Effects of Endemic Microbial Communities on the Rate of Decomposition in the Presence of Shredders

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Large populations and small size allow microbes to be widely dispersed. Yet, geographic barriers and habitat conditions may allow unique species to persist in a confined region. Differences in the composition of stream microbial communities could affect rates of decomposition, but it was hypothesized that any differences in the chemical breakdown will be overshadowed by the mechanical breakdown of shredders. The rate of decomposition produced by microbial communities from 3 streams on green and senescent alder leaves (*Alnus incana*) was measured over 28 days in the presence of amphipods (*Hyallela azteca*). Green leaves exhibited lower remaining leaf mass and higher respiration. No statistical difference in the rate of decomposition was found among streams with the exception of Tenderfoot Creek. Tenderfoot Creek may host a unique microbial community that is capable of higher rates of decomposition in the presence of shredders than the communities found in Brown Creek and Reddington Creek.

Introduction

Micro organisms involved in stream decomposition live in a wide array of geographic locations. They receive carbon and nutrients from feeding on detritus and contribute to plant break down through the use of exoenzymes (Webster and Benfield 1986). In addition to chemical alteration by bacteria and fungi, converting dead organic matter into inorganic nutrients and CO_2 also requires leaching and fragmentation. Each process overlaps and promotes breakdown from the other processes. Many of the soluble compounds and minerals ions leach out of the detritus in the first 24 hours after falling into a body of water. Macro invertebrate shredders contribute by mechanically chewing

organic matter into smaller pieces and exposing greater surface area for microbial attack. All of the processes are necessary for complete decomposition.

It has long been held that fungi and bacteria are ubiquitous and present nearly everywhere on Earth where the habitat is suitable. Unlike macro species which have distributions that are affected by geographic barriers and historical factors, microorganisms smaller than 1mm are considered to be cosmopolitan (Fenchel and Finlay 2003, Finlay and Clarke 1999). The fungi and bacteria involved in the chemical alteration stage of decomposition are also considered to be the same everywhere since they are less than 100 μm in size. The ubiquitous microbe theory proposes that all microbes are present everywhere and only climatic and latitudinal differences affect the concentrations of the different species (Fenchel and Finlay 2004). Because of the small size and large populations of microorganisms, complete extinction of a species is unlikely even in unfavorable habitats (Fenchel and Finlay 2003, Finlay 2002). These characteristics suggest that micro organism communities should by nearly uniform everywhere with only variations in bacteria and fungi density, not species presence.

An alternative view is that the small size of microbes does not preclude them from being constrained by the restrictions experienced by macro organisms. It may be possible that some microbe species are geographically isolated. This is supported by genetic evidence that reveals DNA differences in species that are morphologically similar or nearly identical (Bass et al 2007). Given these genetic differences, it is possible that there are many more microbial species than originally identified under a morphospecies concept. A cryptic species complex, composed of many unique species, may have previously identified the complex members as the same species when compared globally

based on morphological similarity. Unique species may exhibit phenotypic specializations for exploitation of resources in their unique habitats (Fenchel 2005, Fenchel and Finlay 2004, Finlay 2002). Consequently, there may be geographic regions with reproductively isolated species that decompose detritus differently than a morphologically identical species in a different stream.

If bacteria and fungi are not uniformly cosmopolitan, the rate of decomposition among microbe communities may differ. A significant difference in decomposition rates among streams could have important implications for our understanding of stream communities. Researchers have traditionally assumed decomposition by microbe communities to be identical, but differences in the rate of organic material breakdown could affect the composition of other organism populations in the stream that rely on the nutrients and particulate matter released by decomposition (Wagener et al. 1998).

