

**Functional Differences among Microbial Communities of Three UNDERC
Streams and the Effect on Leaf Decomposition**

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Abstract:

Streams communities are often chosen for organic matter decomposition studies as they are characterized by their ease of manipulation, ease of identification of microbial components, and the high rate of decomposition when compared to soil and forest decomposition experiments. These characteristics allow researchers to determine the mechanisms of decomposition and apply them to other environments. The microbial communities of three streams on the UNDERC property were examined for their ability to decompose 0.3 grams of green and senescent alder leaves (*Alnus incana*). The experiment involved 32 mesocosms (four replicates of eight different stream/leaf treatments), over a 28 day period. Amount of leaf decomposition was determined by the remaining AFDM (ash free dry mass) of leaves from the final day of the experiment. Respiration rates (mg/L/g/day) were taken of the leaves on each sampling day to determine the abundance of microbial organisms present on the leaf before drying and ashing. Contradicting the original hypothesis that the communities would be functionally different, there were no statistically significant differences between the remaining AFDM or the respiration rates. Statistically significant results were observed between green and senescent, with green leaves having higher decomposition and microbial respiration rates than senescent leaves. The similarity in functional characteristics shown in the statistics of this experiment lead me to predict that the streams contain a small number of generalist, microbial

species in high abundance. A high abundance of similar species can account for the similarities in respiration rates and remaining AFDM of the leaf packs; however, further DNA analysis of the microbial communities of the mesocosms is needed to support this prediction.

Introduction:

The role of bacteria and fungi in the decomposition of leaves and other vascular plants in fresh water systems has been well documented and examined. There are many factors that are involved in the breakdown of vascular plant tissues in fresh water. Webster and Benfield (1986) identify the species of the plant, the part of the plant (leaf vs. roots vs. stalks), water temperature, dissolved nutrient concentration, dissolved oxygen, presence of macroinvertebrates, acidity of the water, and possibility of disturbance (such as natural or human-induced nutrient runoff) to all affect decomposition.

Webster and Benfield (1986) explain that water temperature can affect the production capability of a microbial population. They describe that the sites studied with higher temperatures display faster breakdown of organic matter and higher microbial production.

Dissolved nutrient concentration is one of the most important aspects of fungal and bacterial decomposition of organic matter. Weyers and Suberkropp (1996) conducted an experiment that compared a hard water stream (high in N

and P) and a soft water stream (lower N and P). Their experiment (as well as many other experiments on the subject, such as Suberkropp and Chauvet (1995)) showed that the stream with higher concentrations of N and P consistently had higher bacterial and fungal biomass and production when compared to streams with lower concentrations of N and P.

Breakdown of leaf material is observed to occur faster in stream systems when compared to lake systems in the same area (Witkamp & Frank, 1969). The faster breakdown can be attributed to the current of the stream water causing lacerations and fragmentation of the leaves (called mechanical abrasion) when leaves contact the jagged rocks in the streambeds. In addition, the decreased breakdown rates in lakes could be attributed to the fact that it is a lotic system in which only part of the leaf surface is exposed to aerobic decomposition (Witkamp & Frank, 1969).

Microbial species up to one millimeter in size are often seen as cosmopolitan and universally ubiquitous since they are rarely bound by geographic barriers as larger animals are (Fenchel & Finlay, 2004, Bass et al., 2007). Because of this cosmopolitan characteristic, I predict that this could result in low species diversity and continuous dispersal among microbial communities. Fenchel & Finlay (2003) explain that small species of microbial organisms (less than one millimeter in size) have a high probability of dispersion and a low probability of local extinction. Despite the ubiquitous descriptions of these

communities, there is substantial evidence supporting the idea that microbial species may be extremely similar morphologically; however, there are habitat-induced functional differences between the species allowing for specialized utilization of local resources (Fenchel & Finlay, 2004).

Species richness also plays a large role in the decomposition process. Bell et al. (2005) show that the richness of a microbial ecosystem can have large effects on the community productivity. Species rich communities are much more productive than communities dominated by one or two species since different microbial species use slightly different resources (Griffiths et al., 2001). Community productivity increases proportionally with species richness in that more of the available resources are used. (Bell et al., 2005). In addition, productivity increases with species richness as there is a higher chance that there will be an abundant species with a large effect on ecosystem function (Bell et al., 2005).

