leaf AFDM remaining over time (k) was used to determine breakdown rates (Benfield 2006). AFDM of organic matter from litter bags used to measure handling losses (not deployed in streams) represented mass at day zero.

Macroinvertebrates

Macroinvertebrates washed off leaves were collected in a 250-µm sieve and preserved in 80% ethanol for sorting and identification to family or genus (except for Oligochaeta and Chironomidae, which were identified to class and family, respectively) and assignment to functional feeding groups (Bott et al. 2012). Functional feeding groups were assigned according to Merritt et al. (2008). Macroinvertebrates from summer deployment were only identified for days 14 and 27 because the remaining days had fewer than 3 bags of each mesh size and leaf species. Macroinvertebrates from the summer deployment were also subsampled due to large numbers of macroinvertebrates in the leaf bags. Mean abundance of macroinvertebrates and shredders (ind/leaf bag) and total macroinvertebrate and shredder density (ind/g AFDM remaining) were calculated for each stream, mesh size, and leaf species within each deployment.

Analysis

Leaf breakdown rates from each stream were compared within each deployment using ANCOVA in SPSS. To assess UNG impact, breakdown rates (of respective bag type) from UNG sites were compared with breakdown rates from non-UNG sites within respective land uses (FrstY v. FrstN, AgY v. AgN, WappAgY v. AgN, DAgY v. DAgN). To assess land use influence, breakdown rates were compared across land uses but within UNG category. All UNG site breakdown rates were compared with one another and all non-UNG site breakdown rates were compared (FrstY v. AgY v. WappAgY v. DAgY and FrstN v. AgN v. DAgN). Relationships between macroinvertebrate variables and leaf decomposition rates were determined by linear regressions. These analyses addressed our hypothesis that UNG presence affects breakdown rates via impact on macroinvertebrate communities. Breakdown rates and macroinvertebrate assemblages were also compared between mesh sizes (CM v. FM, CO v. FO) and leaf species (CM v. CO, FM v. FO), to address our predictions that maple leaves will decompose faster than oak leaves and that leaves in coarse mesh bags will decompose faster than leaves in fine mesh bags. Linear regressions were also done between physical and chemical conditions of the streams and macroinvertebrates and leaf decomposition rates. The study design enables comparison of pairs of sites, one with UNG presence and one without, along a gradient of background land use. Paired t-tests of these site pairs along the landuse gradient were used to determine the impact of UNG activity on physical, chemical, and biological characteristics of the streams.

Results

Leaf breakdown

Summer deployment

All leaf breakdown rates were higher in UNG sites than non-UNG sites across both mesh sizes and leaf types, except for WappAgY CO which was slightly slower than AgN CO (Figure 1). Of the comparisons between UNG sites and non-UNG sites within the same land use, mesh size, and leaf type, 5 out of 16 comparisons were significantly different ($p < 0.05$, Table 3). All significant comparisons were within forested sites CM and FO and dev/ag sites CM, CO, and FO; none of the agriculture sites showed any significant differences in leaf breakdown rates between UNG and non-UNG. Breakdown rates that were significantly higher in UNG sites had rates 2-3 times higher than non-UNG sites. CM rates ranged from -0.0203/d in FrstN to the highest recorded rate of -0.0882/d in WappAgY. FM rates ranged from -0.0202/day in DAgN to -0.0497/d in DAgY. CO rates ranged from -0.0097/day in FrstN to -0.0665/day in AgY. Finally, FO rates were slowest and ranged from -0.0055/day in FrstN to -0.0142/d in AgY. FrstN had the slowest breakdown rates for 3 out of 4 leaf bag types, and agriculture UNG sites had the highest rates for 3 out of 4 types. Though agriculture UNG sites had the highest leaf breakdown rates, these rates were not significantly different than AgN due to high variability of breakdown rates within each bag type in agriculture sites.

High breakdown rates in agriculture sites drove most differences between land use categories within the same UNG category. In sites without UNG presence, AgN CM and CO were significantly higher than FrstN and DAgN CM and CO ($p = 0.006$, $p = 0.012$, $p = 0.012$, and $p = 0.015$, respectfully) (Table 3). FrstN and DAgN had similar breakdown rates, except for FO where DAgN had a significantly faster rate ($p = 0.017$). In UNG sites, there were fewer significant differences between among land use categories. FrstY CO was significantly slower than AgY CO ($p =0.011$), and FrstY FM was slower than WappAgY FM and DAgY FM ($p = 0.027$ and $p = 0.007$, respectively) (Table 3).

Maple leaves decomposed significantly faster than oak in 10 out of 14 comparisons ($p \leq 0.05$) (Figure 1). In coarse mesh bags, maple broke down significantly faster in all sites except for AgY, AgN, and DAgY. In fine mesh bags, maple leaves decomposed significantly faster in all sites except for WappAgY. Breakdown rates between coarse and fine mesh bags within each leaf type were only significantly different in 2 maple comparisons, and none were different in oak comparisons. FrstY CM and WappAgY CM broke down significantly faster than their non-UNG FM counterparts ($p = 0.043$ and $p = 0.004$ respectively) (Figure 1). Though most comparisons were not statistically significant, all coarse mesh bags broke down faster than fine mesh bags with the exception of FrstN FM which was faster than FrstN CM.

Fall deployment

All breakdown rates in forested and dev/ag land uses were higher in UNG site than non-UNG sites. However, in agriculture sites, AgN leaves broke down faster than AgY and WappAgY leaves in FM bags (Figure 2). The only significant differences found between UNG sites and non-UNG sites within same land use, mesh size, and leaf type were in FM leaf packs and, like the summer deployment, occurred in the forested and dev/ag sites ($p = 0.011$ and $p = 0.049$, respectively) (Table 4). Fall deployment CM rates ranged from -0.0167/day in FrstN to -0.0341/day in DAgY. FM rates were slower than CM rates and ranged from -0.0078/day in FrstN to -0.0140/day in AgN.

As in the summer deployment, comparisons between land uses within the same UNG category seemed to be driven by high breakdown rates in agriculture sites. The only significant differences were found in the non-UNG sites where the high rate from AgN outpaced the slower rates from FrstN and DAgN ($p = 0.003$ and $p = 0.005$, respectively) (Table 4). No differences were found among land use categories within UNG sites, almost as if UNG presence negates any land use effects on breakdown rates.

Only maple leaves were used in the fall deployment so there are no leaf species comparisons. Leaves in coarse mesh bags decomposed significantly faster than leaves in fine mesh bags across all sites ($p < 0.05$), except for DAgY (Figure 2).

Water quality and chemistry

Summer deployment

In comparisons between UNG sites and non-UNG sites, only TSS and ammonium were significantly different ($p = 0.002$ and $p = 0.002$) (Table 5). TSS ranged from 0.92 ± 0.2 mg/L in FrstY to 7.63 ± 1.25 mg/L in DAgN and was higher in non-UNG sites within forested and dev/ag land uses. Ammonium ranged from 7.04 ± 2.56 µg/L in FrstN to 16.2 ± 6.99 µg/L in WappAgY and was higher in all UNG sites and significantly so in the forested land use ($p = 0.034$). Though other parameters did not demonstrate significant differences between UNG and non-UNG sites, there were still some noteworthy observations. Temperature (ºC) was generally warmer in agriculture sites than other land uses, ranging from the lowest mean temperature in FrstY (14.91 ± 1.38 ºC) to the highest mean temperature in WappAgY (23.04 ± 0.71 ºC) (Table 5). Dissolved oxygen (DO) was higher in UNG sites than non-UNG sites within forested and dev/ag sites but not agriculture, where AgN had higher DO. AgN and DAgY both had mean discharges over 1000 L/s, which were the highest among all sites.

Ammonium showed a strong positive relationship with breakdown rate. FO breakdown rates were significantly correlated with ammonium ($p < 0.05$), and CM and FM rates were marginally significant ($p < 0.1$) (Figure 3). CO rates demonstrated a slight positive trend with ammonium but were not significant. TSS and specific conductance showed negative and slight positive trends with breakdown rates, respectively, but relationships were not significant.

<u>Fall deployment</u>

Comparisons between UNG sites and non-UNG sites revealed significant difference between temperature, conductivity, and discharge. Temperature was significantly higher in UNG sites ($p = 0.022$), mainly driven by higher temperatures in forested and agriculture UNG sites compared to their non-UNG counterparts ($p = 0.012$ and $p = 0.003$, respectively) (Table 6). Conductivity was highest in agriculture UNG sites ($p = 0.029$). A closer inspection reveals conductivity was only higher in UNG sites within agriculture land use ($p < 0.001$), but in forested and dev/ag land uses, non-UNG sites had higher specific conductance. Sites without UNG presence had significantly higher discharge than UNG sites. This result seemed to be driven by the highest discharge of 2275.26 ± 594.01 L/s observed in AgN which was significantly higher than the two UNG agriculture sites ($p < 0.001$) (Table 6). As in the summer deployment, AgN and DAgY had high discharges. During the fall deployment their discharges, as well as discharge in DAgN, exceeded 1500 L/s. Unlike the summer deployment, ammonium was higher in non-UNG sites rather than UNG sites except for in the dev/ag sites where DAgY had more ammonium than DAgN. TSS was significantly higher in non-UNG forested and dev/ag sites than their UNG counterparts ($p = 0.037$ and $p = 0.026$, respectively). However in the agriculture land use, the UNG sites had significantly higher TSS than AgN mainly driven by high TSS in AgY (4.43 ± 1.37 mg/L) ($p = 0.015$). DAgN had the highest TSS in the fall (6.69 ± 2.19 mg/L), as in the summer deployment (Table 6).

Physicochemical variables had no significant relationships with maple leaf breakdown rates from fall in either mesh size. Ammonium, discharge, DO (% saturation), and DO (mg/L) all had slightly positive trends with maple breakdown rates during fall, and TSS demonstrated a negative trend.

Macroinvertebrates

Summer deployment

Mean macroinvertebrate and shredder abundance and density were higher across all leaf bags in UNG sites than in sites without UNG (Figure 4 and Figure 5). Though they were higher, there were no significant differences in any shredder numbers between sites with and without UNG presence (Table 8). However, in FM bags, macroinvertebrate abundance and density were significantly higher in UNG bags than their non-UNG counterparts ($p = 0.046$ and $p = 0.049$, respectively) (Table 7). CO macroinvertebrate abundance and density were also significant within $p < 0.1$ (Table 7). Within land uses, only FM and CM in FrstY had significantly higher macroinvertebrate abundance than their non-UNG counterparts from FrstN ($p < 0.05$). However, all sites had macroinvertebrates identified from two pickups, except for DAgN which only had macroinvertebrates identified from one pickup; therefore site comparisons including DAgN were not possible. Comparisons across land uses revealed no differences with the exception of WappAgY having significantly higher macroinvertebrate abundance than FrstY in CM bags ($p = 0.002$). Coarse mesh bags had significantly higher macroinvertebrate abundance in both maple and oak bags ($p = 0.0004$ and $p = 0.005$, respectively). However, there was no difference between abundances in maple bags versus oak bags.

There were no significant differences in macroinvertebrate densities within land uses, with the exception of AgY having higher density in CO than AgN ($p = 0.012$). Across land uses however, agriculture sites demonstrated higher densities than forested sites, specifically in the non-UNG sites where AgN had higher densities in FM, FO, and CO than did FrstN ($p= 0.025$, $p = 0.021$, and $p = 0.03$, respectively). Coarse maple bags had higher macroinvertebrate densities than fine maple bags ($p = 0.048$), but there was no difference between CO and FO. Both coarse and fine mesh bags containing maple leaves had higher macroinvertebrate densities than bags containing oak leaves ($p= 0.011$ and $p = 0.017$, respectively).