Since many factors affect stream decomposition, the relative importance of bacteria and fungi in determining a stream's overall rate of decomposition may be negligible. Shredder invertebrates, which mechanically break down leaves, may be responsible for 8-40% of leaf fragmentation (Chergui and Pattee 1991). In streams where shredders were removed by pesticide, leaf decomposition was greatly reduced (Wallace et al. 1995). Alder leaves decompose faster when shredders are present (Ferreria et al. 2006), but the magnitude of shredders' contribution to leaf breakdown is unclear. Not only do microbes rely on shredders to increase leaf surface area for attack, but shredders are also reliant upon microbes. Shredders show a preference for leaves that have been colonized by fungi and bacteria. Shredders may have this preference because exoenzymes that microbes secrete make leaves easier to eat and the microbes provide

additional nutrition for the shredders (Wagener et al. 1998, Graca 2001). Shredders have also been shown to discriminate among leaves based on the species of fungi colonizing the leaves (Graca et al.1993). This suggests that the rate of leaf decomposition may also be affected indirectly by the microbial species composition of a stream based on shredder feeding preferences.

Few studies have investigated the implications of the proposed genetic differences in species that appear the same morphologically. This experiment addressed this issue by observing the rates of decomposition on alder leaves (*Alnus incana*) produced by microbes collected from 3 streams. By studying microbes from different steams the effects of latitude and climate will be held constant so as to observe the effects of any endemic bacteria or fungi that may be present exclusively in a given stream. Since shredders affect decomposition in streams, amphipods (*Hyallela azteca*) were used in this experiment to determine how mechanical breakdown interacts with chemical breakdown. The specific hypothesis being tested was that even if microbes from different habitats decompose organic matter at different rates, the change in decomposition rate as a result of microbe activity would be small enough that there would be no observed differences in decomposition rate. The mechanical breakdown from macro invertebrates would overshadow any chemical breakdown differences from bacteria and fungi. Consequently, it was predicted that the practical implications of any differences in overall decomposition among streams would be insignificant.

Methods

Set-Up

This experiment was conducted at the University of Notre Dame Environmental Research Center (UNDERC) in Land O' Lakes, WI. To control for differences in riparian vegetation, discharge, and substrate the experiment was conducted in 2.4 L mesocosms in the aquatic laboratory. Throughout the experiment all equipment was sterilized with 10% bleach solutions or 70% ethanol solutions to avoid cross contamination.

Water was collected in sterilized carboy containers from each of the three treatment streams: Tenderfoot Creek, Brown Creek, and Reddington Creek (Figure 1). Even though bacteria communities may differ along the length of the stream (Graca et al. 2001), it was assumed that the communities would be similar enough that the location of collection within the stream would not affect comparisons among the streams. All water samples were drawn from the same relative conditions. Water was drawn from the deepest part of the stream, nearest the road. The sediment on the stream bottom was kicked up and the bucket was swept just above the stream bottom to collect bacteria and fungi from the stream bed that had been displaced. The collected water was run through a 500 μm and a 125 μm sieve to remove detritus. Control water from Tenderfoot Lake was collected from the end of the dock at the Aquatic Laboratory. The control water was sterilized by boiling in a microwave for 30 min on high power.

For each inoculate, 50 mL of treatment water was centrifuged at 1000x g for 30 sec and the bottom 5 mL was used as the inoculate. This process concentrates a large sample of microbes from the stream into a small volume of water. By inoculating a large volume of sterile lake water with a small volume of stream water containing live

microbes the differences among streams in water DO, pH, and nutrient content are reduced.

The amphipods used in all the treatments were collected from Brown Creek to control for the microbes that may live on them and affect the treatment communities. To minimize microbial transfer from Brown Creek to the mesocosms, the amphipods were stored in sterile lake water and rinsed with DI water before being added to a mesocosm.

Leaf packets were created by enclosing 0.3000 +/- 0.0100 g of green or senescent leaves into coarse plastic mesh bags tied with nylon string. The green leaves were collected from the UNDERC property in 2007 and the senescent leaves were collected in May 2009.

Phase 1: Decomposition

Added to each bleach sterilized mesocosm was 2L of sterile lake water, 5 mL of the respective inoculate stream water, 40 amphipods (8 amphipods per leaf pack) respectively, and 5 of the respective leaf packets (Table 1). Each of the 8 treatment mesocosms was supported by 4 replicates. The mesocosms were covered to minimize microbial input and aerated with a pump and air stone so as to provide the microbes and the amphipods with oxygenated water as would be found in the streams. Sunlight mimicking light bulbs lit the mescosms for 8 hrs everyday to control for natural daylight in the laboratory.