Stream environments are often utilized for various leaf litter decomposition experiments. Due to the ease of manipulation, ease of identification of microbial components, and the rapid nature of stream decomposition when compared to soil and forest decomposition experiments, stream ecosystems are often viewed as model environments for decomposition experiments (Hieber & Gessner, 2002). Due to the characteristics mentioned above, these experiments can model the mechanisms that drive decomposition, as

well as the importance of certain organisms in the procedure (Hieber & Gessner, 2002).

This decomposition experiment will involve both green and senescent alder leaves. There are differences in chemical content between green and senescent leaves. There are soluble components in green leaves that are not present in senescent leaves resulting in increased leaching and higher mass loss in aquatic environments (Lopez et al., 2001). Approximately 45% to 90% of organic matter entering an allochthonous stream comes from riparian leaf matter, with approximately 6% coming from green leaves (Lopez et al., 2001). Green and senescent leaves often fall into streams through natural litter fall (senescent) or external environmental factors such as animal disturbance, storms, or wind (Lopez et al., 2001).

In order to better understand the role of the microbial community in stream decomposition of alder leaves, this experiment tests whether functional differences in the bacteria and fungal populations of three different streams at UNDERC have an effect on decomposition rates. I hypothesize that the functional differences between the microbial communities of Brown Creek, Tenderfoot Creek, and Reddington Creek will cause differences in leaf decomposition rates when other factors such as nutrient concentration, temperature, and macroinvertebrates are controlled in each stream mesocosm.

Methods:

The aquatic microbial communities of three streams (Brown Creek, Tenderfoot Creek, and Reddington Creek) on the UNDERC property were examined during this experiment by observing the rate of decomposition of green and senescent alder leaves in mesocosms treated with bacteria and fungi of three water treatments. Water samples (5 gallons) were collected from each stream with a carboy sterilized with a 10% bleach solution and filtered through a 500um and a 125 um sieve in order to remove large free-floating organic matter and macroinvertebrates. According to Vannote et al., (1980) streams will vary in microbial productivity with higher productivity in warmer waters, shallow waters, or stretches with increased sunlight penetration. Because of this, stream samples were chosen to have as close to the same relative conditions, such as canopy cover, sunlight, and depth, as possible; however, the sizes of the creeks varied with Reddington Creek being much smaller than Tenderfoot or Brown creeks. The samples were homogenized and fifty milliliters were taken from each stream. The fifty-milliliter sample was centrifuged at 1000-x gravity for thirty seconds in order to concentrate the bacteria and fungi as well as to standardize the nutrient content of each stream. The best way to equalize the amount of nutrients between all the samples was decided to be the addition of the smallest amount of stream water possible. An inoculate of five milliliters of the bottom of the centrifuged bacteria and fungi is added to the mesocosm which is filled to volume (2 liters)

with sterilized water from Lake Tenderfoot. The Tenderfoot lake water was sterilized by microwaving.

Each mesocosm contained five leaf packs of 0.3 grams of air-dried speckled alder (*Alnus incana*) leaves. The stream and control treatments contained two leaf treatments (green and senescent alder leaves) to simulate the types of naturally occurring leaf litter. Each of the three streams will have four replicates as well as a fourth control treatment for a total of 32 mesocosms (sixteen senescent treatments and sixteen green treatments). Each mesocosm contains an airstone for aeration and was covered in order to decrease the probability of contamination across samples. Leaf packs were removed after 2, 7, 14, 21, and 28 days. For each pull day, the respiration rate as well as the Ash Free Dry mass of the leaves was recorded. The respiration rate was determined by placing the leaf pack into a 50mL centrifuge tube with sterile lake water of a known dissolved oxygen content. Approximately 24 hours later, the dissolved Oxygen content is taken again to determine the respiration rate of the microbial community growing on the leaves. In addition to the respiration rates of the microbial communities, the Ash Free Dry Mass (AFDM) is also determined for each leaf pack. AFDM is determined by first drying the leaf packs overnight in an oven at 60° C. The dry weight of the leaves is taken and the leaves are then placed into the muffle furnace at 500° C for 2 hours. The AFDM is calculated by subtracting remaining

ash from the dry weight of the leaf pack and shows how much digestible leaf material was degraded by the microbial communities.

