# Are UNDERC microbial communities from different streams functionally unique?

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ABSTRACT: Until very recently, microbial communities sharing common environments were thought to be functionally equivalent regardless of species composition. Recent work has shown that microbial communities exhibit variation in respiration rates in short (48 hour) incubation experiments on Dissolved Organic Carbon, indicating that microbial communities may vary in regards to decomposition. We tested the functional equivalence of microbial communities across three streams by comparing the total organic matter consumed as well as the respiration within mesocosms inoculated with three distinct microbial communities from three different UNDERC streams across two different experiments. Both experiments tested microbial ability to decompose senesced and green speckled alder leaves, but each respective experiment filled the mesocosms with different gardens of water (Reddington Stream and DI water). We found that for both experiments there was no correlation between leaf type or inoculum regarding both organic matter decomposition and respiration, with one exception. The respiration data for leaf packs in DI water indicates that respiration is significantly higher on green leaves. The total rates of decomposition were faster for the experiment with DI water. Our results seem to suggest that the microbial communities living among these three UNDERC streams are not functionally different, but more tests should be conducted in order to solidify our findings.

INTRODUCTION: Decomposition is an important ecosystem function across many environments. The breakdown of organic matter is a key step in the cycling of nutrients throughout ecosystems (Flanagan et al. 1983). Decomposition also provides

food for plants, thus having an indirect effect on primary production (Suberkropp and Klug 1976, Brinson et al. 1981). In aquatic networks, decomposition drives many abiotic and biotic factors such as the providing of food for many invertebrates and the lowering of pH (Brinson et al. 1981). Allocthonous input (leaf fall) into aquatic systems is the main provider of the raw material for decomposition. Important factors that control decomposition include a host of biotic and abiotic factors. Docherty et al. (2006) have shown that pH, temperature, and dissolved oxygen are all important when considering decomposition rates. Biotic factors include leaf type, invertebrates, and microbial communities residing within the water.

Microbial communities have a large impact on the decomposition of leaf matter (Suberkropp and Klug 1976, Gessner and Chauvet 1994, Findlay 2010). Many physical characteristics of aquatic microbial habitats affect the communities. pH, dissolved oxygen, and temperature can all affect the rate at which microbial communities decompose plant material. Many characteristics of the plant itself can influence microbe decomposition rates (Harrop et al. 2009, Suberkropp et al. 1976). For instance, the amount of nutrients available or the lignin content within the material being decomposed has a large impact (Gessner and Chauvet 1994). It has been shown that in many systems, plant material high in nutrient content has been broken down faster and more completely than material that is nutrient poor.

Fenchel and Finlay (2004) showed that microbial communities exhibit a cosmopolitan distribution. This assertion was applied to microbes that are less than one millimeter in size, thus being able to overcome geographic barriers that would otherwise inhibit the distribution of larger organisms. This distribution has led to

much work on microbial function based on community, with the general assumption that the microbial communities are the same everywhere. This had been a controversial subject, for many studies have found evidence to the contrary. For example, Strickland et al. (2009) found that communities that shared a common history with a given foliar litter exhibited higher decomposition rates when compared to communities foreign to that habitat. Also, it has been shown that the roles of aerobic aquatic heterotrophic bacteria in carbon cycling, as well as the importance of high-molecular-weight Dissolved Organic Matter as a carbon source, may be more complex than is conventionally recognized (Docherty et al. 2006).

In 2009, a student investigated whether microbial communities from three different streams (with a control) broke down different leaves at different rates in a common garden of water from Tenderfoot Lake that had been filtered (63um) and sterilized via boiling. The microbes taken from Tenderfoot Creek broke down the naturally senesced leaves at a much higher rate than any of the other microbes. I hypothesize that the microbial community from Tenderfoot Lake was exhibiting "home-field advantage" because the base water was taken from Tenderfoot Lake. This "home-field advantage" which has been demonstrated in other microbial decomposition experiments, (Reed and Martiny 2007, Strickland et al. 2009, and Docherty et al. 2006), did not occur on the green leaves indicating that the advantage may be context dependent.

Are microbial communities from different streams functionally different? Do microbial communities show increased function, relative to other communities, in their home stream? This experiment tests microbial "home field advantage" further.