One leaf pack was removed from each mesocosm on days 2, 7, 14, 21, and 28. For every leaf pack removed 8 amphipods were also removed to keep the ratio of shredders to leaf packs the same. On days 7, 14, 21, and 28 microbial respiration was measured by taking dissolved oxygen (DO) readings for each leaf pack by filling a 60 mL

centrifuge tube containing a leaf pack with sterile lake water. DO was measured by finding the difference between the initial DO of the sterile lake water and the final DO of the water in each of the centrifuge tubes.

The leaves were dried at 60°C for 48hrs to remove moisture and then weighed to obtain the dried leaf mass. The leaves were muffled at 500°C for 4.5hrs to remove organic matter and leave only inorganic matter to obtain an ash weight. The ash free dry mass (AFDM) of the leaves was calculated by subtracting the leaves' ash weight from the leaves' dried weight.

Phase 2: DNA Analysis

DNA extractions for the second phase of the experiment were preformed on days 1, 14, and 28 using a PowerWater DNA Isolation Kit (Mo Bio Laboratories). On day 0 and day 28 the DNA in the water of 3 of the 4 mesocosm replicates was extracted. On day 14 the DNA of the water and the leaf samples for three of the four replicates was extracted. Leaf samples were measured by including 3 rivet punches with the filtered water. The weight of the leaves after the punches were removed was compensated by adding an average of the punches from the non-extracted samples to the AFDM of the leaves. DNA analysis will be completed in Fall 2009.

Data Analysis

Statistical analyses were performed using ANOVA tests on Systat 12. P-values <0.05 were considered statistically significant. Decomposition among the treatment streams was compared by using the natural logs of the AFDMs on day 28. The AFDM data was linearized with a natural log transformation to compare the rates of decomposition over time. The respiration rates on day 28 were used to compare

microbial respiration. Day 28 was the last day of the experiment that measurements were taken and was used to compare the treatments because any differences in the rate of decomposition among treatments would be most distinguishable after sufficient time had passed for decomposition by microbes and shredders. Post-hoc analysis was performed using Tukey Tests to compare differences among individual treatments.

Results

The interaction of leaf type and water source was found to non-significant for both AFDM and respiration data. To enhance the power of the statistical tests, the test for interaction was dropped.

Leaf type was found to be statistically significant for remaining AFDM (df=1, F=.01137, $p<0.0001$) with green leaves having 40.2% less AFDMs than senescent leaves (Figure 2). The stream from which the microbial inoculate was taken was significant when comparing final AFDM (df=3, F=2.966, $p=0.049$) (Figure 3). Post-hoc analysis revealed Tenderfoot Creek to be the only treatment that differed significantly from Brown Creek and it trended towards having significantly lower AFDM than the other streams. Tenderfoot Creek had 18.9% less AFDM than Brown Creek. All other treatments were found to have AFDMs that were not statistically different from one another.

Leaf type was found to be statistically significant for microbial respiration (df=1, F=.01137, $p<0.0001$) with green leaves having 72.7% higher respiration than senescent leaves (Figure 4). Water source as compared by respiration approached significance (df=3, F=2.785, p=0.059) (Figure 5). Post-hoc analysis again revealed Tenderfoot Creek

to be the only treatment that approached the threshold of having significantly higher respiration than the other creeks. As was observed in the comparison of AFDM, Tenderfoot Creek had statistically higher respiration than Brown Creek, but it was not statistically different from Reddington Creek and the Control. The respiration of Tenderfoot Creek was 26.8% higher than Brown Creek. All other treatments were found to have microbial respiration that did statistically differ from one another.

Discussion

Both final AFDM and respiration as measured by change in DO were considered measurements of decomposition. Final AFDM measured the amount of organic material that had not been decomposed from the starting AFDM (0.2921g) of the 0.3000g leaf packs. Lower final AFDMs indicated greater decomposition by microbes and shredders. Final respiration measured the amount of oxygen used by microbes to break down leaves with higher respiration indicating greater microbial activity.