In addition to the above decomposition rate experiment, a DNA extraction procedure was to be completed three times throughout the study. The three extractions occurred at the beginning (day 1), the middle (day 14), and end of the experiment (day 28) in order to construct a time series of community structure throughout the experiment. Only one DNA extraction per stream sample will be completed (no replicates) due to the high cost of the test. The extracted DNA will be frozen for further research by the supervising graduate student.

In order to minimize the probability of cross-contamination between samples, disposal latex gloves and forceps sterilized with alcohol should be used in the collection of the leaf packs. In addition, every piece of equipment used in this experiment was sterilized with a 10% bleach solution to avoid contamination from bacteria already present in the mesocosm containers.

In order to replicate natural stream conditions for each mesocosm, spotlights with UV-grow lights were placed on timers to simulate sunlight during daylight hours (6am-9pm). In addition to the artificial sunlight, each mesocosm contained an airstone to prevent the water samples from becoming stagnant. Each mesocosm was covered with a Tupperware lid or tin foil in order to reduce the airborne contamination during aeration.

The 32 mesocosms were haphazardly dispersed on the laboratory bench as to not cluster the mesocosm replicates and to account for light or temperature fluctuations in the experiment room.

The data obtained from this experiment was analyzed by log transforming the data as well as ANCOVA and general linear model statistical tests.

Results:

The data was first analyzed using an ANCOVA test in order to determine if a statistically significant difference existed between the ash free dry masses (AFDM) of the different leaf treatments. The statistical analysis of the data displayed a significant difference between the two leaf treatments (green and senescent) when not considering the individual stream treatments (df=1, F-ratio=34.004, $P < 0.0001$). When considering the individual streams, there was no statistically significant difference in the average AFDM of the different treatments (df=3, F-ratio=0.703, $P=0.559$). In addition to the statistical tests performed on the AFDM data, an additional general linear model test was conducted on the respiration rate data. Similar to the results for the AFDM data, there existed a significant difference in the natural log of the average respiration rates from day 28 between the green and senescent leaf treatments when the individual stream treatments were not considered (df=1, F-ratio=34.97, $P=0.0097$). When the individual stream treatments are considered, there is no statistically significant

difference in the natural log of the average respiration rates from day 28 of the different streams ($df=3$, $F\text{-ratio}=0.976$, $P=0.507$). As shown in Table 1, there are visible differences between the three streams used in this experiment. Table 1 displays the large variation in depth of the streams, in addition to the low pH, dissolved Oxygen, and temperature of Reddington creek.

Discussion:

As mentioned in the methods section of the paper, the river continuum concept proposed by Vannote et al., (1980) suggests that a riparian system varies considerably as it flows downstream. According to Vannote et al. (1980), as a river system increases in size, there is less influence from the surrounding vegetation as headwater streams are often allochthonous, relying on external organic matter. As the riparian system increases in size, the system becomes autochthonous in that there is less canopy cover resulting in increased primary production. It is possible that these differences based on the physical parameters of the systems could cause a large difference in the microbial community of the different streams.

The sampling area used in Reddington Creek was much smaller than the sampling areas of Tenderfoot and Brown creeks (shown in Table 1). Reddington creek had much more canopy cover and a much lower level than either Tenderfoot creek or Brown creek. As discussed in Webster and Benfield (1986),

dissolved Oxygen, pH, and temperature can have a large effect on the microbial community of a stream. When collecting the site evaluations of the three streams, it was predicted that the low pH, temperature, and dissolved Oxygen of Reddington would have a visible effect on the microbial community when compared with Tenderfoot and Brown creeks. As displayed in the results section, the only statistically significant difference between the average AFDMs of the eight treatments was seen in the leaf treatments (shown in Figure 2). This result was expected in that green leaves contain a higher concentration of digestible material in the form of Nitrogen and Phosphorous than senescent leaves leading to increased leaching and decomposition rates (Lopez et al., 2001).