I placed microbial communities from Tenderfoot, Brown, and Reddington Creeks into mesocosms that were filled with water from Reddington Creek. My hypothesis is that due to the "home-field advantage" concept, the microbial communities from Reddington Creek will break down the naturally senesced leaves faster and more completely, but the effect will not occur on green leaves, similar to the 2009 experiment. In a common garden of DI water (a neutral source) the experiment was run again. For this experiment, I hypothesize that all bacteria will have equal rates of decomposition and with equal effectiveness.

MATERIALS AND METHODS: This experiment examined the differences in the microbial communities of three UNDERC streams (Brown, Tenderfoot, and Reddington) with respect to their abilities to decompose both naturally (brown) and manually (green) senesced speckled alder (*Alnus incana*) leaves. Speckled alder is the dominant riparian species at UNDERC and therefore gave the best representation of field habitat. Five-gallon carboys sterilized with 10% bleach solution were used to gather the water for both the common water garden and for the microbial inoculums. For each experiment, a total of six carboys were used: three containing the water garden and three containing the inoculums. For the common water garden (Reddington and DI water), the water was run through a .2-µm pore in order to remove all microbes and bacteria. This differed from last year, when the water gardens were sterilized via boiling. To create the bacterial inoculums, water from the Reddington, Brown, and Tenderfoot carboys was agitated to ensure homogeneity, and then passed through a 125-µm sieve. This pore size removed fine particulates, but

allowed microorganisms to pass through. 400 ml was then extracted and poured into eight separate 50-ml falcon tubes that were placed into a centrifuge (four at a time) and run at 1000 x gravity for 30 seconds. This produced a pellet that contained concentrated amounts of microbes. Five ml were extracted using a micropipette. My technique removed as much of the pellet as possible with as little stream water. This was applied to better equalize the amount of stream nutrients in each mesocosm. Each inoculum was stored in a 50-ml bottle.

32 two-liter mesocosms were sterilized with 10% bleach solution and filled with approximately one liter of the common water garden (Reddington or DI). Five leaf packs were then added to each mesocosm. The leaf pack was the functional unit for this project and was packed with either brown or green leaves. To prepare a leaf pack, I obtained .3 grams of dry leaf mass (+ .01g) and placed it in a mesh citrus sack. The inoculums were then added. All mesocosms were aerated with air from the UNDERC compressed air system via an airstone and given light at 12-hour intervals to mimic natural conditions. The lids of the mesocosms were closed to help prevent contamination. In order to prevent clustering, the mesocosms were placed evenly apart from each other and in a predetermined order (chart 1).

After the experiment had begun, leaf packs were pulled on days 2, 6, 12, 18, and 24. One these select days, leaf packs were removed by a pair of forceps sterilized with 20% bleach solution and placed into a 50-ml falcon tube. The tube was then filled with water of a known volume, temperature, and dissolved oxygen. The time was noted when adding this water. The leaf packs were then incubated for two hours in a refrigerator; light was absent and the temperature was controlled. Dissolved

oxygen and temperature were then measured again within the tube to measure respiration over that time period. Respiration was expressed as change in DO per unit time per liter water per dry weight (g) leaf mater. The leaves were removed from the mesh sack, placed on a tin of a known weight, and dried in an oven at 60°C for two days. After two days, the tins were weighed again, yielding the dry mass of the leaf matter and the tin. The tins were then baked in a muffle furnace at 550°C for two hours then removed and weighed, giving us the Ash Free Dry Mass (AFDM). The AFDM is calculated by subtracting the remaining ash from the dry weight of the leaf matter and gave us a measurement of the amount of organic leaf matter consumed.

We log transformed the AFDM of remaining leaf matter and calculated the K-value as FORMULA □=□□−□□ where K is the constant rate of decomposition for each replicate. We then performed a 2x2 ANOVA using K-value as the dependent variable and leaf type (2 levels, brown and green) and microbial inoculums (4 levels, Reddington, Tenderfoot, Brown, and Control) as the factors (8 treatments, n=4 replicates) for each experiment. We performed a repeated measures ANOVA on the respiration data through time (8 treatments, 6 time points, n=4 replicates).