Shredder abundance and density demonstrated no significant differences within and across land uses, between mesh type and between leaf species (Table 8). Macroinvertebrate abundance was significantly correlated with breakdown rates in 2 of 4 bag types, and density was significantly correlated with breakdown rates in 3 out of 4 bag types ($p < 0.05$) (Figure 6); CO was an exception in both measurements (Figure 7), and macroinvertebrate abundance in CM was not significantly correlated with breakdown rate ($p = 0.052$) (Figure 6).

Fall deployment

Much like the summer deployment, leaf bags in UNG sites had higher numbers of macroinvertebrates and shredders than did non-UNG sites in the fall (Figures 8 and 9). Macroinvertebrate abundance and density in FM bags were significantly higher in UNG sites ($p <0.001$). Macroinvertebrate abundance in CM was also significantly higher in UNG sites ($p = 0.011$), and density was different within $p < 0.1$ ($p= 0.074$) (Table 9).

Within land use categories, forested and dev/ag CM bags demonstrated higher macroinvertebrate abundances in UNG sites than their non-UNG counterparts ($p = 0.01$ and $p = 0.006$, respectively). Comparisons across land uses yielded no significant differences in macroinvertebrate abundance between forested, agriculture, and dev/ag. Coarse mesh bags had significantly higher abundances than did fine mesh bags ($p < 0.0001$).

Comparisons of macroinvertebrate densities between sites within land use categories (FrstY v. FrstN, AgY v. AgN v. WappAgY, DAgY v. DAgN) demonstrated differences in macroinvertebrate density only at $p < 0.1$. FrstY CM and FM had higher macroinvertebrate densities than FrstN ($p = 0.063$ and $p = 0.057$, respectively). WappAgY FM also had higher macroinvertebrate density than AgY ($p = 0.084$). The only significant difference in macroinvertebrate density within UNG sites across land uses was higher density in WappAgY CM than FrstY CM ($p = 0.018$). Coarse mesh bags again had higher macroinvertebrate density than fine mesh bags ($p = 0.003$).

The fall deployment, in comparison with the summer, had higher overall numbers of shredders. Shredder abundance and density were significantly higher in UNG sites in both CM and FM bag types ($p = < 0.001$, $p = 0.006$, $p = 0.01$, and $p = 0.003$) (Table 10). WappAgY had significantly higher shredder abundance and density than AgN in CM ($p = 0.006$ and 0.025, respectively) and different within $p < 0.1$ in FM ($p = 0.052$ and $p = 0.076$). AgY also had slightly higher shredder density than AgN ($p = 0.056$). A low shredder count in AgN was the driver of differences within land use categories as well as across land uses because both FrstN and DAgN contained higher numbers of shredders, significantly so in CM ($p = 0.008$ and $p = 0.09$, respectively). FrstY CM also had higher shredder density than FrstN CM ($p= 0.005$). Coarse mesh again had a higher abundance of shredders than fine mesh ($p < 0.001$), but when it came to shredder density, no significant difference was observed between the two.

Like the summer deployment, the fall macroinvertebrates demonstrated a positive trend with breakdown rate. That being said, the strength of this relationship is not as significant as it was in the summer. Macroinvertebrate density in FM yielded a significant relationship with leaf breakdown rate within p

< 0.1 ($p = 0.076$); in CM the relationship with breakdown rate was significant at $p = 0.05$ (Figure 10). Shredder density in CM bags also demonstrated a slight positive relationship with breakdown rate ($p= 0.031$) (Figure 11).

Seasonal comparisons

A paired t-test between CM and FM breakdown rates from summer, and CM and FM breakdown rates from fall revealed summer breakdown rates were significantly faster than fall ($p < 0.001$). Another paired t-test between combined UNG sites from summer and fall and combined non-UNG sites from summer and fall within FM and CM demonstrated that UNG sites had significantly faster leaf decomposition than non-UNG sites ($p = 0.003$).

Ammonium, phosphorus, and discharge were significantly higher during fall than in summer ($p < 0.001$, $p < 0.001$, and $p = 0.009$, respectively), and water temperature was significantly colder in fall ($p < 0.0001$). In both summer and fall, AgY had the highest specific conductance and lowest discharge, DAgN had highest TSS, and AgN, DAgY, and DAgN had higher discharges than other sites. Ammonium showed a positive trend and TSS negative but not significant trends with breakdown rate in both summer and fall deployment.

A paired t-test between summer and fall macroinvertebrate abundance in CM and FM leaf bags revealed much higher macroinvertebrate abundance in summer than in fall ($p < 0.001$). On the other hand, a paired t-test of shredder densities in CM and FM bags between summer and fall showed fall having significantly higher density than summer ($p = 0.01$).

Site characteristics and leaf breakdown

The mean slope of each watershed basin was quantified using USGS StreamStats, in an attempt to further explain the breakdowns rates we observed. There was a positive relationship between mean slope of the basin (degrees) and breakdown rates. In the summer deployment, this trend was not significant, although breakdown rates still showed positive relationships with slope. In the fall deployment, FM and CM breakdown rates were positively correlated with mean slope of the watershed ($p = 0.034$ and 0.035, respectively) (Figure 12).

Discussion

Leaf decomposition

The objective of this research was to examine whether leaf breakdown as a measure of ecosystem function was significantly different in streams whose watersheds were impacted by UNG activity than those without UNG presence. Leaf breakdown across all sites was higher in UNG sites than in non-UNG sites during summer, and most UNG sites in the fall showed this same trend, except for sites in agriculture land use. This completely rejects our prediction that UNG impairment would slow rates of leaf decomposition due to negative impacts on biota associated with leaf decomposition. Maple leaf breakdown rates we observed (-0.0202/d to -0.0882/d in summer and -0.0078/d to -0.0341/d in fall) were above average in comparison with other studies and average breakdown rates for maple leaves. Breakdown of leaves in three studies ranged from -0.004/d to -0.014/d for maple species and from -0.002/d to -0.004/d for oak species (Bott et al. 2012, Wallace et al. 1982, Webster and Benfield 1986) but rates in our study were 2 – 20 times faster for maple leaves and 2 – 15 times faster for oak leaves. We expected increased sediment from UNG development to slow leaf decomposition rates by burying leaf material or physically removing microbial and macroinvertebrate communities from leaf packs. However, we also found in both summer and fall that UNG sites had significantly more macroinvertebrates and shredders than sites without UNG presence, in contradiction to our predictions. So what does this tell us, if anything, about the effects of UNG activity on aquatic ecosystems?

Leaf breakdown rates tend to correlate with shredding invertebrates, implying that these consumers are responsible for much of the leaf mass loss and therefore have a strong influence on decomposition (Benfield and Webster 1985, Hagen et al. 2006, Sponseller and Benfield 2001). However, our breakdown rates in summer demonstrated no trend with shredders, and our fall breakdown rates only showed slight positive correlation with shredders. Fall shredder density in CM was the only significant correlation between shredder density and breakdown rate ($p = 0.031$). The observations that breakdown rates were faster in summer than fall but that streams contained fewer shredding macroinvertebrates in summer than in fall suggest that perhaps something other than shredding insects is having a larger impact on breakdown rate. That being said, the role of macroinvertebrates should not be dismissed because total macroinvertebrate abundance and density were strongly correlated with summer and fall breakdown rates and again were generally higher in UNG sites rather than non-UNG sites. This observed pattern of higher breakdown rates and higher macroinvertebrate abundance and density in UNG sites than in non-UNG sites is suggestive of an influence of UNG activity on the aquatic ecosystems. Though that result is interesting, several other factors in addition to macroinvertebrates may have influenced our faster and highly variable leaf decomposition rates and might be related to UNG activities or land use.

Higher water temperatures in the summer along with providing leaves as a food source during a time of year when leaves are less available in streams may have created nutrient hot spots where we placed leaf bags in these streams leading to higher numbers of insects observed in leaf packs during summer compared to the fall (Benfield and Webster 1985, Hagen et al. 2006). However, shredder counts were higher in fall than summer, most likely due to life cycles of shredders and their congruence with autumn leaf fall. So, if patterns in shredder density do not explain the high leaf breakdown rates in summer and were only slightly correlated with breakdown rates during fall, what might be influencing leaf breakdown? Benfield and Webster (1985) state that in streams where shredders are numerically unimportant or absent, leaf processing appears to occur as a function of microbial and physical factors.

Ammonium was positively correlated with breakdown rate, in summer and fall. Higher ammonium concentrations could enhance microbial growth, which would increase microbial processing of leaf litter. Enrichment of nitrogen and phosphorus can increase leaf decomposition rates in streams due to positive effects on microbial productivity (Gulis and Suberkropp 2003), which can also benefit macroinvertebrates (Paul et al. 2006). This could explain why both ammonium and macroinvertebrates are positively related to breakdown rates.

Ammonium concentration was also higher in all UNG sites in the summer but not in the fall, which might have contributed to higher leaf breakdown rates in UNG sites during summer.

UNG activity might influence leaf breakdown in streams, but there are several other factors that could also affect decomposition in similar ways making it hard to attribute these impacts to UNG activities alone. In addition, variability across sites and between summer and fall in breakdown rates, discharge, water chemistry, temperature, and flow-related disturbance, as well as in bag specific breakdown rates, make it difficult to pinpoint UNG presence as having a major influence on leaf decomposition. The other variables that may explain the variance in breakdown rates across sites and time include different nutrient concentrations, flow regimes, regional hydrology, pre-existing land uses, as well as many other local factors including different riparian communities. Anthropogenic disturbances in riparian corridors, whether from UNG activity, agriculture, or development, might influence breakdown by altering sediment inputs (Sponseller and Benfield 2001). Variability in leaf pack processing could be attributed to patch-specific community dynamics that are governed by relative distribution of sediment particles and food resources associated with them (Reice 1974, Sponseller and Benfield 2001). Therefore, differences of land use across our sites and unintended local dynamics within each land use may have stronger influences on breakdown than the addition of UNG activity in the watershed.

Hagen et al. (2006) and Sponseller and Benfield (2001) found riparian land use is related to leaf breakdown rates, but land use at the catchment scale is not. Different sites within a land use category may behave differently due to dissimilarities in finer-scale local influences despite broad-scale similarity in land use patterns at the watershed scale. So in context with this study, the incongruences of riparian vegetation and land use but not necessarily UNG activity within our study watersheds may have bigger influences or increase variability of measured leaf breakdown rates.

One of our predictions was that sedimentation would negatively influence breakdown rates. Our results are consistent with this statement in that TSS measurements were negatively correlated with breakdown rates. However, TSS was lower in UNG sites than non-UNG sites, which rejects our hypothesis of UNG sites having more sedimentation. This result also contradicts our hypothesis that macroinvertebrates would be negatively impacted by sedimentation via UNG activity because streams with UNG presence had lower TSS and higher macroinvertebrate abundance than non-UNG. This contradiction led us to think that perhaps breakdown rates were being influenced more by preexisting land use factors and only minimally influenced by UNG activity.