The statistical analysis for the AFDM data was carried out using the data day 28 of the experiment. This decision was justified by the prediction that a difference in the decomposition rate between stream treatments would be most apparent in the final day of the experiment where the leaf packs had the longest exposure time to the respective microbial communities. It was found that there is no statistically significant difference in the rate of leaf degradation between the four stream treatments. Despite the lack of a statistically significant difference between the stream water treatments, there are trends in the AFDM data that suggest that statistically significant results may be found if one increased replication or increased duration of the experiment. This trend is displayed in Figure 3. On average, the remaining AFDM on day 28 was highest in the control

treatment and lowest in the Tenderfoot treatment. This finding shows that the control treatment had the lowest decomposition rates and the Tenderfoot treatments had the highest decomposition.

In addition, there was no statistically significant difference in the average respiration rates of the microbial communities present on the leaf packs when comparing the stream treatments. Similar to the statistical results for average decomposition rates (AFDM), the only statistically significant result from the respiration data is the significant difference between green and senescent leaf pack treatments (shown in Figure 4). Also similar to the decomposition data, the data used for the statistical analysis was taken from the final day (day 28) of the experiment with the justification that if differences existed between the microbial abundance present on the leaf packs it would be most apparent on the last day of the experiment. As expected, the respiration rates increased as the experiment progressed and the abundance of the microbes increased within each mesocosm. Despite the lack of a statistically significant difference between the stream treatments, there were some trends visible when examining the average respiration rate for both green and senescent leaves of the final day of the experiment (displayed in Figure 5). As shown in Figure 5, the control treatment had the lowest average respiration rate on the final day of the experiment, which was expected as the control should have contained the least abundant microbial community. Figures 3 and 4 show the exponential increase in respiration rate over

the duration of the experiment. It is impossible to make sure the control is completely sterile at the beginning of the experiment as there is no way to sterilize the alder leaves without destroying them. Leaf packs from the Brown, Reddington, and Tenderfoot creek treatments had similar respiration rates with microbial communities from the Tenderfoot creek leaf packs having the highest respiration rates of the four treatments.

Even though there is no statistically significant difference between the decomposition rates or respiration rates of the microbial communities from the three streams sampled at UNDERC, our hypothesis is not completely refuted. The original hypothesis stated that there is a functional difference between the microbial communities of the three UNDERC streams examined in this experiment. These statistical tests only explain that there is no difference in the decomposition and respiration rates of the microbial communities; however, there may still be large genetic differences between the microbial communities of the different streams.

As with any scientific experiment, there is potential for this experiment to be improved upon. As mentioned above, it was nearly impossible to keep the control from being contaminated. Also mentioned above, it is impossible to sterilize the leaf packs before placing them in the mesocosms without destroying the leaves. For this reason, it is likely that the control treatment mesocosms were contaminated with bacteria already present on the leaves. The initial setup of the

experiment includes 32 airstones with only a few available motors. Since there were few motors with several splitters running from them, some mesocosms received less airflow than other mesocosms received. This difference was minute, but might be improved upon with a more advanced system of air pumps. The mesocosms were stored at room temperature. It is understood that room temperature was often warmer than the stream conditions these microbial communities were used to, but as we were testing differences in the relative decomposition rates the lack of realism was considered unimportant. In addition, as mentioned above, this experiment shows that the mesocosm communities are functionally similar; however, it does not conclusively tell if the communities are genetically similar. It is possible that the communities are genetically different, but species abundance of certain species in response to the physical characteristics of the host stream creates similar functional abilities. As mentioned in Bell et al. (2005), species rich communities of microbes function better than communities dominated by one or two species. However, it is not a linear relationship. Synergistic or non-synergistic aspects of different microbial species can improve or degrade the productivity of the community (Bell et al., 2005). When considering the results of this experiment, it is possible that the communities are genetically different with rare microorganisms specialized to each stream environment. However, the similar functional characteristics shown in the statistics of this experiment lead me to predict that the streams contain a

small number of generalist, microbial species in high abundance. A high abundance of similar species can account for the similarities in respiration rates and remaining AFDM of the leaf packs; however, this prediction may or may not be supported by DNA analysis of the mesocosm microbial communities.

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Table 1: Physical parameters of the three streams used in this experiment.

