Differences between two methods measuring the decomposition of organic matter in

an aquatic system

by

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ABSTRACT

The decomposition of organic matter in aquatic systems is a critical component of ecosystem health and can be used as an indicator of water quality, water chemistry, fish and microorganism community function. The deployment of leaf packs is a common method used to measure the decay rate of organic matter in aquatic systems. Recent studies have begun to use cotton strips as a surrogate for leaf packs, which have been used in the study of organic decomposition in soils. This study aimed to quantify any differences and the relationship between leaf packs and cotton strips, with an additional comparison of caged and uncaged cotton strips. Percentage mass loss per degree day was used to compare leaf packs and cotton strips, and tensile strength was used to determine the difference between caged and uncaged strips. A total of 378 samples (128 for leaf packs, 128 for caged cotton strips and 128 for uncaged cotton strips) were placed in three watersheds, and each watershed had six sites. There were significant differences between leaf packs and cotton strips, and site had the strongest effect, which may reflect confounding factors such as water velocity, temperature, pH, and variability in water chemistry. There was no significant difference between caged and uncaged cotton strips, which indicated that the cage used in the study had no impact on the decomposition of cotton strips. Also, tensile strength was shown as another valid way to determine the decay rate of cotton strips, as a measure of stream function. For future research, the uncaged cotton strips would be the best method, saving both time and labour.

Key words: leaf pack, cotton strips, organic matter, decomposition, stream system, percentage mass loss per average degree day, tensile strength

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Introduction

Leaves and twigs are the most common organic matter found in rivers (A. J. B. Wallace et al. 2008), which is not only a suitable substrate for leaf-colonizing fungi and bacteria (dos Santos Fonseca et al. 2013), but also forms critical structural material of stream beds (Bilby and Likens 1980). This allochthonous, or terrestrially derived organic matter also serves as a food source for leaf-shredding stream invertebrates (Erdozain et al. 2018) and is decomposed into smaller elements and entrained into the stream food web (Benkf and Bruce Wallace 1997). The amount of this organic matter are cellulose (Chaukura et al. 2018). The amount of organic matter present can be an indicator of the health of streams, which plays an important role in environmental monitoring and assessment.

Leaf packs have been used as a common method (Mark O Gessner, Chauvet, and Dobson 2018; PETERSEN and CUMMINS 1974) to help assess the condition of the stream system, by inferring decomposition rates as a measure of stream function, and allowing researchers to compare ecosystem function across different sites. However, leaf packs can have plenty uncontrollable variabilities (A. S. D. Tiegs et al. 2017), such as differences of leaf quality (J. B. Wallace, Grubaugh, and Whilles 1996), tree species (M. O. Gessner and Chauvet 1994), and growth condition. These variables cannot be avoided due to the selective expression of genes and sometimes can have huge impact on the decomposition rate (A. J. B. Wallace et al. 2008).

As a alternative, cotton strips have been used in soil research for many years (Gestel, Kruidenier, and Berg 2003; Rabinovich, Melnik, and Bolobova 2002), since the major constituent of cotton strips is cellulose, same as leaf packs. Cotton strips may offer a solution to the shortcomings with leaf variability when using leaf packs to measures decomposition of organic matter. Both methods can indicate the condition of the stream ecosystem through mass loss as a measure of decay rate. Decay rate of cotton strips can be measured by determining weight loss (Reeves 2011), but more commonly, the loss in tensile strength is used as the indicator variable (Newman et al. 2001; Roberts and Rowland 1998). In contrast to leaves litter, cotton strips can be standardized, which is especially useful when comparing differences among multiple sites, reducing the overall variability in sample quality. It also reduces the time and laboratory on preparations before deployment, since all work can be easily done in the laboratory within a shorter period. The differences in tensile strength (Roberts and Rowland 1998) can be easily determined, which would be considered a more efficient method when doing aquatic ecosystem assessments.

This study was designed to measure the differences between the decomposition of leaf packs and cotton strips in freshwater aquatic systems because leaf packs have been used in this area for a very long time to simulate the natural decomposition process. I predicted that the cotton strips would yield similar results to the leaf packs, providing another view of organic matter decomposition, and demonstrating that the cotton strips are a suitable surrogate.