Leaves deployed during summer experienced periods of heavy rains, which greatly increased runoff and discharge in the study streams. The hydrology and steepness of certain sites (specifically AgN, WappAgY, DAgY, and DAgN) could have allowed for flashy water flows and turbulent waters, potentially resulting in physical fragmentation and apparent high breakdown rates. Physical evidence, such as torn bags, tattered leaves, and even bent rebar, suggests that the sites mentioned appeared to be affected most by altered hydrology. AgN always had the highest discharge causing fast turbulent flows, WappAgY had the third highest mean slope of the watershed, DAgY was visibly the steepest on a local scale and also had the second highest mean slope. Lastly, during our study period, a beaver dam was constructed in DAgN greatly slowing the flow through the study reach. Eventually, water broke through the dam causing a large pulse of water to move through the system. This dam construction and removal may have caused unintended hydrologic effects on breakdown rates in this site. High flows can increase leaf fragmentation and breakdown rate (Paul et al. 2006, Pozo et al. 2011, Webster and Waide 1982), which could explain our observed faster and more highly variable breakdown rates in summer than in fall. Rueda-Delgado et al. (2006) found that irregular hydrological pulses in streams can have significantly stronger impacts on breakdown rates than leaf-associated invertebrates. Hagen et al. (2006) also mentioned that physical breakage and fragmentation may dominate leaf breakdown in agricultural streams. So unlike our prediction of sedimentation lowering leaf breakdown rates, it might have increased leaf breakdown by increasing physical fragmentation, especially when accompanied by high flows. Continuous discharge measurements could have provided hydrologic evidence of stream "flashiness," but the small streams we used for our study were ungaged. As a result, we sought alternative traits of the watersheds that might cause more rapid delivery of runoff to streams and therefore enhance physical fragmentation of leaves. Mean slope of the watershed influences how quickly water is delivered to stream channels during storms and showed a positive relationship with leaf breakdown rates. Runoff reaches streams with steeper slopes faster, promoting flashier response to storms, which could ultimately lead to increased physical breakdown of leaves (Pozo et al. 2011).

Implications of altered leaf breakdown rates

Our results indicate faster breakdown rates in streams with UNG presence in comparison to non-UNG streams, as well as faster breakdown rates compared with other studies. So clearly there is alteration in "normal" breakdown rates, potentially caused by UNG activity. What implications will this have on stream ecosystems? The standout implication of altered breakdown rates is the loss and lack of natural retention patterns of organic matter, which might affect insect survival and secondary production (Paul et al. 2006). Cummins et al. (1989) stated that many aquatic insects have evolved life history strategies that involve timing larval development to natural organic matter cycling in streams. So to alter natural breakdown regimes could potentially have negative impacts on the health and hardiness of macroinvertebrate communities in these streams. High precipitation in summer due to storm runoff could lead to accelerated leaf breakdown and might significantly reduce organic matter storage in affected streams, potentially influencing other important stream processes and functions (Paul et al. 2006). Increased exposed surfaces and newly constructed dirt roads associated with UNG development might increase runoff and sediments associated with runoff by modifying hydrology of these watersheds, though the hydrological impact may only be observed locally around the UNG developments.

UNG signature

Land-cover patterns are not longitudinally homogeneous (Reice 1974), and the extent of land use impacts on stream ecosystems may depend on spatial distribution of development in the watershed and the spatial scale at which this distribution is evaluated (Sponseller and Benfield 2001). This land-cover and spatial distribution also applies to UNG impacts, which could explain why UNG presence might not have had a very strong effect on leaf breakdown rates in our study streams. The UNG signature in our study watersheds might have been too diffuse to detect a signature via leaf breakdown. The highest recorded UNG pad density among our study sites was less than 0.6 pads per square mile which is 2 – 7 times less than UNG pad densities necessary to see a turbidity increase in a study by Entrekin et al. (2011). So UNG pad densities within our sites may have been too low to influence leaf breakdown in our streams. Another aspect related to UNG pads is their proximity to our study reaches. Only two sites had a well pad visible from the study reaches. These two observations suggest that UNG impact was too diffuse throughout the watersheds to cause a major impact on in-stream leaf decomposition at our study sites. UNG well pad maturity could also play a role on influences of UNG activity on aquatic ecosystems. A newer active well will likely contribute more sediment to streams due to active construction and development of the infrastructure and increased traffic at these sites (Burton et al. 2014). Once a well is completed and activity diminishes, the input of sediment from UNG development may greatly decrease. Potential impacts of UNG may decline over time due to less activity at an old well site and because of management and containment strategies utilized at finished well sites to aid in the prevention of sedimentation and contamination from UNG presence.

Effects of mesh size and leaf species on breakdown rates

We predicted that leaves in coarse mesh bags would breakdown faster than leaves in fine mesh bags due to exclusion of larger macroconsumers, particularly shredders, from fine mesh bags. Leaves of both species decomposed faster in coarse mesh bags than in fine mesh bags across all sites in the summer, with the exception of maple leaves in FrstN. The same result was found in the fall. However, breakdown rates from the fall deployment demonstrated more significant differences between mesh types than rates from summer, most likely due to higher shredder densities and abundance in fall. The higher shredder numbers in fall allowed for significantly faster breakdown in coarse mesh than in fine mesh. This relationship was still present in summer, but lower shredder densities might have caused breakdown rates to be more similar between mesh sizes. Also, due to higher temperatures, microbial activity might have been higher during summer and could have compensated for loss of macroinvertebrate breakdown and led to similar leaf breakdown rates in fine and coarse mesh bags.

Leaf breakdown rates also might have been slower in fine mesh bags as a result of protection from high flows and mechanical breakdown. Heiber and Gessner (2002) point out this limitation in fine mesh bags, stating that fine mesh might cause an unnatural (and unintended) reduction in physical leaf fragmentation and abrasion, as well as alter water circulation patterns, potentially trapping sediment and hindering nutrient and oxygen exchange. Shredder exclusion could have also led to differing microbial decomposer assemblages or abundances between the two mesh types (Heiber and Gessner 2002, Howe and Suberkropp 1994).

Like we predicted, maple leaves decomposed faster than oak leaves, as demonstrated in many other studies (Thompson and Bärlocher 1989, Wallace et al. 1982, Webster and Benfield 1986). Maple leaves have higher quality carbon and maintain higher microbial biomass, making them more desirable and palatable to macroinvertebrates than more recalcitrant oak leaves (Gulis and Suberkropp 2003, Steffen et al. 2007).

Land use

Land use affected leaf breakdown rates in streams, regardless of the presence of UNG. However, in the absence of UNG, land use had more of an influence than sites with UNG. In comparisons across land uses, leaf breakdown rates were higher in agriculture sites than in forested and dev/ag sites. AgN specifically had much higher rates than FrstN and DAgN in the summer. AgN had higher recorded discharge in summer which might have led to fast turbulent flows and increased physical fragmentation of leaves. Also, AgN had significantly higher macroinvertebrate density than FrstN, which might also have contributed to higher breakdown rates in AgN than forested and dev/ag sites without UNG.

Despite the strong influence of land use observed in streams without UNG, breakdown rates were more similar among land use types for streams with UNG activities in their watersheds. This result suggests that if land use did have any effect on breakdown rates across our sites, it seemed to be nullified with the presence of UNG. Specifically, leaf breakdown in UNG agricultural streams was not significantly faster than in UNG forested or developed/agriculture streams. This similarity could be caused by the presence of UNG activities by causing streams in forested watersheds and developed/agricultural watersheds to behave more like agricultural streams. Hydrologic alterations associated with UNG development in forested landscapes could result from increased exposed surface, dirt roads, and pipelines associated with UNG activity. Increased runoff from these features, along with potential dissolved contents in runoff, may stimulate faster breakdown rates in UNG sites, regardless of preexisting land use. This result could also indicate that the influence of UNG activity on stream ecosystems may be equivalent to changes observed from agricultural land use.

Conclusions

In conclusion, our results show no definitive UNG impact on leaf breakdown rates in streams, at least no more than preexisting land uses and disturbances. Leaf breakdown was not a useful measure of UNG impacts on streams in this study because high flow variability and differences in temperature and nutrients among sites were not strongly linked with UNG presence yet still likely influenced leaf breakdown. The effectiveness of leaf breakdown as a measure of UNG impairment may have been limited due to influences of preexisting land uses both at the local and catchment scale (Hagen et al. 2006) and inconsistent distribution of UNG pads in the study watersheds. Variability between breakdown rates of summer and fall also adds to the inconclusiveness of our results because we found no reliable pattern of UNG impact on leaf breakdown.

More replicates and a simpler study design with fewer variables would be helpful in a future study. Rather than exploring UNG impact across many land uses, it may have been more beneficial to have more replicates within a single land use to simplify the study design and potentially have stronger, more significant comparisons and results. Fewer variables within leaf packs themselves would have also simplified the study design. Using only one leaf type and one mesh size would have allowed for more replicates and more focus on the broad question of whether an UNG signature can be detected by measuring leaf breakdown. Land use and leaf pack variables are important and provide necessary and valuable insights into a very complicated process, but within this study, given the time and personnel restraints, a simpler more concise design may have yielded better comparisons.

Inclusion of sediment characteristics of streams, such as mean substrate size and sediment size, could also help determine sedimentation impacts on breakdown in these sites. A reduction in substrate particle size, through sedimentation, can limit accumulation and retention of leaf material in streams thus preventing the development and maintenance of local shredder populations (Reice 1974, Sponseller and Benfield 2001, Webster and Benfield 1986). Different sizes and distribution of sediment particles could explain some variability in leaf pack processing. Better site selection with more congruent local land use variables could provide better control and lessen natural variability among sites. Sites chosen within each land use category were similar at the catchment scale, but locally, the streams, riparian quantity and diversity, and adjacent land uses were different and could have allowed for unintended differing influences within land use categories. Smaller stream more proximal to UNG pads could also be beneficial in detecting an UNG signature due to increased pad density in a smaller watershed.

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Table 1. Study sites categorized by presence or absence of UNG and percentage of respective land use and respective pad number.

Land Use	Stream Code	Site Name	%Stable V eg	% Ag	% Dev	Number of Well Pads	Drainage Area (sq. mi)	Pad Dens. (pad/sq. mi.)
Forest	FrstY	Grays Run	99.89	0.03	0.01	9	16.23	0.55
Forest	FrstN	Loyalsock Creek	97.91	1.04	0.3	0	27	0
Agriculture	AgY	Apalachin Creek	94.35	4.73	0.81	11	43	0.26
Agriculture	AgN	Bowman Creek	94.59	4.9	0.5	0	54	0
Agriculture	WappAgY	Wappasening Creek	94.5	4.81	0.66	23	47	0.49
Dev/Ag	DAgY	Blockhouse Creek	92.22	6.05	1.62	10	38	0.26
Dev/Ag	DAgN	Nanticoke Creek	86.43	11.92	1.55	0	48	0

Table 2. Watershed characteristics quantified using USGS StreamStats (USGS 2016).

Site	Drainage Area (sq. mi)	Stream Length (mi)	Stream Density (mi/sq. mi)	Mean slope (degrees)	Mean Precip. (inches)
FrstY	16.2	24.39	1.5	12.4	37
FrstN	27	54.6	2.02	4.2	41
AgY	43.4	78.5	1.83	7.4	37
AgN	38.9	54.59	1.4	9.4	43
WappAgY	56	120.37	2.15	6.5	36
DAgY	37.8	67.51	1.79	11.4	36
DAgN	48.2	92.13	1.91	5.9	37

Table 3. Summer deployment breakdown rates by mesh size and leaf type. P-values are from ANOVA comparisons between UNG sites and non-UNG sites within a land use category. * in land use column indicates significant difference between UNG and non-UNG sites within land use (p-value column). N-land use column indicates breakdown rate comparisons across land uses within non-UNG sites and bag type. Y-land use column indicates breakdown rate comparisons across land uses within UNG sites and bag type. Different letter indicates significant difference.