	Depth (m)	Temp (°C)	DO (mg/L)	pH	Conductivity (ms)
Average Tenderfoot	0.265	30.125	8.135	7.305	0.2
Average Brown	0.81	27.8	7.5275	6.84	0.2
Average Reddington	0.075	24.75	2.99	4.6	0.1

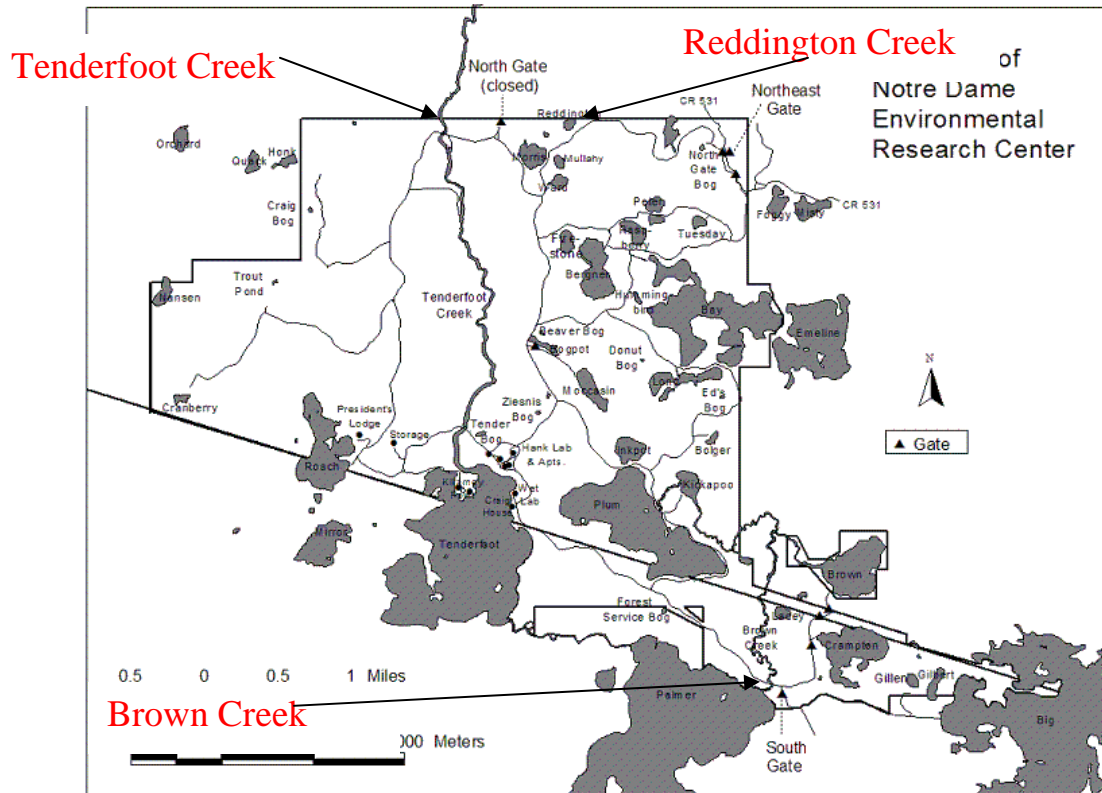


Figure 1: Map of the UNDERC property with the three stream sampling sites labeled.