As a secondary methodological assessment, this study also examined differences in decomposition between two types of cotton strip treatments: caged and uncaged. As in previous studies in leaf packs(A. S. D. Tiegs et al. 2017; S. D. Tiegs et al. 2013), a coarse mesh cage was placed around half of the cotton strips to keep benthic macroinvertebrates off of the cotton strip surface, so that only the fungi and bacteria decompose the cotton strips, and to add protection from physical abrasion in the streams. To my knowledge, there has not been a direct comparison for caged and uncaged cotton strips before.

Method

Study area

The study was conducted in three catchments (Upper Restigouche (NBI), Upper Quisibis (NBE) and Charlo (NBR)) of similar size (~ 200 km^2) located in northern New Brunswick (Figure 1). The Upper Restigouche and Upper Quisibis (~ 20%) (hereafter Restigouche and Quisibis, respectively) include private industrial freehold land, owned and managed by J.D. Irving Ltd. (JDI). The Quisibis and Charlo catchments are largely contained within provincial Crown land. Within the Restigouche and Quisibis catchments (early Devonian), deep water clastic is the most common type of surficial geology, while shallow water clastic (early Silurian) in Charlo (GeoNB, New Brunswick Department of Energy and Resource Development). Restigouche had intensive forest harvest management, Quisibis had extensive forest harvest management, and Charlo had minimal management. This area rains from April to October and snows between November to March with peak discharge rates occurring during spring melt and fall rains and low flow events occurring between August and September (Environment Canada 2017).
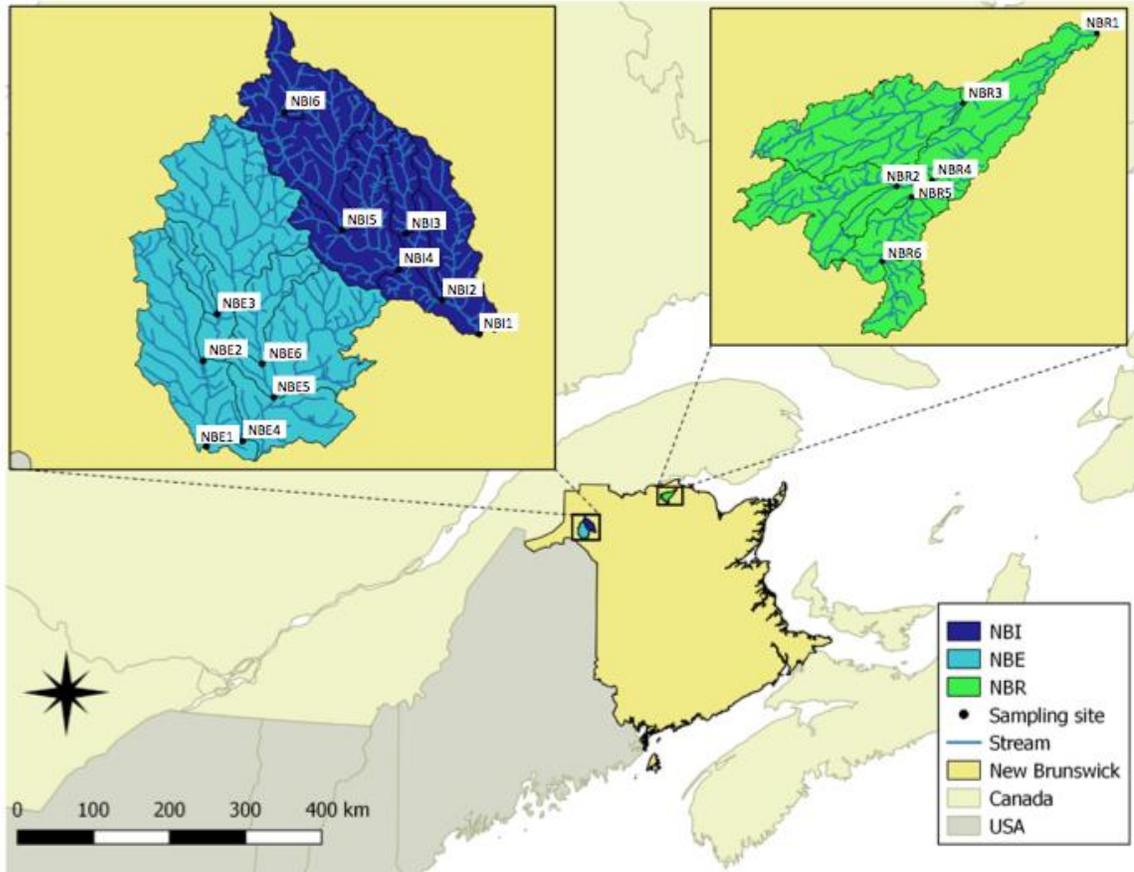
Figure 1. Study sites in northern New Brunswick where leaf pack and cotton strips were deployed from September to October 2018.