RESULTS: The ANOVA of experiment one (Reddington water) K-Values yielded no significant difference between either leaf treatment (df=2, F=2.425, *P=0.132*) or stream inoculum (df=3, F=0.193, *P=0.900*) (figure 1). A 2x2 ANOVA with K-Values as the dependent variable and and leaf type (2 levels, brown and green) and microbial inoculums (4 levels, Reddington, Tenderfoot, Brown, and Control) as the factors (8 treatments, n=4 replicates) showed no significant relationship (df=3,

F=0.334. *P=0.801*). A repeated measures ANOVA on the respiration data for experiment one showed a significant difference between leaf treatments (df=4, F=2.642, *P=0.07*). No significant difference was found between inoculums (df=12, F=0.912, *P= 0.553*) (figure two).

The ANOVA of experiment two (DI water) K-Values again yielded no significant difference between either leaf treatment (df=1, F=0.001, *P=0.982*) or stream inoculum (df=3, F=0.873, *P=0.469*) (figure 3). A 2x2 ANOVA with K-Values as the dependent variable and leaf type (2 levels, brown and green) and microbial inoculums (4 levels, Reddington, Tenderfoot, Brown, and Control) as the factors (8 treatments, n=4 replicates) showed no significant relationship (df=3, F=0.494. *P=0.690*). A repeated measure ANOVA on the respiration data for experiment two showed no significant difference between leaf treatments (df=3, F=1.533, *P=0.234*) or between inoculums (df=9, F=0.901, *P= 0.531*) (figure 4).

DISCUSSION: My hypotheses were partially based upon the results of an experiment performed last year, in which a student found that there was a significant difference in both decomposition and respiration rates between leaves collected off the forest floor and green leaves. It has been shown that nutrition content is an important factor when considering leaf decomposition by microbes. Leaves that are high in nutrients are broken down faster and more completely than leaves poor in nutrients (Webster and Benfield, 1986). This did not occur in my experiments. When comparing the leaves used between the 2009 experiment and mine, it was found that the brown leaves from the past year differed from the brown

leaves from this year. The leaves used last year were picked up off of the forest floor in the spring and were thus subject to six months of decomposition. By the time they were used in the experiment, the leaves were surely devoid of much of their nutrient content and probably consisted of material difficult to decompose (cellulose). This could explain the difference in both the decomposition and respiration rates for that experiment in regards to leaf type. In contrast, my leaves were collected during leaf fall in October 2009 and subject to almost no decomposition. Therefore, it is possible that the nutrition contents between the two leaf treatments were too similar to have shown any significant differences in the decomposition and respiration data. I also assert that the way in which my experiment was performed in regards to leaf type was more ecologically accurate for UNDERC stream systems. As leaves fall from their trees and land in streams, they should contain more nutrients than those that had been decomposing on the forest floor all winter. Leaves with the nutrient content from the 2009 experiment should match said leaf type. Those leaves would also be subject to decomposition from microbes not found within streams. Therefore, leaves taken just before falling in autumn should be better representatives of the leaves found decomposing in streams.

As previously mentioned, the statistical analysis of the decomposition and respiration data showed no differences between streams or between leaf treatments. Assuming that the methods used for this experiment were good indicators of streams in the field, it is logical to assume that the microbes in UNDERC streams show no ecologically important functional differences. Thus,

"home-field advantage" for UNDERC stream microbial communities does not appear to exist in a biologically important way. This could be because the streams share similar riparian environments and are used to decomposing similar types of leaves. Also, this similarity could be attributed to the wide distribution of these microbial communities, as demonstrated by Fenchel and Finlay (2004).

As stated above, Docherty et al. showed that pH is a critical factor when examining leaf decomposition rates (2006). When examining the leaf matter decomposition graphs for experiments one and two, while we notice that there were no significant differences between leaf treatment or stream inoculums for either experiment, we do see an interesting trend. The rates of decomposition for experiment one (Reddington water) were much slower than those for experiment two (DI water); approximately half (graph 3). The pH for Reddington stream was 4.3, while the DI water garden was 7.1. This stark difference in pH could potentially explain the large difference in total decomposition rates for the two experiments.