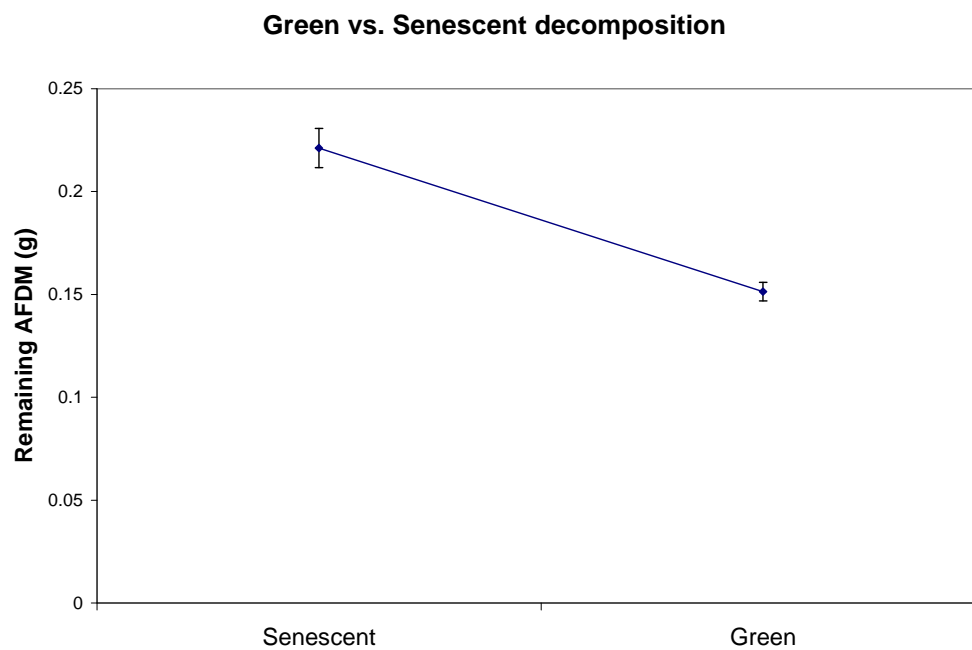


Figure 2: Final remaining AFDM (in grams) after 28 days for senescent and green leaf treatments. The data are only separated by leaf treatments and the stream treatments are not considered. The error bars show the standard error in the leaf decomposition data.

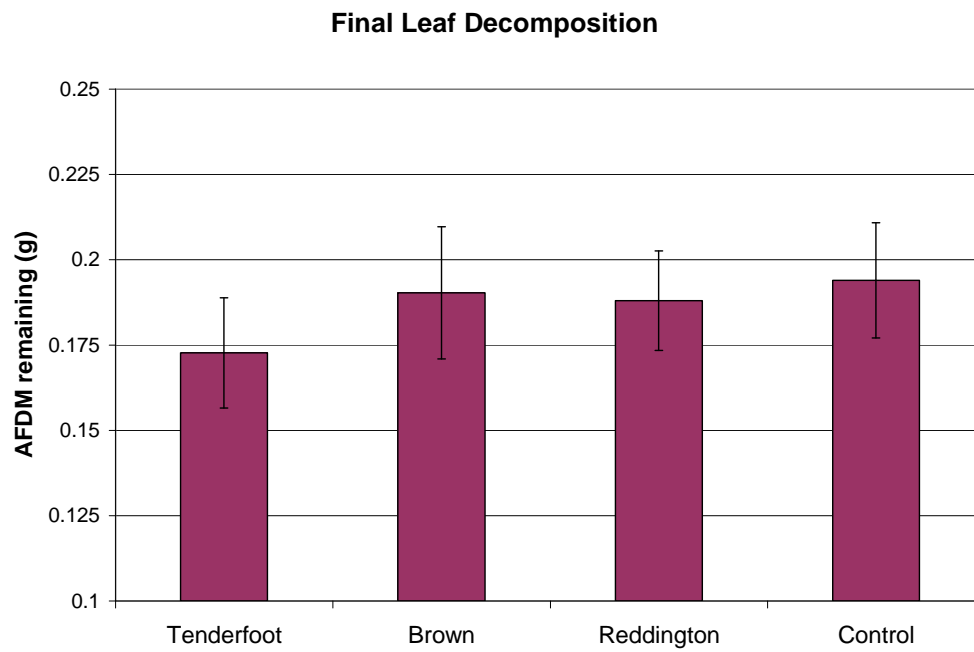


Figure 3: Average leaf decomposition data taken on the final day of the experiment displayed in grams of AFDM (ash free dry mass) remaining after 28 days. Error bars represent the standard error of the individual stream treatment data.