The Upper Restigouche catchment (NBI) has a cool climate with relatively abundant precipitation and the hilly landscape of this area is shaped by tributaries of Saint John River. Mid slopes and lower slopes are always covered by softwood community such as balsam fir, white spruce, and red spruce. However, as the elevations rises, the coniferous community increases forming a mixed forest[8]. The Upper Quisibis catchment (NBE) has a continental climate, with less precipitation because of its lower elevation, which leads to the warm and dry summers. It has a higher incidence of wildfire. Tree species here are similar to the Upper Restigouche. The average annual temperature of the Charlo

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catchment is warmer, due to lower elevations and the effect of the Chaleur Bay(Society 2019).

Study design

The purpose of this study was to compare different methods for estimating decomposition rates in streams, including the traditional leaf pack method and cotton strips, the latter method both with and without a protective cage. To assess the variation between leaf packs and cotton strips, a comparative catchment study design was made. This study also focused on the impact of different treatments (caged and uncaged) employed only in the cotton strip group.

In this study, we selected three catchments with six sites in them. The catchments were located at similar latitudes to reduce the variation in forest composition. At each of the six sites selected in each catchment, we deployed seven leaf packs, seven caged cotton strips, and seven uncaged cotton strips. A total of 126 leaf packs were prepared and 378 strips were cut with an additional seven strips per treatment serving as procedural controls.

Preparing and deploying samples

After Erdozain et al (2018), senescent speckled alder leaves (Alnus incana) were collected in Sault Ste. Marie, Ontario by the Canadian Forest Service and then air-dried. Leaves were weighed into 4.0 ± 0.1 g groups and placed in mesh bags with a 5 mm x 10 mm mesh size and a 15-cm diameter metal wire frame inserted to maintain the shape and minimize leaf clumping (Erdozain et al. 2018). Leaf packs were tied to bricks placed on

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the stream bottom. In September 2018, seven leaf packs were distributed within each 60-m reach at each site and incubated in the streams for an average of 34 days, with all sites within 2 - 4 days of one another depending on their respective collection date.

The cotton strips were cut from a bolt of unprimed heavyweight 12 oz. cotton artist canvas (Style # 548, Fredrix Lawrenceville, GA, USA). Strips were measured out on the bolt of fabric so that the 8 cm (long edge) was on the uncut or long edge of the fabric and the 2.5 cm (short edge) was cut along the width of the fabric. All edges of each strip of fabric were frayed to 3 mm width to prevent further fraying once deployed(S. D. Tiegs et al. 2013).

For the caged method, plastic poultry fencing with 1 cm^2 opening was cut into 10 x 12 cm rectangles. The rectangles were then duct taped together and further secured using heavy duty staples. One edge was left open, so the strips could be inserted.

For both treatments, cotton strips were labeled and weighed ($\pm$ 0.001g). Caged treatments were given an identifier e.g., NBI-1 C to NBI-7 C, and bare strips a double letter number identifier e.g., NBI-1 UC to NBI-7 UC. Strips were dried in an oven at 105˚C for 20 hours, then they were removed from the oven, placed in a desiccator for 15 to 30 mins to cool and weighed again(A. S. D. Tiegs et al. 2017; S. D. Tiegs et al. 2013).

Strips were grouped by site (seven per site) and wrapped in tinfoil and autoclaved for 30 mins at 121ºC. Strips were kept in sealed plastic bags prior to deployment. The day

before deployment, the cages for the strips were sterilized using 70% ethanol, and the top portion of the cage was duct taped shut following insertion of the cotton strip. A puncture was made through the caged and uncaged strips 3mm from the top and a zip tie was placed through the strip or the strip and cage. Both treatments were attached using zip ties to a length of plastic-coated clothesline. The caged treatment was always placed first on the line and then the treatments were alternated (e.g. NBI-1 C, NBI-1 UC, NBI-2 C, NBI-2 UC). Strips were placed approximately 10 cm from each other. The clothesline was secured to rebar and weighed down using rock, so that the strips sat on or close to the streambed.