Every scientific experiment can be improved upon and this one is no different. First, contamination from the laboratory environment was nearly impossible to prevent. Changes in methods to include DNA testing may help to assure no contamination occurred. Also, the issue of microbes already present on the leaf may have confounded our results. The leaves themselves were not sterilized before the experiment in order to preserve the integrity of the leaf. Thus, the control mesocosms would be subject to those microbes already on the leaf. Due to spatial constraints, aeration and lighting was inconsistent. During both experiments, it was noted that some mesocosms received more lighting and air than

others. This change between mesocosms was indeed small, but nonetheless may have contributed to some significant changes in data, as light and chemical nutrients are major factors affecting decomposition rates (Docherty et al. 2006, Gessner and Chauvet 1994, Findlay 2010).

The results obtained from these two experiments help give us a better idea of how the microbial communities in UNDERC streams function. For example, we might expect different outcomes, when examining leaf decomposition in regards to different factors. pH could potentially limit the amount of decomposition taking place (graph 3). The amount of nutrients found within the leaf matter could also be a large factor in microbial decomposition. The UNDERC stream microbial communities as a whole should be considered an intricate complex of organisms that are affected by many different abiotic and biotic factors, most prominently pH and nutrient availability.

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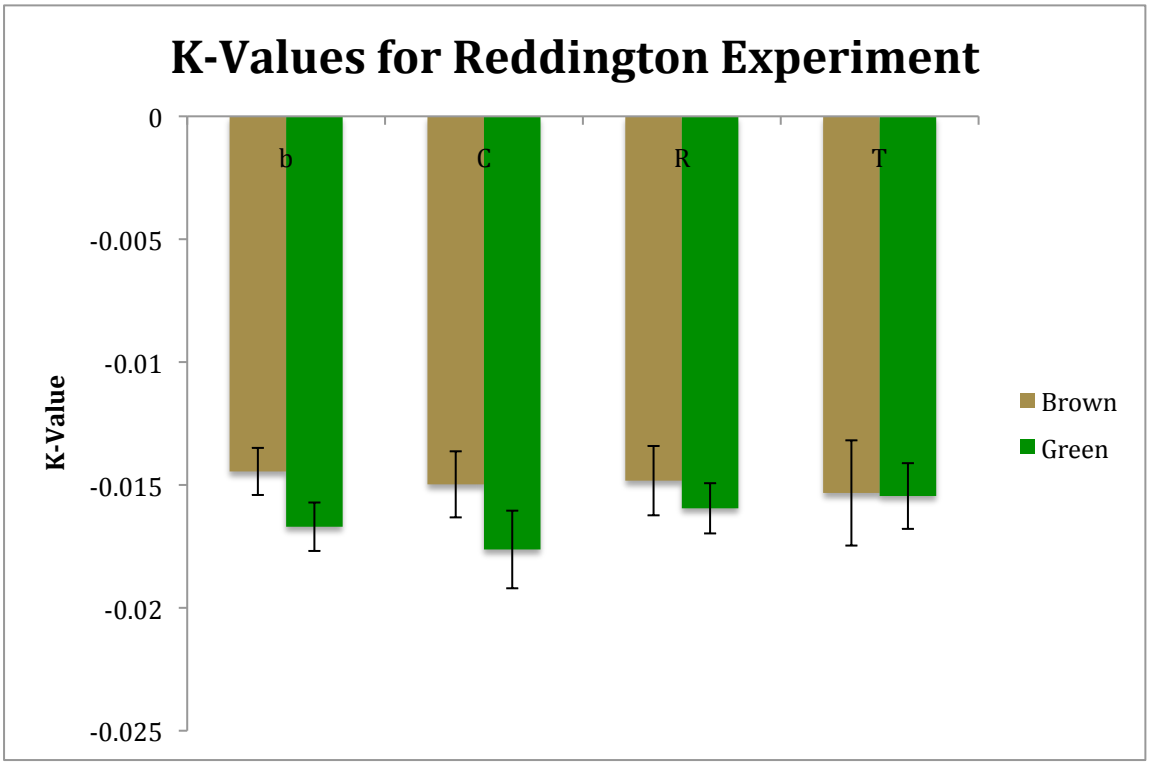


Figure 1: Comparing the K-Values from experiment one (Reddington water) against leaf-type (brown v green) and inoculums (Brown, Control, Reddington, Tenderfoot). No significant difference was found between any of the treatments or leaf-types.