Green leaves showed significantly higher decomposition than senescent leaves as shown by lower AFDM and higher respiration. Decomposition may have been greater on green leaves because of their higher nutritional value (Graca 2001). Senescent leaves dropped by alder trees have lower nutrition because the tree reabsorbs many of the leaves' nutrients. The availability of high nutrition detritus may promote larger and more active microbial communities that may be responsible for the increase in decomposition. Shredders may also benefit from the higher nutrition content and increase their feeding accordingly. However, green leaf matter, which falls into streams during high wind

storms, only makes up about 6% of total allochthonous organic matter that enters a stream (Lopez 2001). The majority of decomposition is done on senescent leaves.

Of the three treatment water sources, only Tenderfoot Creek exhibited a significant difference in decomposition rate. Tenderfoot's rate of decomposition was statistically higher than Brown Creek, but it trended towards being higher than Reddington and the Control. This may indicate that the bacterial and fungal communities in Tenderfoot and Brown are different enough to create different rates of decomposition. This claim is supported by the combined results of a lower final AFDM and higher respiration. DNA analysis of the microbial communities in the second phase of this experiment should confirm or deny perceived differences in the taxonomic groups present in the mesocosms.

If the communities are different, it may be that shredders do not disguise differences in microbial communities, but rather they make the differences more pronounced. If the microbial species present in Tenderfoot are better able to colonize the leaves, then shredders may be more apt to feed on those leaves, thereby increasing the rate of decomposition. If microbes and amphipods decompose 18.9% more detritus in Tenderfoot Creek than Brown Creek over the same time period, that could be an explanatory factor for differences in the streams' water clarity and substrate. Knowing that decomposition may be slower in Brown Creek than in Tenderfoot Creek may be important for making comparisons of productivity and species diversity between the creeks. Further studies of allochthonous inputs and outputs for Tenderfoot Creek and Brown Creek may reveal more about the implications of this experiment's findings.

The water collection points in Tenderfoot and Brown are in the same water flowage and are separated by approximately 7km. The discovery of unique microbial communities in Tenderfoot and Brown would support research that shows communities can change along a stream gradient (Graca et al. 2001). Future experiments should investigate the abiotic characteristics of Tenderfoot Creek to determine what factors influence the composition of the microbe community.

The control treatment was intended to serve as a baseline for microbial decomposition since it was expected that the sterile lake water would have no living bacteria or fungi. The control treatment was not statistically lower than any of the stream treatments which were inoculated with microbes. While it is possible that microbes were able to colonize the control treatments from the air despite the mesocosm covers, all treatments were equally susceptible to microbial contamination from the laboratory environment and should have been affected equally. It is unlikely that cross contamination with other treatments was an issue since the equipment used was well sterilized with ethanol between uses. One explanation is that the leaves may have harbored microbial communities before they were introduced to the mesocosms (Osono 2002). But, leaves in all treatments would have had equal exposure to microbes before the experiment began and therefore any one treatment should not have been affected more than another. Sterilization of the leaves prior to running the experiment would have been difficult without compromising the integrity of the leaves. Furthermore, sterilization of the leaves was not undertaken because microbial communities would have already colonized leaves before they would enter streams naturally. Another possibility is that in the presence of shredders, stream microbial communities do not statistically

increase or decrease the rate of decomposition from the rate that would be produced by the communities present on the leaf prior to the leaves entering the stream.

The data shows that decomposition rates for the treatments diverged as the experiment progressed. Other decomposition research has found bacterial communities to be largest after 8 weeks (Hieber and Gessner 2002). Repeating this experiment for a longer period of time until decomposition is complete may reveal whether the rates of decomposition are the same or if there is greater differentiation in the rates.

Leaf pack mass loss and respiration measurements indicate that there is no significant difference between Reddington Creek and Brown Creek. This may indicate that there is no difference in microbial communities or it may indicate that the presence of shredders disguised the difference. Regardless, the rate of decomposition for practical purposes is the same. Tenderfoot Creek showed a higher rate of decomposition than Brown Creek in spite of the presence of shredders, and more extensive testing may reveal that Tenderfoot Creek supports endemic microbial life.