Retrieval and processing of deployed samples

After approximately four weeks, the cotton strips and leaf packs were collected from each site of deployment. Leaf packs were retrieved, and the contents emptied into containers filled with stream water and then preserved in 37% formaldehyde. In the lab, containers were emptied into 1 mm and 250 µm sieves under the fume hood and then leaves were washed individually with water. Using a dissecting microscope, invertebrates were picked from the material collected in the sieves and stored in 70% ethanol. Residual leaf material was dried in the oven at 60°C for 48 h, cooled in a desiccator and weighed. Dried leaves were finally ashed in a muffle furnace at 500°C for 2 h, cooled in a desiccator, and ash-free dry mass (AFDM) calculated by subtracting ash mass from dry mass(Erdozain et al. 2018). Percent AFDM lost was calculated by subtracting the AFDM at the end of the incubation period from the starting AFDM (which was calculated to be 95.2% of the starting dry mass during a preliminary study).

The uncaged cotton strips were cut from the clothesline and placed in a pan containing river water and gently cleaned with a soft toothbrush to remove any debris that was attached(A. S. D. Tiegs et al. 2017; S. D. Tiegs et al. 2013). The general condition of the strips was noted. Strips in mesh cages were gently removed from the mesh cages using forceps and placed in the pan of river water and cleaned of any debris. Cleaned strips were then placed in 95% ethanol for 1 hour to kill any microbes and limit further decay. Strips were then wrapped individually in aluminum foil and placed in a zipper seal plastic bag. Collected strips were kept cool until they were transported to the laboratory. Collected strips were dried in the laboratory at 105°C for 16 h and then weighed ($\pm$ 0.001g). Strips were handled using forceps to reduce the likelihood of contamination or handling loss(A. S. D. Tiegs et al. 2017; S. D. Tiegs et al. 2013). Strips were then sealed in a Ziploc bag until tensile strength could be measured.

Two sets of procedural controls were prepared prior to the tensile strength measurements. The first set of procedural controls was prepared in the same ways as the uncaged strips placed in the stream but was placed in a sealed container containing distilled water. The container was placed in the laboratory fridge for the same duration as the cotton strips were deployed. The procedural control was then dried and weighed, and tensile strength was tested. The second set of procedural controls was simply cut cotton strips, weighed, immersed in ethanol (95%), placed in a drying oven for 16h, reweighed after drying, and then processed to determine tensile strength.

Calculating percentage mass loss per average degree day

We calculated percentage mass loss to compare cotton strips with leaf packs. Percentage mass loss represents gave the precise amount of mass lost in a standard unit, which was good for comparing every sample (Egglishaw 1972).

$$\text{Percentage mass loss (\%)} = ((A - B) / A) * 100$$

Where A = dried weight (g) before deploying

B = dried weight (g) after collecting

We then calculated the percentage mass loss per degree day to account for differences in water temperatures among sites. Some studies have noted that water temperature (Piggott et al. 2015) can affect the decomposition condition. This was calculated as:

$$\text{Percentage mass loss per average degree day} = \text{Percentage mass loss} * \text{Average Degree day}$$

Where Average Degree day = T1 + T2 +T3 + ……. + Ti

Ti = average water temperature (℃) calculated for each day from the date of deployment (T1) to date of collection (Ti)

Determining tensile strength

Tensile strength, the maximum tension that tears cotton strips apart, was used as a response valuable for cotton strips. Prior to measuring the tensile strength, the cotton strips were placed in a desiccator with silica drying medium for one to two days. The tensile strength of the cotton strips was measured using a Mark 10 Series 5 force gauge mounted on a motorized test stand. The force gauge and stand were equipped with wedge

grips that held the cotton strip securely during force testing. The force gauge speed was set to 2 cm/ min and the break detection was measured in peak Newton strength (N).