Landuse	Stream name	Stream code	Mesh size/Leaf Type	Breakdown rate (/d)	N-land use	Y-land use	p-value
Forested*	Gray's Run	FrstY	CM	-0.05587		A	< 0.001
	Loyalsock Creek	FrstN	CM	-0.0203	A		
Agriculture	Apalachin Creek	AgY	CM	-0.08076		A	0.728
	Bowman Creek	AgN	CM	-0.07158	B		
	Wappasenning Creek	WappAgY	CM	-0.0882		A	0.561
Dev/Ag*	Blockhouse Creek	DAgY	CM	-0.07531		A	0.013
	Nanticoke Creek	DAgN	CM	-0.02592	A		
Forested	Gray's Run	FrstY	FM	-0.02774		A	0.364
	Loyalsock Creek	FrstN	FM	-0.02414	A		
Agriculture	Apalachin Creek	AgY	FM	-0.0454		AB	0.292
	Bowman Creek	AgN	FM	-0.03084	A		
	Wappasenning Creek	WappAgY	FM	-0.04076		B	0.185
Dev/Ag	Blockhouse Creek	DAgY	FM	-0.04966		B	0.058
	Nanticoke Creek	DAgN	FM	-0.02017	A		
Forested	Gray's Run	FrstY	CO	-0.01854		A	0.075
	Loyalsock Creek	FrstN	CO	-0.00973	A		
Agriculture	Apalachin Creek	AgY	CO	-0.06646		B	0.244
	Bowman Creek	AgN	CO	-0.03275	B		
	Wappasenning Creek	WappAgY	CO	-0.02569		AB	0.646
Dev/Ag*	Blockhouse Creek	DAgY	CO	-0.02541		AB	0.008
	Nanticoke Creek	DAgN	CO	-0.01011	A		
Forested*	Gray's Run	FrstY	FO	-0.01261		A	0.001
	Loyalsock Creek	FrstN	FO	-0.00549	A		
Agriculture	Apalachin Creek	AgY	FO	-0.01422		A	0.371
	Bowman Creek	AgN	FO	-0.00752	A		
	Wappasenning Creek	WappAgY	FO	-0.0135		A	0.421
Dev/Ag*	Blockhouse Creek	DAgY	FO	-0.019		A	0.004
	Nanticoke Creek	DAgN	FO	-0.00849	A		

Table 4. Fall deployment breakdown rates by mesh size and leaf type. P-values are from ANOVA comparisons between UNG sites and non-UNG sites within a land use category. * in land use column indicates significant difference between UNG and non-UNG sites within land use (p-value column). N-land use column indicates breakdown rate comparisons across land uses within non-UNG sites and bag type. Y-land use column indicates breakdown rate comparisons across land uses within UNG sites and bag type. Different letter indicates significant difference.

Landuse	Stream name	Stream code	Mesh size	Breakdown rate (/d)	N-land use	Y-land use	p-value
Forested	Gray's Run	FrstY	CM	-0.028266		A	0.681
	Loyalsock Creek	FrstN	CM	-0.016739	A		
Agriculture	Apalachin Creek	AgY	CM	-0.028073		A	0.635
	Bowman Creek	AgN	CM	-0.0249	A		
	Wappasenning Creek	WappAgY	CM	-0.023477		A	0.903
Dev/Ag	Blockhouse Creek	DAgY	CM	-0.034096		A	0.238
	Nanticoke Creek	DAgN	CM	-0.02534	A		
Forested	Gray's Run	FrstY	FM	-0.013697		A	0.011
	Loyalsock Creek	FrstN	FM	-0.007833	A		
Agriculture	Apalachin Creek	AgY	FM	-0.013309		A	0.78
	Bowman Creek	AgN	FM	-0.013989	B		
	Wappasenning Creek	WappAgY	FM	-0.013772		A	0.86
Dev/Ag	Blockhouse Creek	DAgY	FM	-0.016019		A	0.049
	Nanticoke Creek	DAgN	FM	-0.01058	A		

Table 5. Summer deployment mean (± 1 SE) chemical and physical characteristics of streams in each land use category. P-values are from paired t-tests within in land use between UNG and non-UNG. Total UNG v non-UNG p-value from t-test between all UNG sites and non-UNG sites.

	Forest			Agriculture				Dev/Ag			Total UNG v non UNG
	FrstY	FrstN	p-value	AgY	AgN	WappAgY	p-value	DAgY	DAgN	p-value	p-value
Temp (°C)	14.91 ± 1.38	19.36 ± 0.57	0.005	21.76 ± 0.60	18.50 ± 0.55	23.04 ± 0.71	< 0.001	15.73 ± 0.28	20.50 ± 0.85	0.005	0.553
pH	7.74 ± 0.12	7.99 ± 0.23	0.308	7.67 ± 0.07	8.01 ± 0.14	7.98 ± 0.05	0.223	8.06 ± 0.15	8.07 ± 0.16	0.467	0.237
DO %	94.4 ± 1.36	95.04 ± 0.84	0.915	94.48 ± 2.61	98.43 ± 1.00	101.78 ± 1.06	0.914	99.58 ± 0.65	101.62 ± 3.49	0.062	0.666
DO (mg/L)	9.5 ± 0.34	8.76 ± 0.09	0.010	8.30 ± 0.21	9.23 ± 0.18	8.74 ± 0.20	0.042	9.88 ± 0.03	9.15 ± 0.27	0.017	0.712
Cond (µS/cm)	34.88 ± 5.96	32.80 ± 1.97	0.677	207.75 ± 4.07	57.75 ± 23.24	109.50 ± 3.17	0.006	145.25 ± 13.87	205.00 ± 25.59	0.132	0.067
Discharge (L/s)	699.19 ± 302.26	587.11 ± 130.33	0.988	340.54 ± 49.11	1028.57 ± 461.05	467.96 ± 59.14	0.056	1018.49 ± 241.24	765.37 ± 272.15	0.847	0.072
SRP (µg/L)	3.79 ± 0.96	4.65 ± 1.05	0.248	4.63 ± 1.04	3.96 ± 1.17	5.32 ± 1.76	0.280	8.99 ± 2.37	6.76 ± 1.69	0.181	0.096
NH4 - N (µg/L)	12.07 ± 2.74	7.04 ± 2.56	0.034	10.94 ± 0.84	7.6 ± 1.55	16.2 ± 6.99	0.157	14.31 ± 5.31	8.26 ± 2.14	0.105	0.002
TSS (mg/L)	0.92 ± 0.2	3.13 ± 0.41	0.001	1.80 ± 0.48	2.27 ± 0.39	2.70 ± 0.45	0.961	2.97 ± 0.37	7.63 ± 1.25	0.073	0.002

Table 6. Fall deployment mean (± 1 SE) chemical and physical characteristics of streams in each land use category. P-values are from paired t-tests within in land use between UNG and non-UNG. Total UNG v non-UNG p-value from t-test between all UNG sites and non-UNG sites.

	Forest			Agriculture				Dev/Ag			Total UNG v non UNG
	FrstY	FrstN	p-value	AgY	AgN	WappAgY	p-value	DAgY	DAgN	p-value	p-value
Temp (°C)	6.35 ± 0.71	4.03 ± 1.25	0.012	7.79 ± 2.06	6.36 ± 1.09	8.85 ± 1.20	0.003	5.72 ± 0.90	6.12 ± 1.75	0.206	0.022
pH	7.75 ± 0.17	7.76 ± 0.21	0.988	7.69 ± 0.16	7.68 ± 0.21	7.62 ± 0.08	0.927	7.78 ± 0.27	8.12 ± 0.14	0.222	0.473
DO %	94.65 ± 0.50	91.13 ± 0.63	0.007	92.05 ± 3.08	95.66 ± 0.96	97.25 ± 1.74	0.492	97.14 ± 1.84	90.73 ± 2.00	0.145	0.239
DO (mg/L)	11.69 ± 0.22	12.00 ± 0.38	0.200	11.15 ± 0.84	11.85 ± 0.26	11.31 ± 0.28	0.072	12.18 ± 0.16	11.40 ± 0.73	0.154	0.656
Cond (µS/cm)	31.83 ± 0.54	34.00 ± 4.29	0.595	234.00 ± 29.96	29.60 ± 2.71	142.75 ± 5.33	< 0.001	116.60 ± 13.04	169.00 ± 24.26	0.016	0.029
Discharge (L/s)	963.38 ± 249.42	1190.33 ± 230.29	0.168	513.45 ± 156.82	2275.26 ± 594.01	706.60 ± 273.25	< 0.001	1583.72 ± 414.66	1807.32 ± 535.63	0.391	< 0.001
SRP (µg/L)	6.74 ± 2.78	10.09 ± 5.45	0.286	7.61 ± 2.85	9.29 ± 5.44	11.53 ± 7.57	0.711	11.91 ± 4.54	13.01 ± 5.30	0.709	0.460
NH4 - N (µg/L)	117.27 ± 21.24	162.84 ± 19.72	0.076	94.12 ± 28.87	140.29 ± 21.49	128.91 ± 43.66	0.874	201.31 ± 33.29	88.34 ± 20.87	0.136	0.591
TSS (mg/L)	0.22 ± 0.06	1.50 ± 0.54	0.037	4.43 ± 1.37	0.45 ± .07	0.73 ± .08	0.015	1.12 ± 0.15	6.69 ± 2.19	0.026	0.498

Table 7. Mean (± 1 SE) summer deployment macroinvertebrate abundance (ind/bag) and density (ind/g AFDM). P-values from paired t-test between UNG sites and non-UNG sites.

UNG	Landuse	Stream name	Stream code	Mesh size	Macro Abundance (ind/bag)	p-value	Macro Density (ind/g AFDM)	p-value
Y	**Forested**	Gray's Run	FrstY	CM	386.80 ± 34.22		234.30 ± 75.52	
	Agriculture	Apalachin Creek	AgY	CM	2110.00 ± 372.26		811.79 ± 235.56	
	Agriculture	Wappasenning Creek	WappAgY	CM	2343.00 ± 400.05		1482.91 ± 491.51	
	Dev/Ag	Blockhouse Creek	DAgY	CM	2056.00 ± 478.67	0.110	1659.48 ± 680.62	0.150
N	**Forested**	Loyalsock Creek	FrstN	CM	1001.50 ± 283.17		284.30 ± 100.42	
	Agriculture	Bowman Creek	AgN	CM	960.67 ± 294.04		555.68 ± 331.27	
	Dev/Ag	Nanticoke Creek	DAgN	CM	346.00 ± 68.2		97.71 ± 23.62	
Y	**Forested**	Gray's Run	FrstY	FM	511.50 ± 71.69		263.71 ± 64.91	
	Agriculture	Apalachin Creek	AgY	FM	1151.50 ± 369.29		526.21 ± 157.43	
	Agriculture	Wappasenning Creek	WappAgY	FM	1065.83 ± 322.41		664.69 ± 242.47	
	Dev/Ag	Blockhouse Creek	DAgY	FM	1507.00 ± 548.70	0.046	1247.45 ± 606.37	0.049
N	**Forested**	Loyalsock Creek	FrstN	FM	277.00 ± 55.80		119.94 ± 29.41	
	Agriculture	Bowman Creek	AgN	FM	472.75 ± 61.14		227.42 ± 39.79	
	Dev/Ag	Nanticoke Creek	DAgN	FM	108.00		42.09	
Y	**Forested**	Gray's Run	FrstY	CO	356.00 ± 88.55		55.98 ± 17.41	
	Agriculture	Apalachin Creek	AgY	CO	2439.00 ± 509.58		365.28 ± 95.88	
	Agriculture	Wappasenning Creek	WappAgY	CO	2736.00 ±640.15		439.89 ± 93.74	
	Dev/Ag	Blockhouse Creek	DAgY	CO	2599.33 ± 873.78	0.092	511.02 ± 183.98	0.070
N	**Forested**	Loyalsock Creek	FrstN	CO	854.17 ± 187.21		117.20 ± 29.96	
	Agriculture	Bowman Creek	AgN	CO	796.83 ± 125.94		144.95 ± 25.80	
	Dev/Ag	Nanticoke Creek	DAgN	CO	448.50 ± 96.10		64.18 ± 15.62	
Y	**Forested**	Gray's Run	FrstY	FO	315.40 ± 88.73		93.90 ± 28.74	
	Agriculture	Apalachin Creek	AgY	FO	1331.6 ± 611.03		345.18 ± 151.49	
	Agriculture	Wappasenning Creek	WappAgY	FO	785.2 ± 176.67		224.56 ± 48.31	
	Dev/Ag	Blockhouse Creek	DAgY	FO	1549.83 ± 581.292	0.189	559.89 ± 219.884	0.166
N	**Forested**	Loyalsock Creek	FrstN	FO	247.83 ± 50.90		67.74 ± 15.05	
	Agriculture	Bowman Creek	AgN	FO	514.33 ± 86.56		149.89 ± 30.89	
	Dev/Ag	Nanticoke Creek	DAgN	FO	98.50 ± 39.5		24.73 ± 10.98	

Table 8. Mean (± 1 SE) summer deployment shredder abundance (ind/bag) and density (ind/g AFDM). P-values from paired t-test between UNG sites and non-UNG sites.