Acknowledgements

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Tables

Table 1. Combinations of experiment treatments. Each treatment combination of leaf type and water source was replicated 4 times for a total of 8 treatments and 32 total mesocosms.

Amphipods Present (P)	Green (G)	Tenderfoot Creek (T)
		Brown Creek (B)
		Reddington Creek (R)
		Control (Tenderfoot Lake) (C)
	Senescent (S)	Tenderfoot Creek (T)
		Brown Creek (B)
		Reddington Creek (R)
		Control (Tenderfoot Lake) (C)

Figures

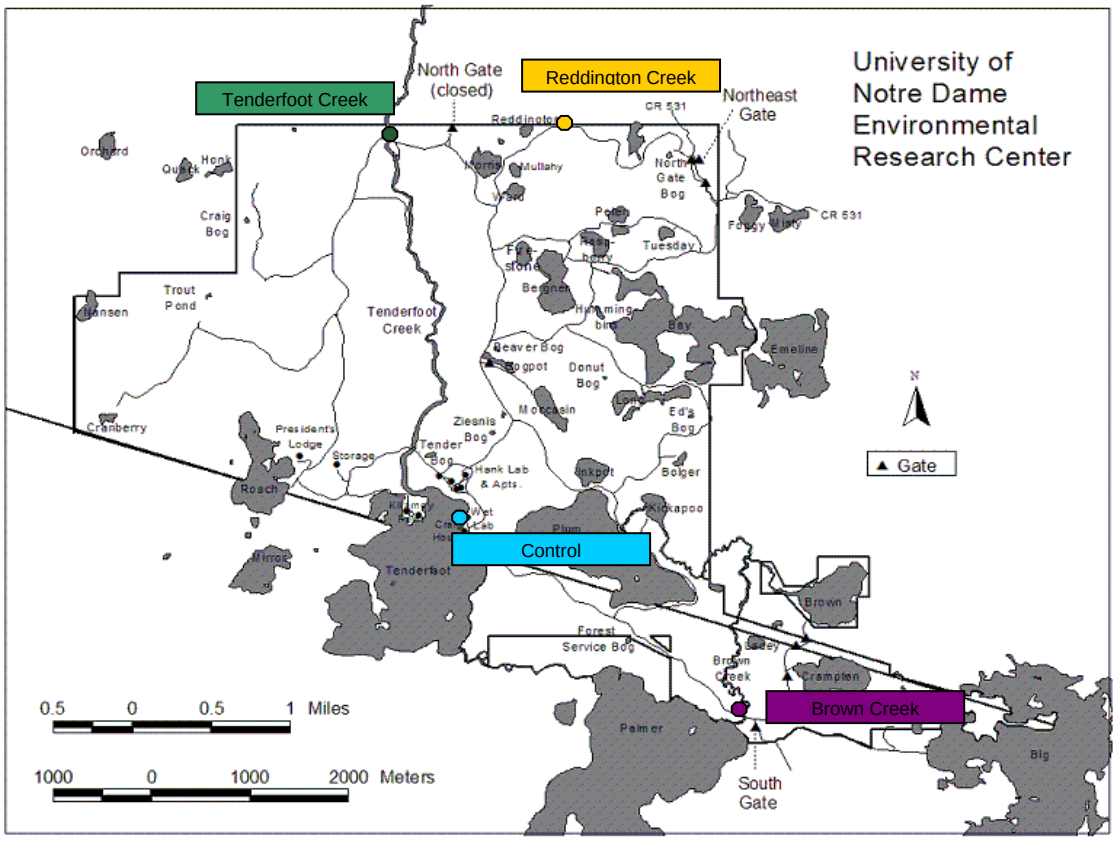

Figure 1. Water source stream locations. Water for the 3 treatment creeks and a lake water control was collected at the locations marked on the map.