Statistical analysis

All analyses were conducted in R (Version 3.5.2 GUI 1.70) and RStudio (Version 1.1.463), an integrated development environment for R, a programming language for statistical computing and graphics. The R packages that used in this study included *GAD* (Sandrini-Neto and Camargo 2014), *ggplot2* (Wickham 2011) *multcomp* (Hothorn et al. 2014), *GGally* (Wickham 2011), *car*(Fox and Weisberg 2013), *Nortest* (Logallo et al. 2014), *plyr* (Logallo et al. 2014), *Rmisc* (Logallo et al. 2014), *olsrr,* and *corrplot*(Wei and Simko 2016).

Two-way nested Analysis of Variance (ANOVA)

Two-way nested ANOVA was used to compare the means of PMLAD between treatments (leaf packs and cotton strips) and among watersheds (NBE, NBI, and NBR) and sites (6 sites nested in each watershed). The null hypotheses were that there was no difference in PMLAD between leaf packs and cotton strips, and there was no difference in PMLAD among watersheds.

The model tested was:

$$PMLAD = treatment + watershed + watershed/site + treatment*watershed + treatment*watershed/site$$

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In this model, the treatment and watershed were fixed factors, and the site was a random factor nested in watershed. The "treatment * watershed" and "treatment *watershed/site" terms were the interactions. If the interaction terms were not significant, a reduced model was run:

$$PMLAD = treatment + watershed + watershed/site$$

If the treatment*watershed interaction was significant, the main effects were disentangled by comparing means of treatment separately for each level of the watershed:

$$PMLAD = treatment + watershed/site + treatment * watershed/site$$

If the treatment*watershed interaction was not significant, then ran the reduced model:

$$PMLAD = treatment + watershed/site$$

If only one of the two factors had a statistical significance, then the factor would be the main effect causing the difference of PMLAD. However, if none of the factors was important or both treatment and watershed were important, then interpreting main effects and doing multiple comparisons would be processed.

Variance components analysis

Variance components analysis was used to quantify the amount of random variation in the dependent variable that was associated with the random-effects variables in the model. Random effects in this model were sites nested in watersheds.

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Concordance Correlation

The concordance correlation coefficient measures the agreement between two variables. In this study, concordance correlation was used to compare tensile strength between caged and uncaged cotton strips to find if there is a difference between caged and uncaged cotton strips. The concordance correlation coefficient (r_c) would be 1 for a perfect positive agreement, -1 for a perfect negative agreement, and 0 for no agreement at all. The concordance correlation coefficient would measure the accuracy by comparing the 1:1 line with the distribution of the tensile strength from caged and uncaged cotton strips. The location shift was the tension deviation from accuracy, and the scale shift was how close the tensile strength to the 1: 1 line. Small location and scale shift indicated the distribution is very close to the 1:1 line, which suggested the high similarity between caged and uncaged cotton strips (Lin 1989; Zar 1999).