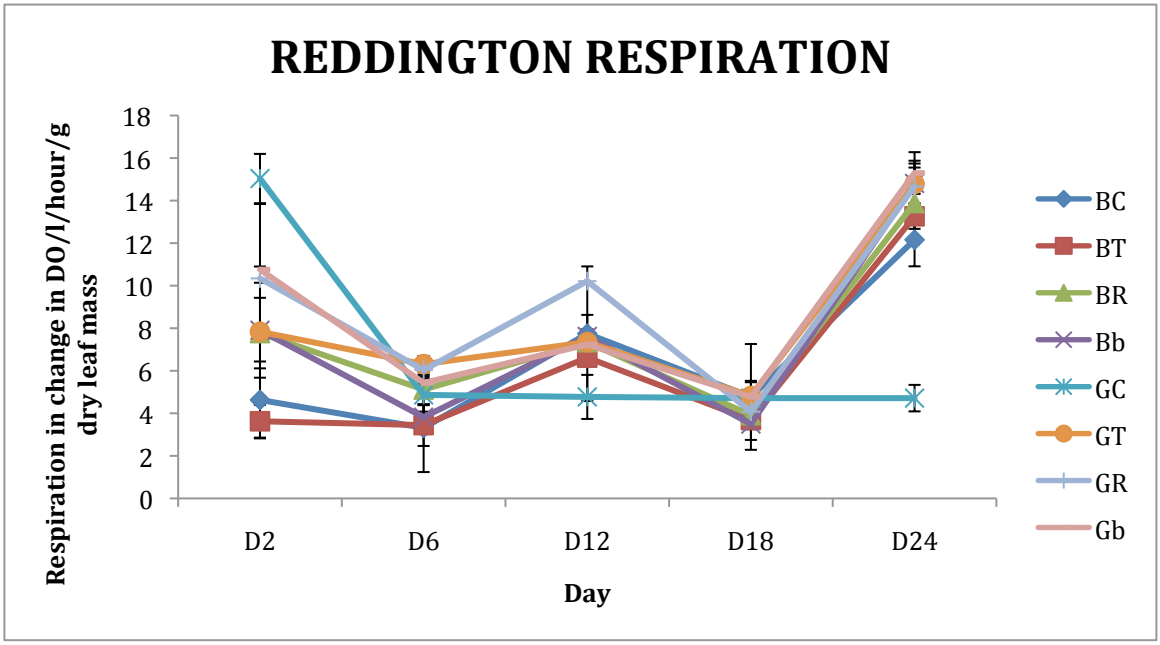


Figure 2: Respiration data over time between treatments for experiment one (Reddington water). No significant differences were found.