UNG	Landuse	Stream name	Stream code	Mesh size	Shredder Abundance (SH ind/bag)	p-value	Shredder Density (SH ind/g AFDM)	p-value
Y	**Forested**	Gray's Run	FrstY	CM	77.00 ± 19.80	0.348	56.29 ± 22.43	0.518
	Agriculture	Apalachin Creek	AgY	CM	2.00 ± 1.26		0.73 ± 0.57	
	Agriculture	Wappasenning Creek	WappAgY	CM	0.33 ± 0.33		0.06 ± 0.06	
	Dev/Ag	Blockhouse Creek	DAgY	CM	8.83 ± 4.89		5.35 ± 3.05	
N	**Forested**	Loyalsock Creek	FrstN	CM	11.50 ± 4.76		2.48 ± 1.22	
	Agriculture	Bowman Creek	AgN	CM	8.00 ± 3.97		7.74 ± 4.96	
	Dev/Ag	Nanticoke Creek	DAgN	CM	0.50 ± 0.29		0.10 ± 0.06	
Y	**Forested**	Gray's Run	FrstY	FM	18.00 ± 8.76	0.811	10.31 ± 5.42	0.906
	Agriculture	Apalachin Creek	AgY	FM	0.00		0.00	
	Agriculture	Wappasenning Creek	WappAgY	FM	0.00		0.00	
	Dev/Ag	Blockhouse Creek	DAgY	FM	13.00 ± 9.15		10.94 ± 8.02	
N	**Forested**	Loyalsock Creek	FrstN	FM	1.33 ± 0.99		0.56 ± 0.43	
	Agriculture	Bowman Creek	AgN	FM	3.75 ±2.84		2.37 ± 1.98	
	Dev/Ag	Nanticoke Creek	DAgN	FM	0.00		0.00	
Y	**Forested**	Gray's Run	FrstY	CO	70.33 ±24.36	0.547	11.53 ± 4.52	0.661
	Agriculture	Apalachin Creek	AgY	CO	0.33 ± 0.33		0.04 ± 0.04	
	Agriculture	Wappasenning Creek	WappAgY	CO	1.33 ± 1.33		0.34 ± 0.34	
	Dev/Ag	Blockhouse Creek	DAgY	CO	3.00 ± 1.61		0.55 ± 0.31	
N	**Forested**	Loyalsock Creek	FrstN	CO	13.67 ± 7.84		1.80 ± 1.08	
	Agriculture	Bowman Creek	AgN	CO	12.33 ± 4.72		2.79 ± 1.17	
	Dev/Ag	Nanticoke Creek	DAgN	CO	0.25 ± 0.25		0.04 ± 0.04	
Y	**Forested**	Gray's Run	FrstY	FO	25.80 ± 8.64	0.807	7.79 ± 2.88	0.777
	Agriculture	Apalachin Creek	AgY	FO	0.40 ± 0.40		0.10 ± 0.10	
	Agriculture	Wappasenning Creek	WappAgY	FO	0.00		0.00	
	Dev/Ag	Blockhouse Creek	DAgY	FO	24.50 ± 11.85		8.94 ± 4.31	
N	**Forested**	Loyalsock Creek	FrstN	FO	3.33 ± 1.15		0.89 ± 0.32	
	Agriculture	Bowman Creek	AgN	FO	9.83 ± 4.74		2.86 ± 1.45	
	Dev/Ag	Nanticoke Creek	DAgN	FO	0.00		0.00	

Table 9. Mean (± 1 SE) fall deployment macroinvertebrate abundance (ind/bag) and density (ind/g AFDM). P-values from paired t-test between UNG sites and non-UNG sites.

UNG	Landuse	Stream name	Stream code	Mesh size	Macro Abundance (ind/bag)	p-value	Macro Density (ind/g AFDM)	p-value
Y	**Forested**	Gray's Run	FrstY	CM	265.20 ± 30.46	0.011	204.22 ± 62.51	0.074
	Agriculture	Apalachin Creek	AgY	CM	240.33 ± 58.10		193.91 ± 86.93	
	Agriculture	Wappasenning Creek	WappAgY	CM	165.53 ± 28.36		139.26 ± 60.70	
	Dev/Ag	Blockhouse Creek	DAgY	CM	223.54 ± 32.38		335.93 ± 146.20	
N	**Forested**	Loyalsock Creek	FrstN	CM	125.33 ± 22.2		107.10 ± 53.51	
	Agriculture	Bowman Creek	AgN	CM	162.92 ± 39.61		98.44 ± 24.76	
	Dev/Ag	Nanticoke Creek	DAgN	CM	92.33 ± 14.72		45.13 ± 12.05	
Y	**Forested**	Gray's Run	FrstY	FM	75.33 ± 13.17	< 0.001	57.29 ± 12.65	< 0.001
	Agriculture	Apalachin Creek	AgY	FM	99.53 ± 16.80		55.82 ± 9.75	
	Agriculture	Wappasenning Creek	WappAgY	FM	95.20 ± 18.38		61.82 ± 15.04	
	Dev/Ag	Blockhouse Creek	DAgY	FM	93.29 ± 22.59		61.63 ± 15.84	
N	**Forested**	Loyalsock Creek	FrstN	FM	51.87 ± 8.00		23.83 ± 3.79	
	Agriculture	Bowman Creek	AgN	FM	36.73 ± 8.39		25.09 ± 6.68	
	Dev/Ag	Nanticoke Creek	DAgN	FM	53.60 ± 11.13		26.55 ± 4.92	

Table 10. Mean (± 1 SE) fall deployment shredder abundance (ind/bag) and density (ind/g AFDM). P-values from paired t-test between UNG sites and non-UNG sites.

UNG	Landuse	Stream name	Stream code	Mesh size	Shredder Abundance (SH ind/bag)	p-value	Shredder Density (SH ind/g AFDM)	p-value
Y	**Forested**	Gray's Run	FrstY	CM	59.07 ± 10.01	< 0.001	25.75 ± 3.37	0.006
	Agriculture	Apalachin Creek	AgY	CM	98.73 ± 23.57		47.60 ± 12.86	
	Agriculture	Wappasenning Creek	WappAgY	CM	66.87 ± 8.81		32.28 ± 6.35	
	Dev/Ag	Blockhouse Creek	DAgY	CM	85.08 ± 10.62		102.84 ± 37.26	
N	**Forested**	Loyalsock Creek	FrstN	CM	28.20 ± 4.08		11.79 ± 2.38	
	Agriculture	Bowman Creek	AgN	CM	20.83 ± 4.42		13.39 ± 2.99	
	Dev/Ag	Nanticoke Creek	DAgN	CM	40.80 ± 8.78		18.44 ± 4.64	
Y	**Forested**	Gray's Run	FrstY	FM	27.07 ± 7.32	0.01	18.69 ± 5.67	0.003
	Agriculture	Apalachin Creek	AgY	FM	80.27 ± 15.42		44.28 ± 8.74	
	Agriculture	Wappasenning Creek	WappAgY	FM	82.07 ± 16.93		52.29 ± 13.16	
	Dev/Ag	Blockhouse Creek	DAgY	FM	40.43 ± 6.92		28.10 ± 5.89	
N	**Forested**	Loyalsock Creek	FrstN	FM	24.20 ± 4.73		11.68 ± 2.39	
	Agriculture	Bowman Creek	AgN	FM	12.40 ± 3.54		8.47 ± 2.85	
	Dev/Ag	Nanticoke Creek	DAgN	FM	33.93 ± 7.07		17.30 ± 3.38	

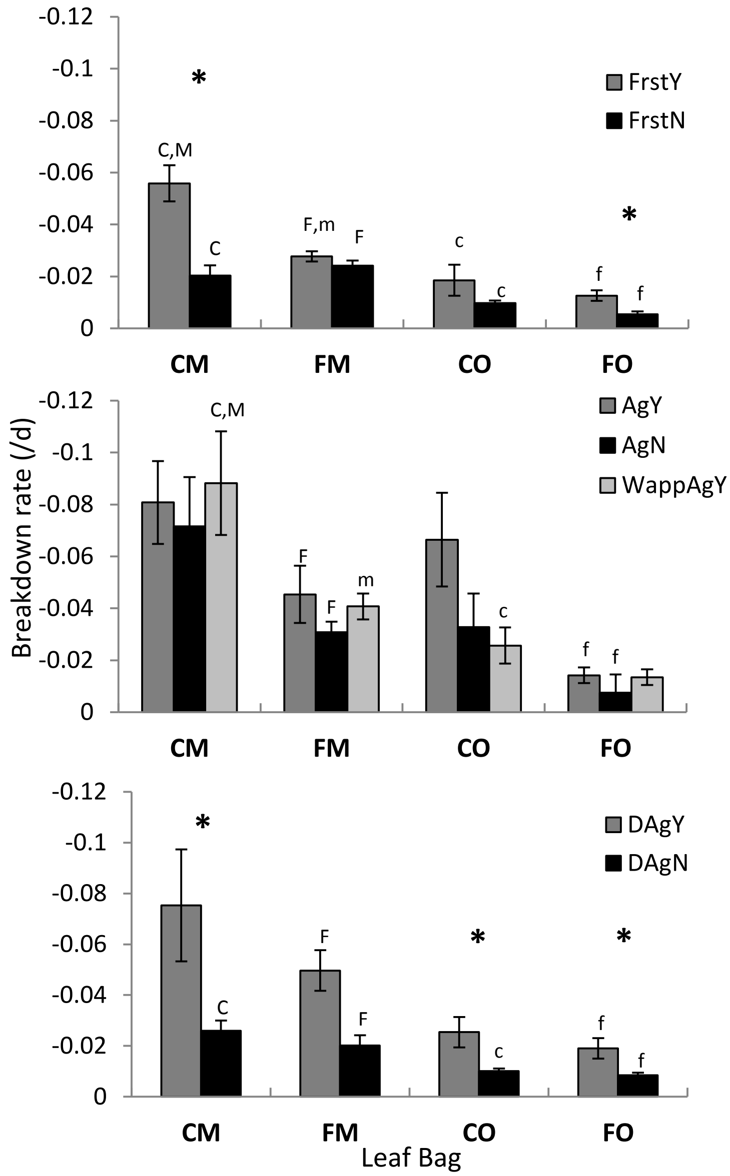

Figure 1: Summer deployment breakdown rates (/d) in each leaf bag. Only significant differences are shown, * = UNG v. non-UNG within each land use and bag type. C = CM v. CO, F = FM v. FO, M = CM v. FM, O = CO v. FO. All mesh size and leaf type comparisons are within site. Different letter case indicates significant difference.