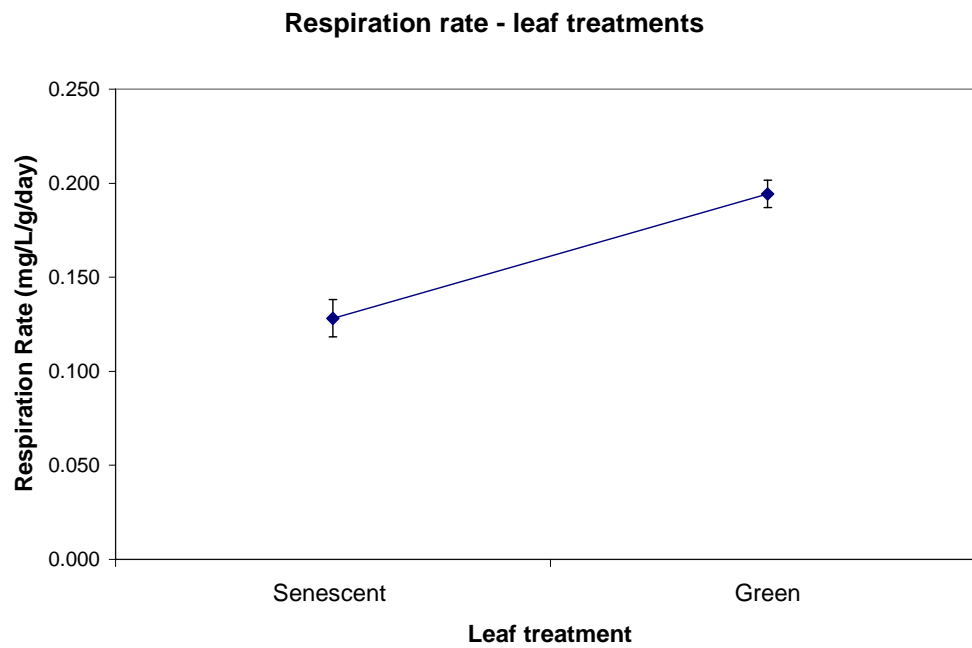


Figure 4: The respiration rates for both green and senescent leaf treatments after 28 days. Respiration rate measured in mg/L/g/day. The data are only separated by leaf treatments and the stream treatments are not considered. The error bars show the standard error in the leaf decomposition data.

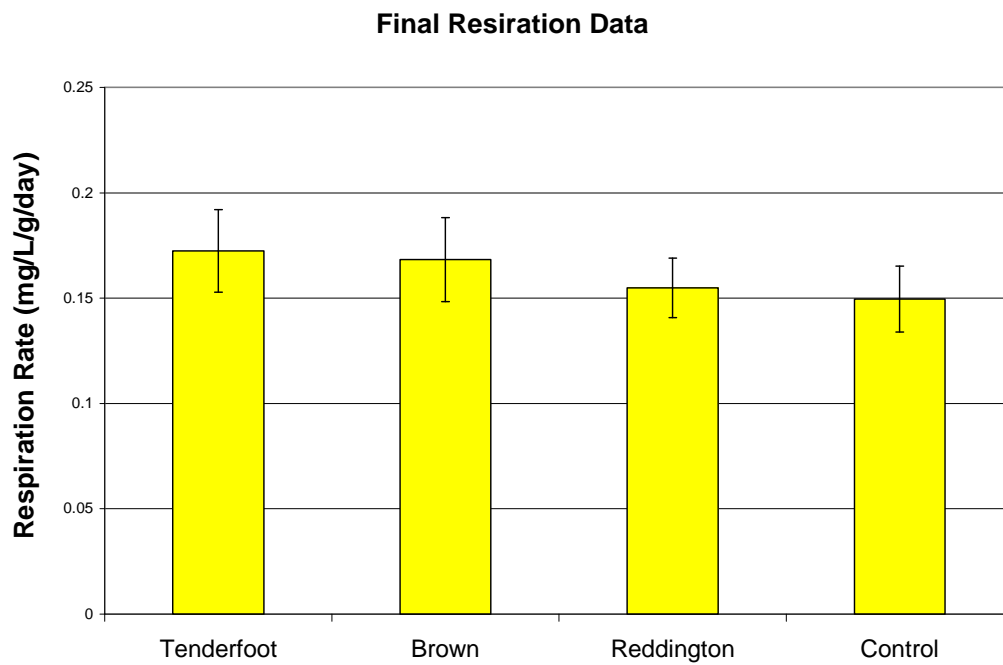


Figure 5: Average respiration rate data taken on the final day of the experiment. Data for respiration rate(mg/L/gram/day) after 28 days exposure to stream mesocosms displayed by stream treatment. Error bars represent the standard error of the individual stream treatment data.