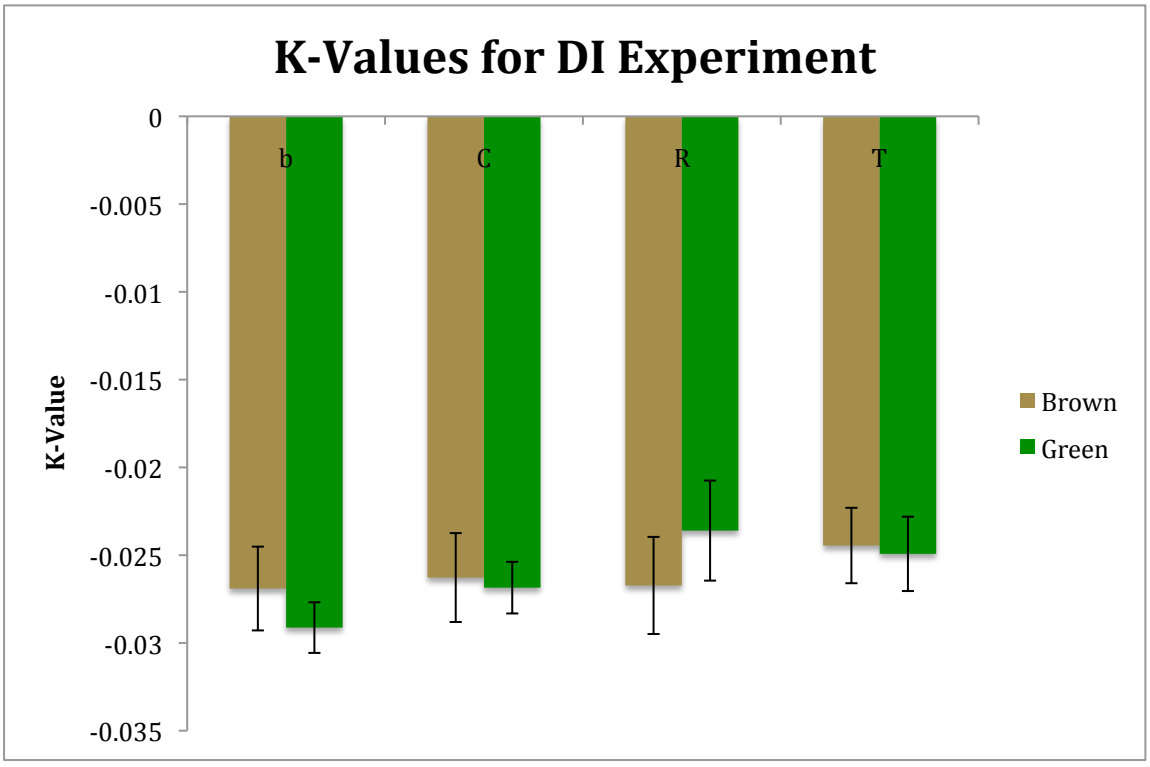


Figure 3- Comparing the K-Values from experiment two (DI water) against leaf-type (brown v green) and inoculums (Brown, Control, Reddington, Tenderfoot). No significant difference was found between any of the treatments. A significant difference was found for leaf type however.

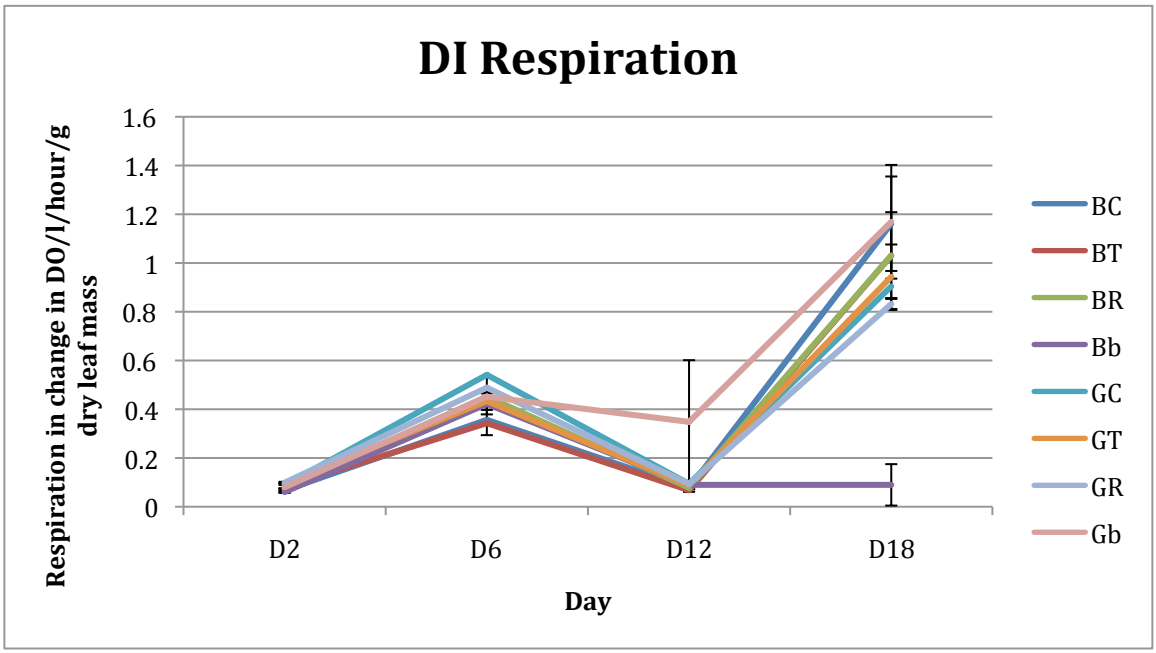


Figure 4: Respiration data over time between treatments for experiment two (DI water). Leaf-type was the only significant difference found.