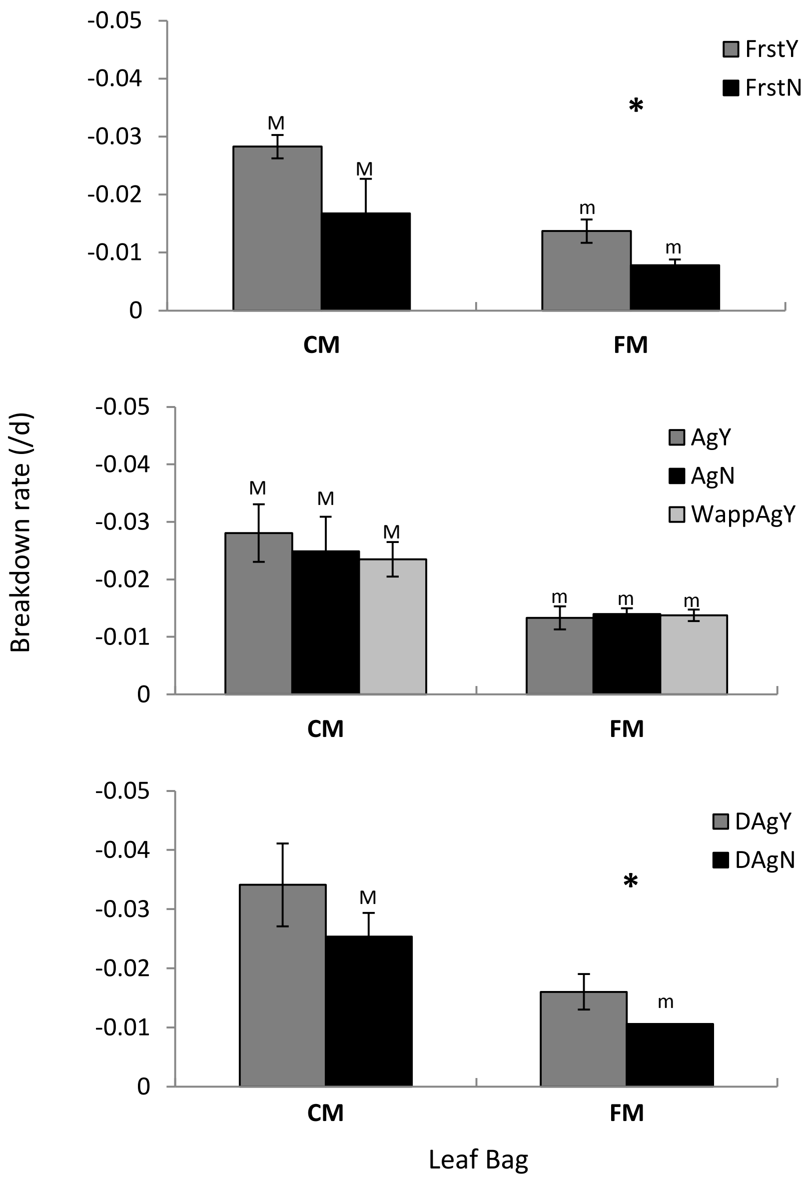

Figure 2. Fall deployment breakdown rates (/d) in each leaf bag. Only significant differences are shown, * = UNG v. non-UNG within each land use and bag type. M = CM v. FM. All mesh size comparisons are within site. Different letter case indicates significant difference.

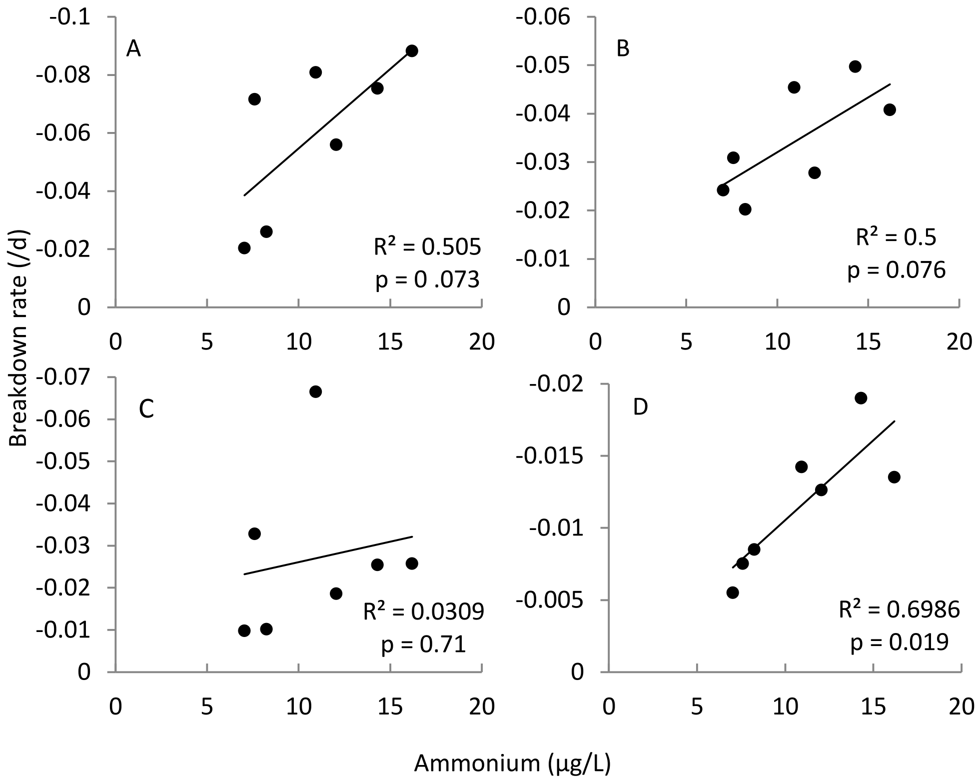

Figure 3. Linear regression of summer deployment breakdown rates and ammonium (µg/L). CM (A) FM (B) CO (C) FO (D). Note different y-axis values for different leaf species.

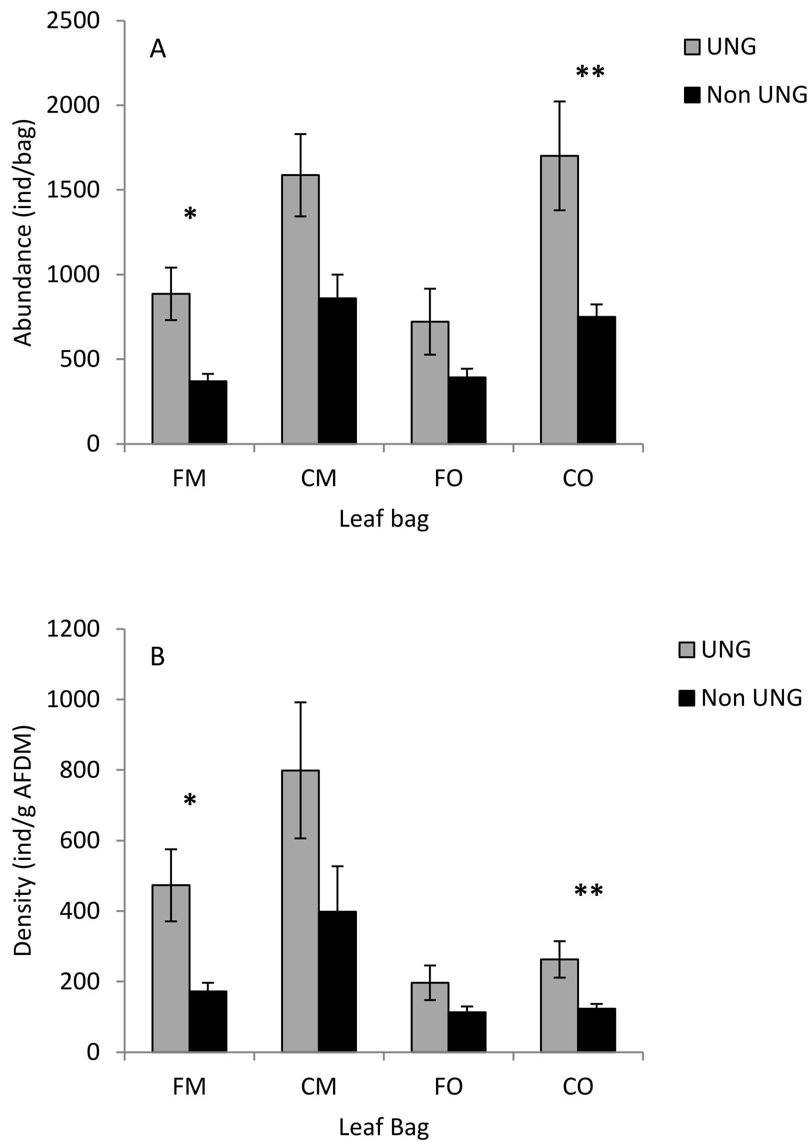

Figure 4. Summer deployment mean (± 1 SE) macroinvertebrate abundance (A) and density (B) in each leaf bag. * indicates significant difference between UNG and non-UNG sites at $p < 0.05$. ** indicates significance at $p < 0.1$. P-values from paired t-tests of total means.

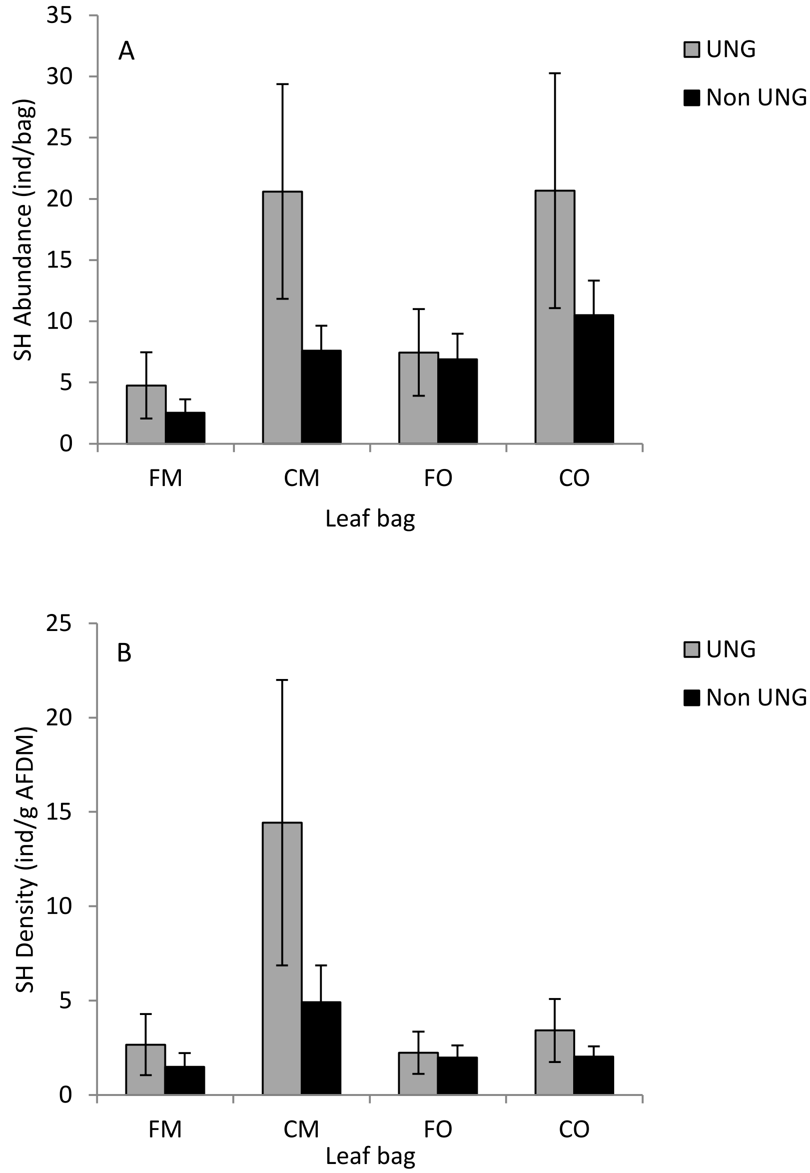

Figure 5. Summer deployment mean (± 1 SE) shredder abundance (A) and density (B) in each leaf bag.