Results

Two-way Nested ANOVA

Leaf packs lost much more mass than cotton strips in 34 (average) days in the watershed (Figure 2). Across the three watersheds, the average PMLAD was 11.24% (SD = 0.028) for leaf packs and 0.3% (SD = 0.0002) for all cotton strips. The PMLAD for caged and uncaged cotton strips were within 0.01% of one another. NBE and NBI demonstrated greater variability both among- and within-site for both caged and uncaged cotton strips as compared with NBR.
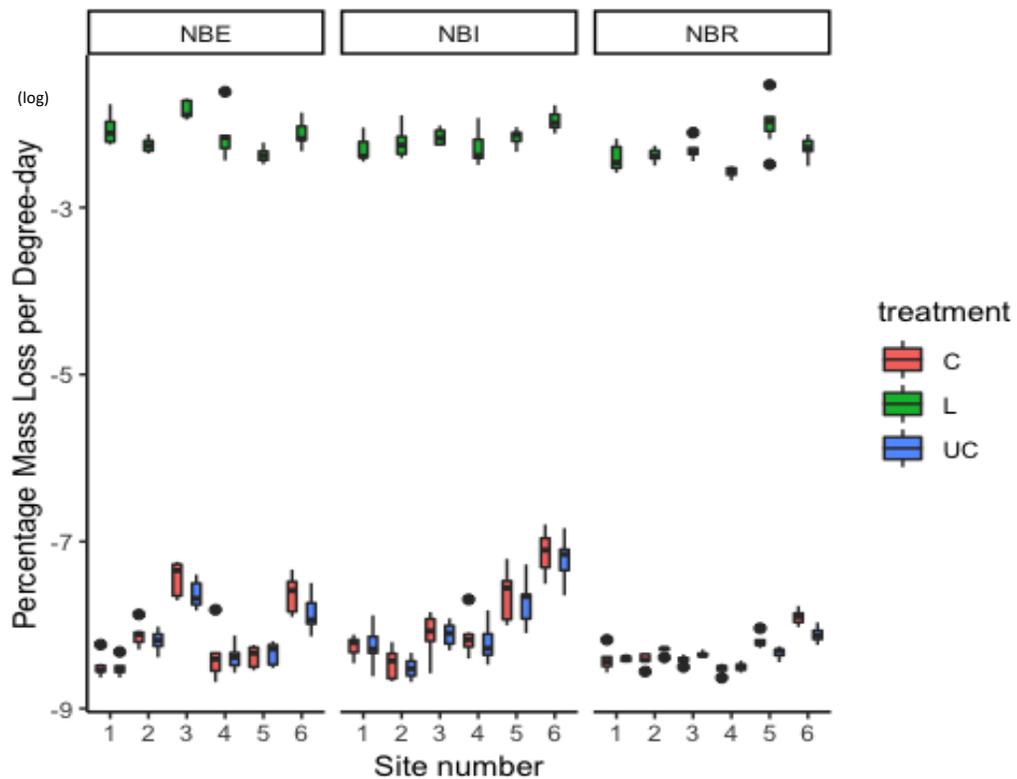
Figure 2. Box plots of PMLAD (log transformed) for sites (1-6) and watersheds (NBE, NBI, and NBR), with boxes coloured by treatment: caged cotton strips (C), leaf packs (L) and uncaged cotton strips (UC).

Significant interactions can be found between treatment and watershed (F value = 6.4, P value < 0.001) and between treatment and site nested in watersheds (F value = 25.7, P value < 0.001). So, the null hypothesis was rejected due to the significant interactions,

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then this study broke data by watershed to continue determining the difference between leaf packs and cotton strips in individual watershed.

Table 1. Results of a 2-way nested ANOVA for leaf packs and cotton strips testing the model: PMLAD = treatment + watershed + watershed/site + treatment*watershed + treatment*watershed/site where the treatment and watershed were fixed factors, and the site was a random factor nested in watershed. The "treatment * watershed" and "treatment *watershed/site" terms were the interactions.

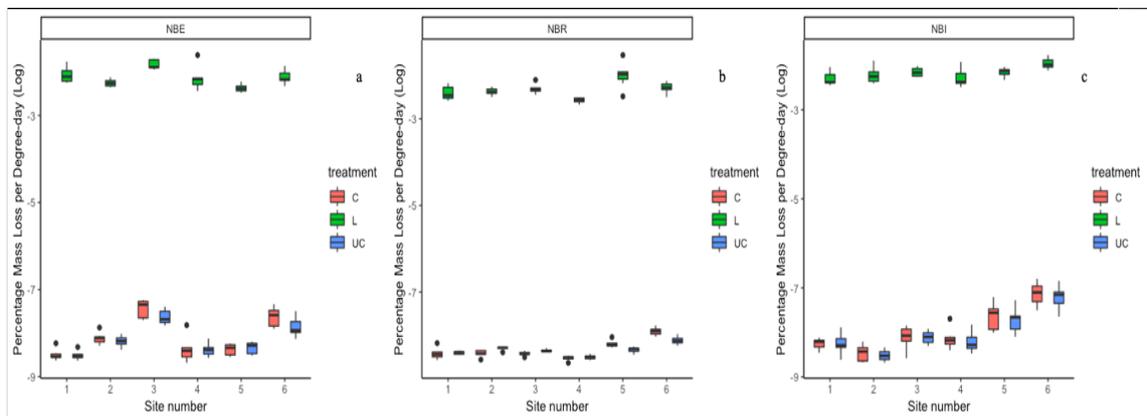|	Df	Mean Sq	*F* value	*P* value
Treatment	2	1476.1	53807.0	<0.001
Watershed	2	2.5	921	<0.001
Treatment: watershed	4	0.1	6.4	<0.001
Treatment: site/watershed	45	0.7	25.7	<0.001
residual	324	0.03		