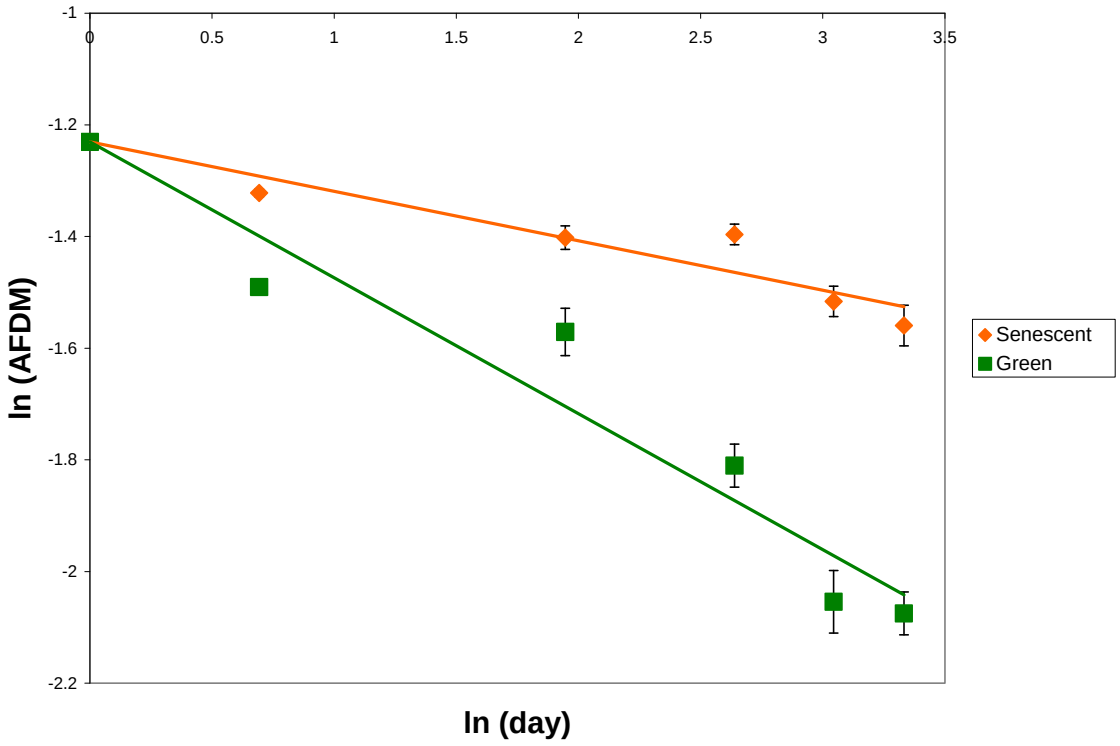

Figure 2. Remaining AFDM of green and senescent alder leaves linearized by natural log transformation. AFDM was calculated from dried and ashed weights of leaf packs after decomposition in mesocosms containing amphipod shredders and different stream microbial communities. Green leaves exhibited statistically lower AFDM compared to senescent leaves ($p<0.0001$).