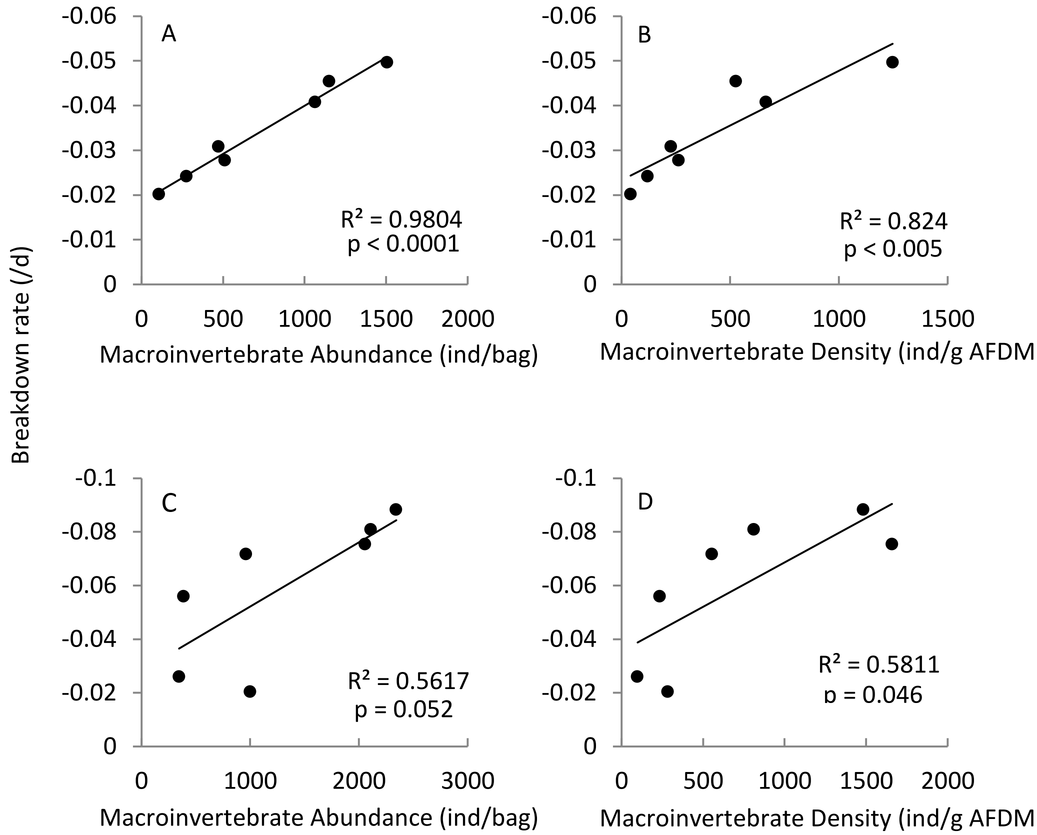

Figure 6. Linear regression of summer deployment leaf breakdown rates and FM macroinvertebrate abundance (A) and density (B) and CM macroinvertebrate abundance (C) and density (D). AFDM = ash-free dry mass. Note difference in x-axis scales between FM and CM regressions.

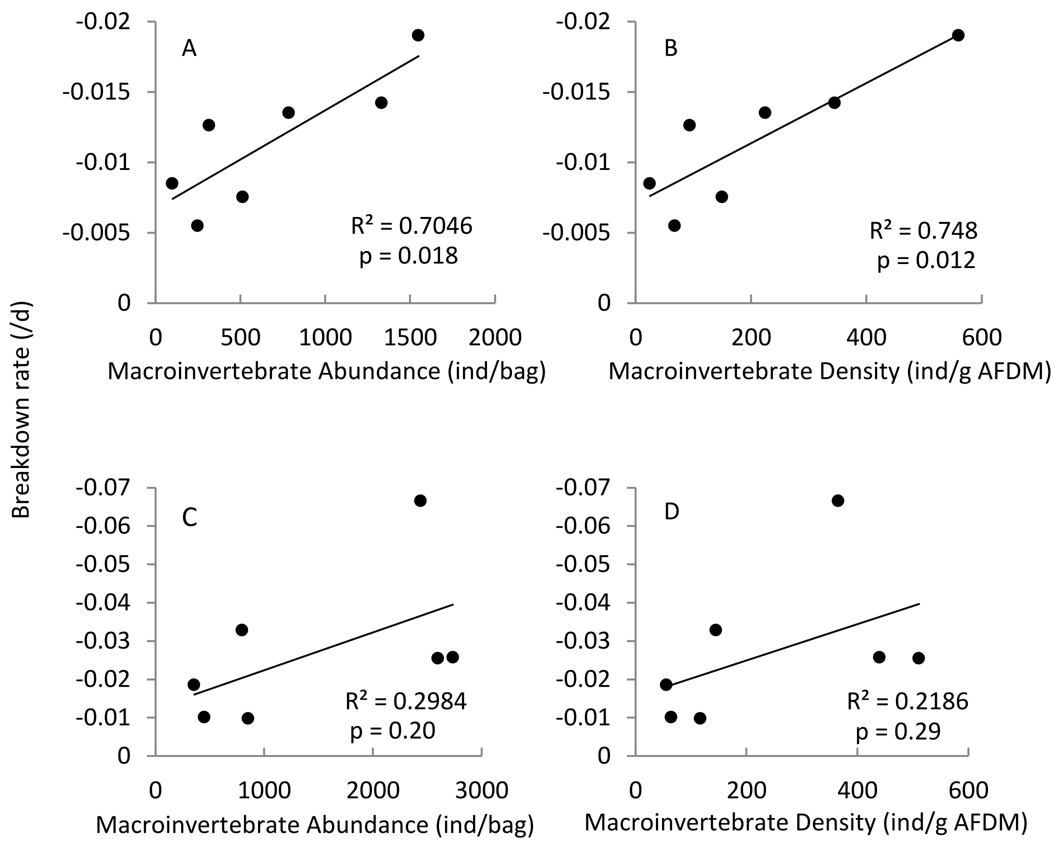

Figure 7. Linear regression of summer deployment leaf breakdown rates and FO macroinvertebrate abundance (A) and density (B) and CO macroinvertebrate abundance (C) and density (D) AFDM = ash-free dry mass. Note difference in x-axis scales between FO and CO regressions.

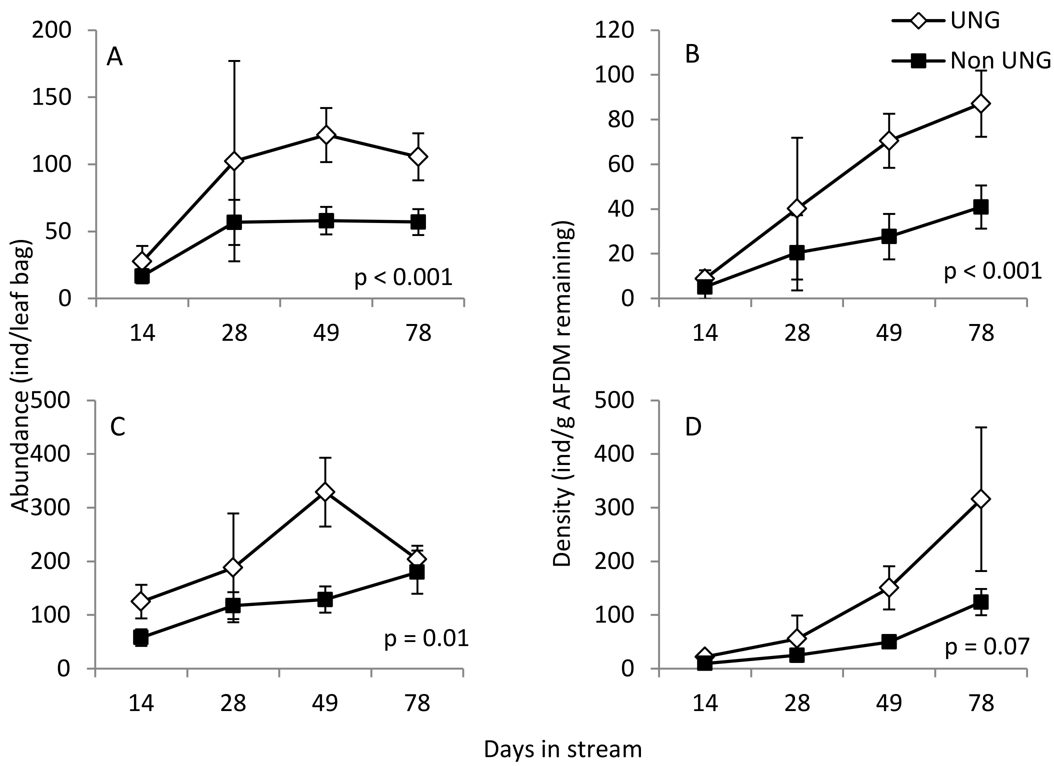

Figure 8. Fall deployment mean (± 1 SE) FM macroinvertebrate abundance (A) and density (B) and CM macroinvertebrate abundance (C) and density (D) over time in streams with and without UNG. Error bars on some dates are too small to be seen. AFDM = ash-free dry mass. P-values from paired t-test of total means.