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Figure 3. The individual distribution of PMLAD after log transfrmation related to watershed and site for each watershed (a-c)

In each watershed, caged and uncaged cotton strips seemed to have similar patterns overall (Figure 3 a-c). In watershed NBI, the same site had the highest PMALD for leaf packs and cotton strips (Site 6). However, the site with the minimum value differed between methods. In watershed NBE, the highest PMALD was at the same site for leaf packs, caged, and uncaged cotton strips (Site 3). However, the site with the minimum PMLAD for leaf packs (Site 5) differed from that of the cotton strips (Site 1). In watershed NBR, the site with the minimum PLADM was the same for leaf packs and cotton strips, while the site with maximum PLADM differed between methods. Due to the significant interactions, this study broke down the data by watershed to determine the difference between leaf packs and cotton strips in each watershed. (Tables 2-4).

Table 2. Results of the ANOVA model for watershed NBE: PMLAD = treatment + watershed/site + treatment*watershed/site where the treatment was fixed factors, and the site was a random factor nested in watershed. The "treatment *watershed/site" term was the interactions.

	Df	Mean Sq	*F* value	*P* value
Treatment	2	0.60	15.22	<0.001
sitenumber	5	0.20	60.19	<0.001
Treatment: site/watershed	10	0.04	11.84	<0.001
residual	108	0.003		

Table 3. Results of the ANOVA model for watershed NBI: PMLAD = treatment + watershed/site + treatment*watershed/site where the treatment was fixed factors, and the site was a random factor nested in watershed. The "treatment *watershed/site" term was the interaction.

	Df	Mean Sq	*F* value	*P* value
Treatment	2	1.05	10.90	<0.001
sitenumber	5	0.46	58.22	<0.001
Treatment: site/watershed	10	0.10	12.20	<0.001
residual	108	0.007		

Table 4. Results of the ANOVA model for watershed NBR: PMLAD = treatment + watershed/site + treatment*watershed/site where the treatment was fixed factors, and the site was a random factor nested in watershed. The "treatment *watershed/site" term was the interaction.

	Df	Mean Sq	*F* value	*P* value
Treatment	2	0.30	46.11	<0.001
sitenumber	5	0.03	69.30	<0.001
Treatment: site/watershed	10	0.007	17.80	<0.001
residual	108	0.0003		

Overall, there still were significant interactions between sites nested in watershed and treatment among three watersheds (NBE: F value = 15.22, P value < 0.001; NBE: F value = 10.90, P value < 0.001; NBE: F value = 46.11, P value < 0.001). As the distribution

showed above (Figure 2b-2d), cotton strips shared a similar trend in each watershed, increasing and decreasing among same sites. However, leaf packs sometimes showed different trends. In watershed NBE, the PMLAD (log) of leaf packs dropped a little in site 2 while cotton strips increasing.

Variance components analysis

The results of Variance Component Analysis indicated that in watershed NBE, 63.76% of site variance was attributable to treatment difference, which was less than NBI and NBR. (Table 5) NBI had the highest value, that 94.53% of site variance was attributable to treatment difference and in watershed NBR, 70.57% of site variance was attributable to treatment difference.

Table 5. Variance component analysis output for leaf packs and cotton strips in each watershed.

Watershed	MS(sitenumber (watershed))	MS(Residual)	n	MS(sitenumber (watershed))- MS(Residual)/ n	Variance Component Analysis (%)
NBE	0.03882	0.00328	7	0.00507	63.76
NBI	0.96043	0.00787	7	0.13608	94.53
NBR	0.00655	0.000368	7	0.00883	70.57

Concordance Correlation Coefficient

For both treatments, the average tension was 171.62 (N), and caged and uncaged groups reached similar tension at each watershed (Table 6). The cotton strips in watershed NBR always had higher tension than watershed NBE and NBI, which meant strips had a lower decay rate in watershed NBR.

Table 6. The mean value of cotton strips for both caged and uncaged among watersheds.