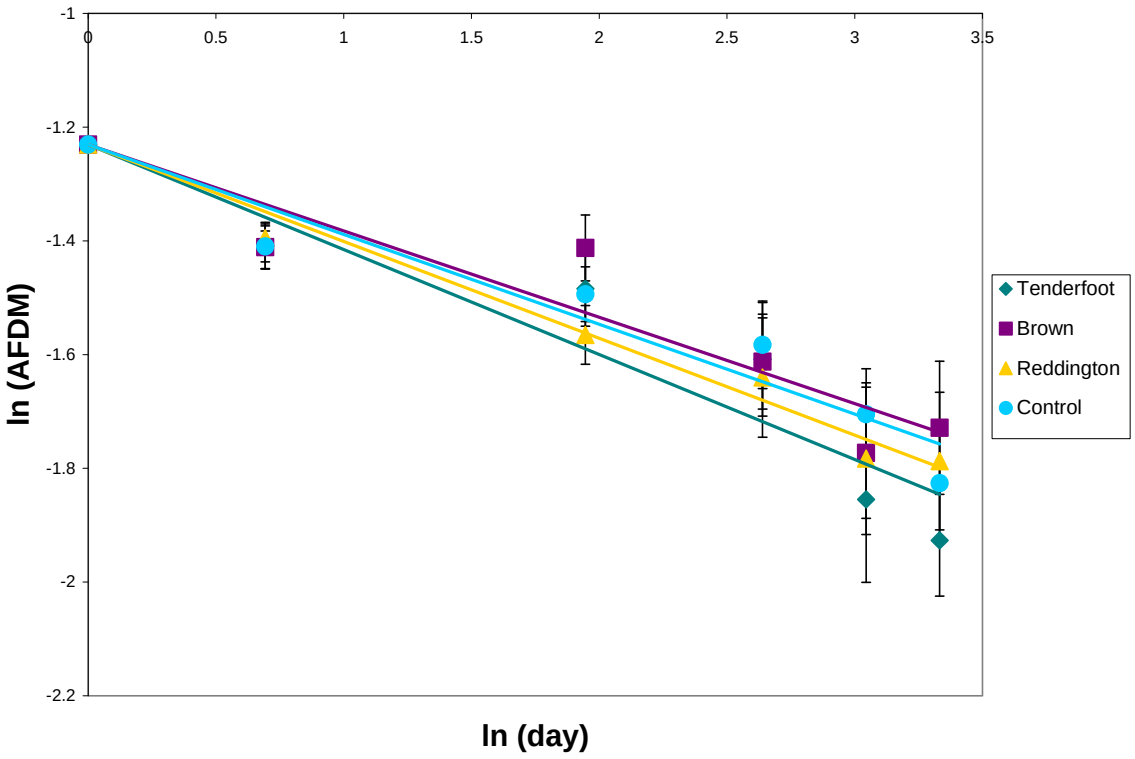

Figure 3. Remaining AFDM of alder leaves linearized by natural log transformation for Tenderfoot Creek, Brown Creek, Reddington Creek, and Control. AFDM was calculated from dried and ashed weights of leaf packs after decomposition in mesocosms containing amphipod shredders and different stream microbial communities. There was no observed statistical difference in AFDM with the exception of Tenderfoot, which exhibited lower AFDM compared to Brown ($p>0.0351$).