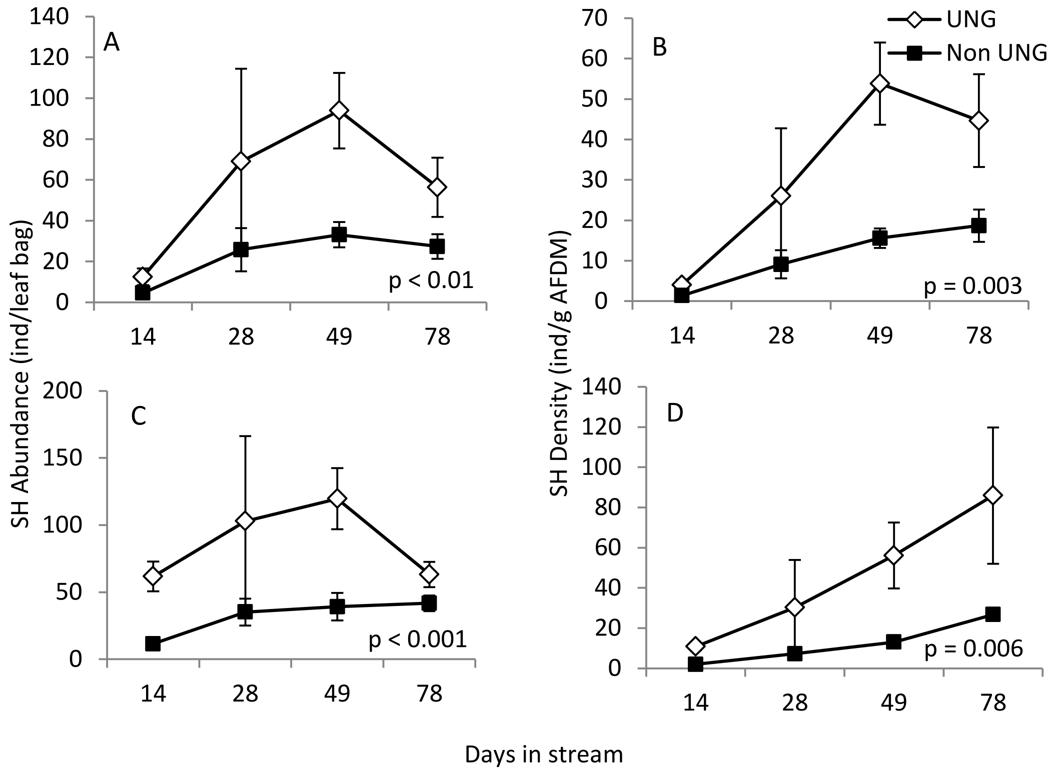

Figure 9. Fall deployment mean (± 1 SE) FM shredder abundance (A) and density (B) and CM shredder abundance (C) and density (D) over time in streams with and without UNG. Error bars on some dates are too small to be seen. AFDM = ash-free dry mass. P-values from paired t-test of total means.

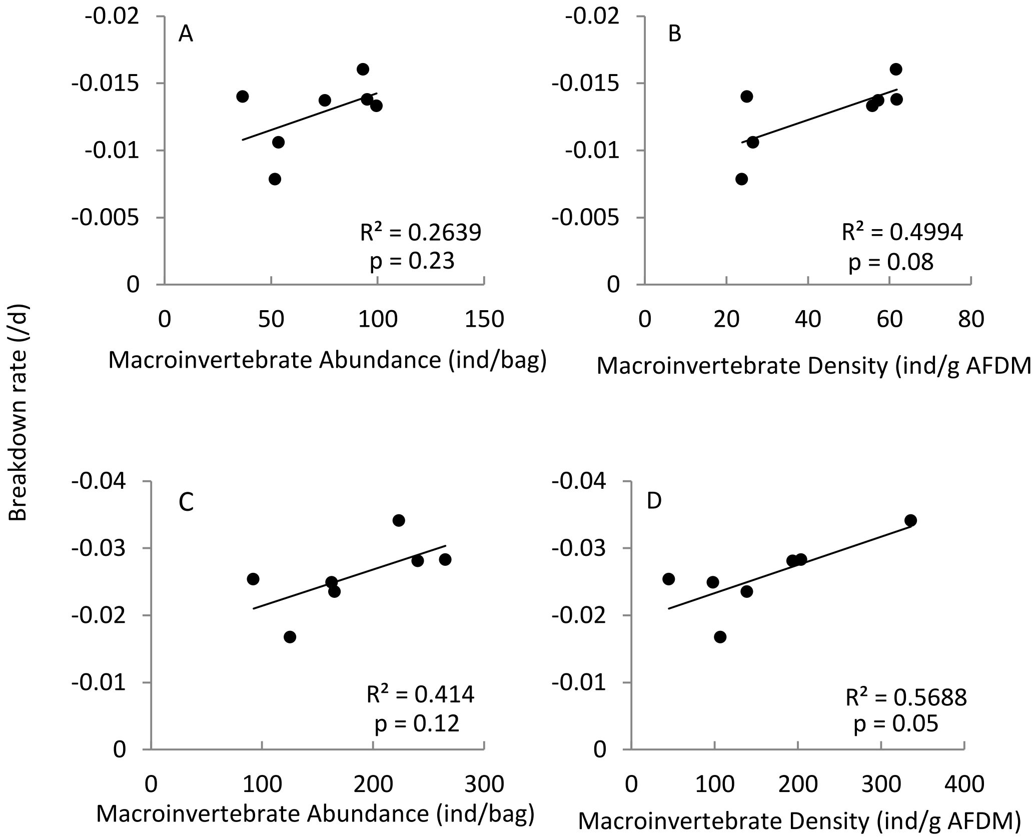

Figure 10. Linear regression of fall deployment leaf breakdown rates and FM macroinvertebrate abundance (A) and density (B) and CM macroinvertebrate abundance (C) and density (D). AFDM = ash-free dry mass. Note difference in x-axis scales between FM and CM regressions.

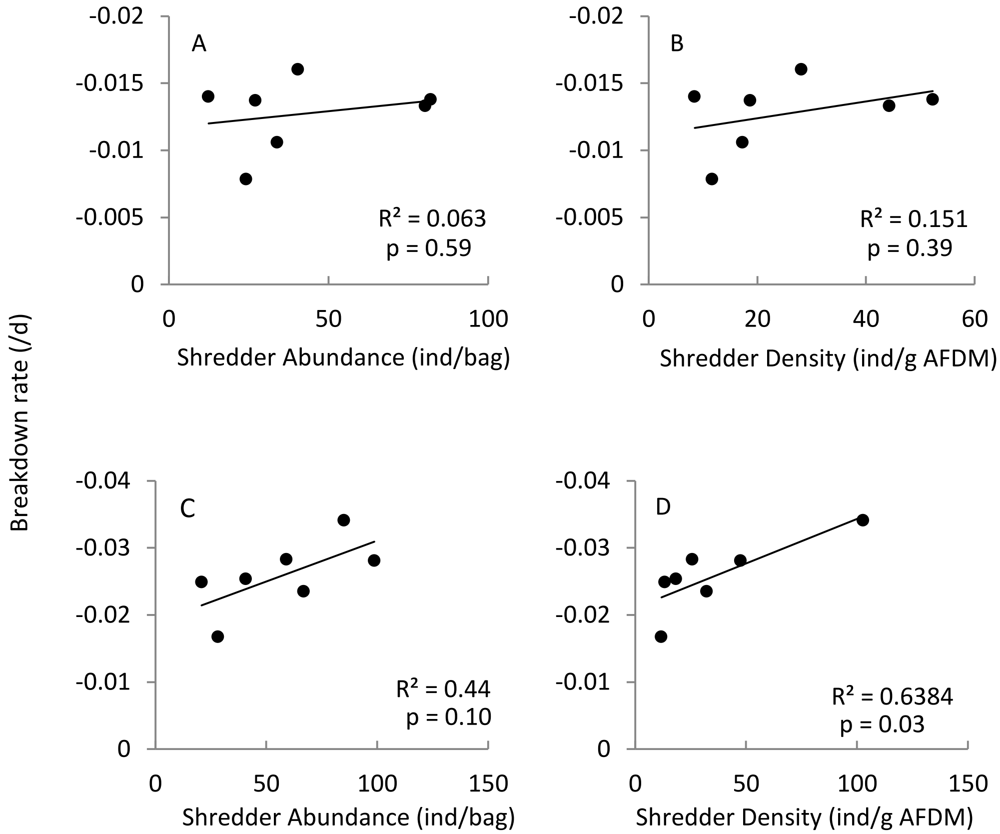

Figure 11. Liner regression of fall deployment leaf breakdown rates and FM shredder abundance (A) and density (B) and CM shredder abundance (C) and density (D). AFDM = ash-free dry mass. Note difference in x-axis scales between FM and CM regressions.

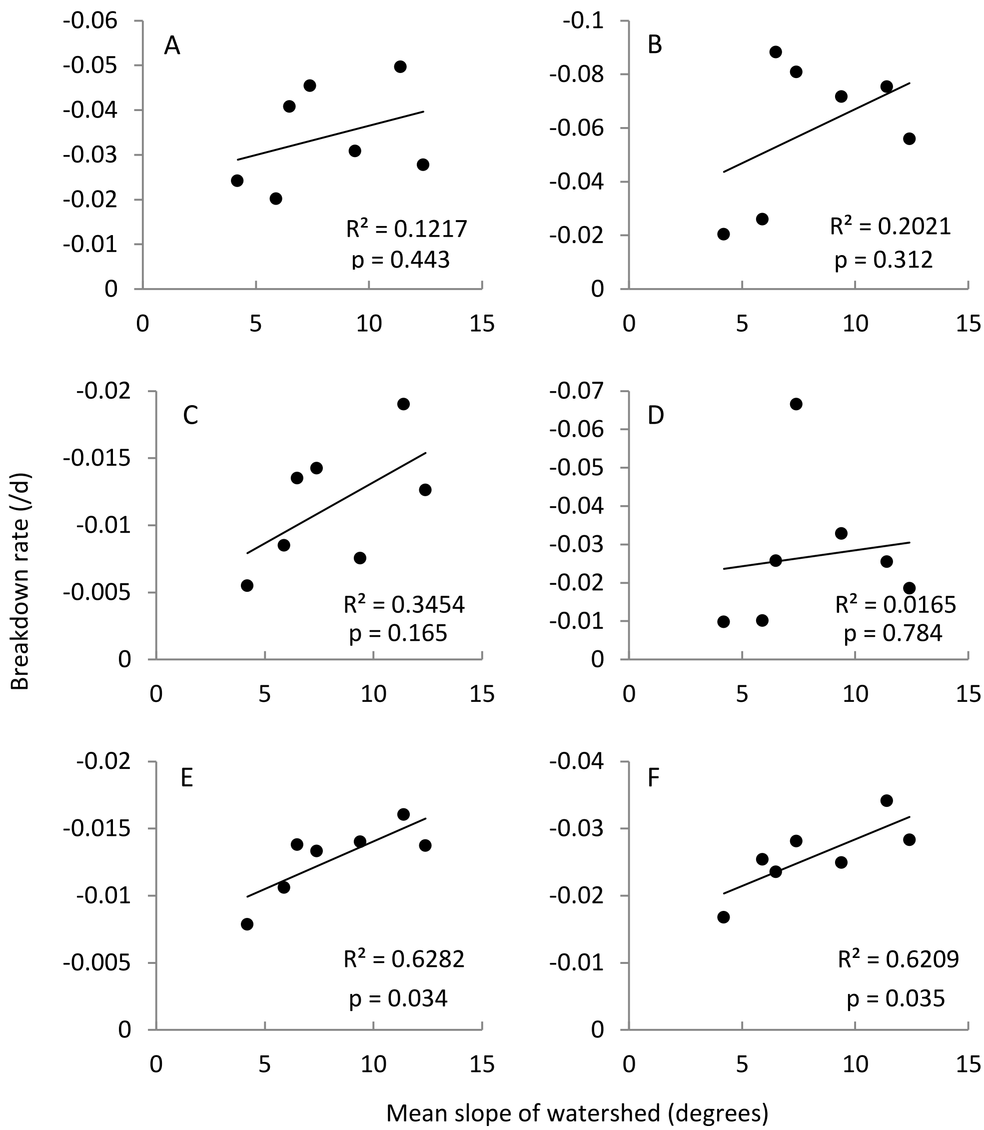

Figure 12. Linear regressions of mean slope of the each watershed (degrees) and breakdown rates (/d). Summer FM (A), Summer CM (B), Summer FO (C), Summer CO (D), Fall FM (E), and Fall CM (F). The mean slope of each watershed basin was quantified using USGS StreamStats (http://water.usgs.gov/osw/streamstats/).