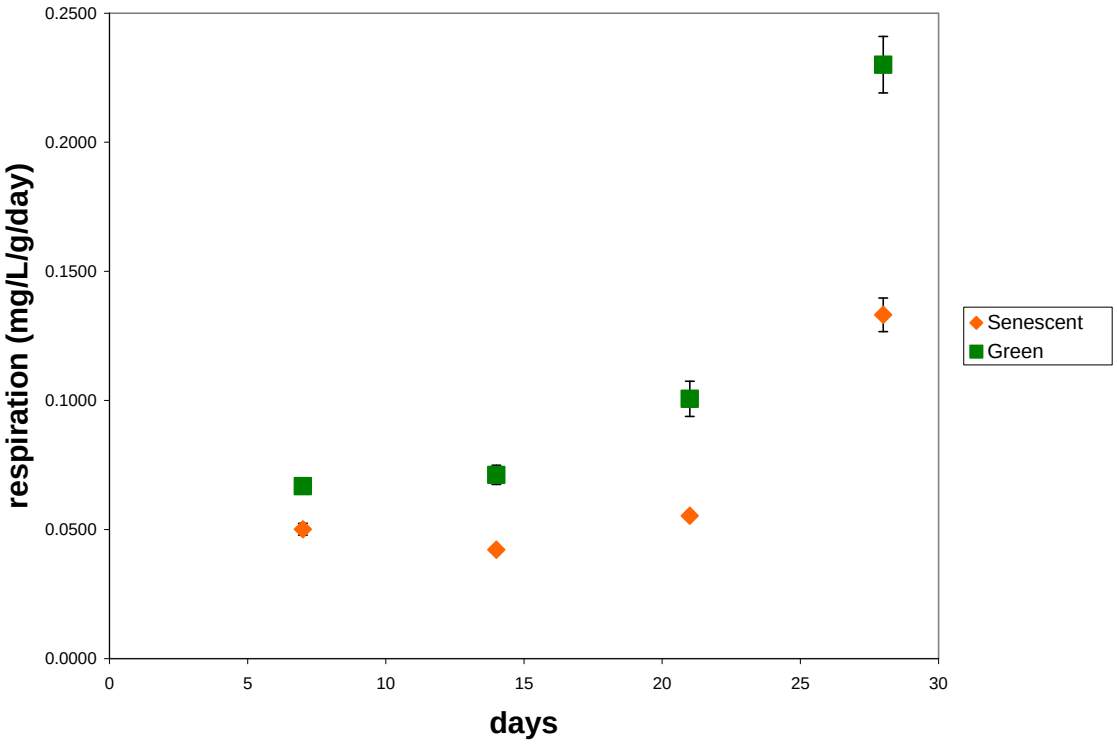

Figure 4. Microbial respiration on green and senescent alder leaves. Respiration measurements were calculated from initial and final DO measurements taken of decomposing alder leaves that had been placed in a centrifuge tube with 60mL of sterile lake water for approximately 24hrs. Green leaves show statistically higher respiration compared to senescent leaves ($p<0.0001$).

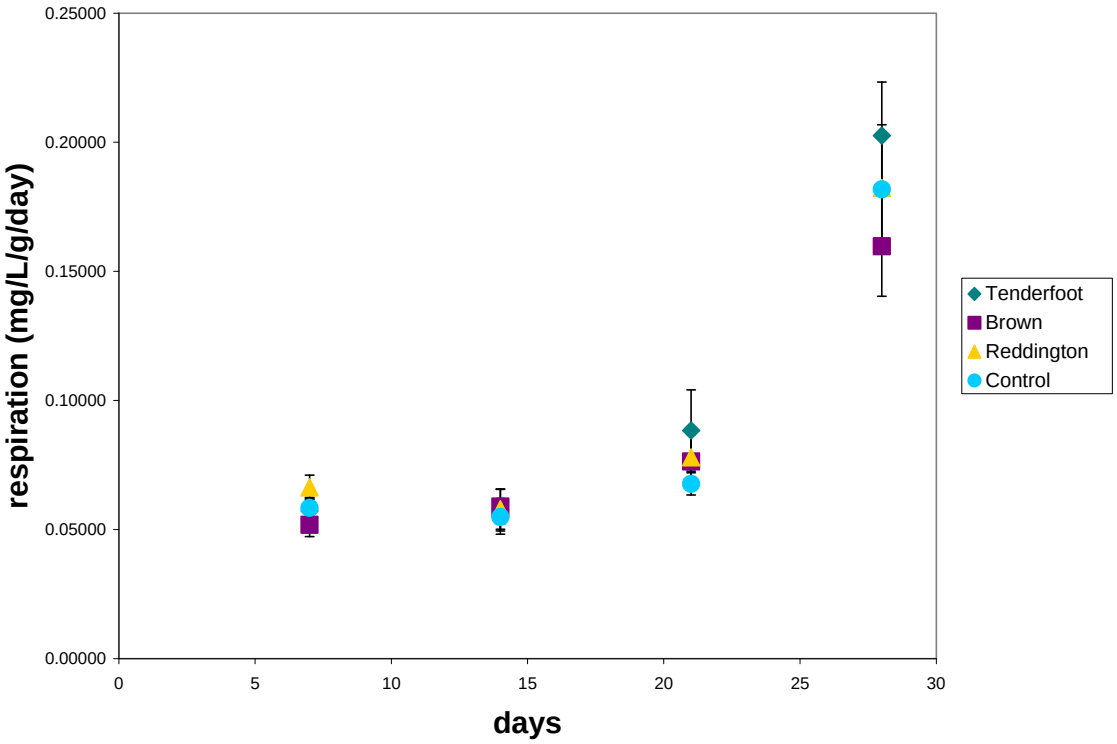

Figure 5. Microbial respiration on alder leaves for Tenderfoot Creek, Brown Creek, Reddington Creek, and Control. Respiration measurements were calculated from initial and final DO measurements taken of decomposing green and senescent leaves that had been placed in a centrifuge tube with 60mL of sterile lake water for approximately 24hrs. Measured respiration shows the greatest differentiation on day 28. There was no observed statistical difference in respiration among streams with the exception of Tenderfoot, which exhibited higher respiration than Brown ($p>0.0356$).