Treatment	Caged			Uncaged		
Average tension (N)	171.62			170.80		
SD	52.06			51.72		
watershed	NBE	NBI	NBR	NBE	NBI	NBR
Average tension (N)	155.76	144.83	219.39	148.68	144.68	215.99
SD	50.24	40.89	27.39	47.43	41.56	27.83

Figure 4. The distribution of tensile strength of caged and uncaged cotton strips among sites in each watershed.

The Concordance Correlation Coefficient was used to test the relationship between caged and uncaged cotton strips. The scale shift was 0.84 indicating that the distribution of the points was around the 1:1 line with few points away from the 1:1 line. Also, the location shift was -0.14 which indicated the average distance from each point to the 1:1 line was very small. The R_c was 0.81, very close to 1, suggesting that there was a high similarity

between caged and uncaged cotton strips.
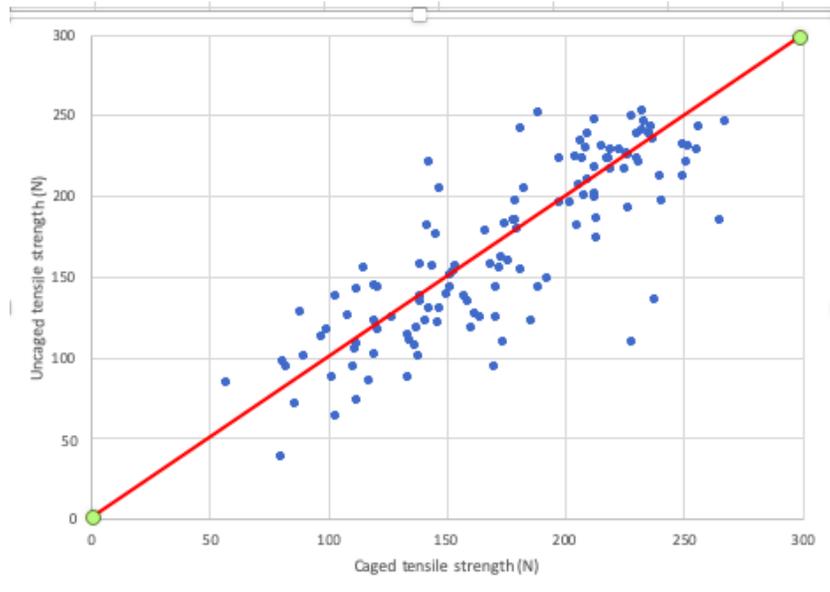
Discussion

This study rejected the null hypothesis that there is no difference between leaf packs and cotton strips. The results above showed that significant interactions can be found in 2-way nested ANOVA model, and the interactions still existed while doing separate ANOVA model by each watershed. However, there was little difference between caged and uncaged cotton strips based on the concordance correlation coefficient, which indicated that caged and uncaged cotton strips showed a high similarity.

Leaf packs and cotton strips

There was a large difference in the percentage of mass loss per average degree day (Figure 3 a-c) between leaf packs and cotton strips, as leaf packs consistently lost far

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greater mass. Although they differed, there were some similarities. For all methods among three watersheds, site-6 seemed had a higher PMLAD value than other sites, except site-3 in NBE (for both leaf packs and cotton strips), site-5 in NBR (for leaf packs only)(Roberts and Rowland 1998). Furthermore, this study did find few differences between caged and uncaged cotton strips, which suggests the cage that used in this study had no significant impact on the decomposition of cotton strips Thus, both caged and uncaged cotton strips can offer the same results to measure the decay rate.

The large difference in mass loss in this study made it hard to compare whether leaf packs and cotton strips provided similar results. The leaf packs lost 35.61% mass during this study (29 days, which was three times more than cotton strips (which had a mass loss of 10.00%). It was not unexpected for organic matter to have such a fast decay rate , other researches also showed the weak correlation between cotton strips and leaf packs (A. S. D. Tiegs et al. 2017). Across the catchments in our study, there were some similarities between methods in terms of which sites had the highest or lowest mass loss. However, interpreting the results of the method comparison was complicated by differences among sites and among watersheds, which led to significant interaction terms. Other research may offer some variables that could help explain.

Temperature effects

A study by Vyšná et al. (2014) indicated that diel temperature was the most important predictor of decomposition rates, accounting for approximately 20% of the variability in decomposition12. Water temperature could affect the metabolic rate of bacteria and fungi,

which could have impacts on the decay rate (Vyšná et al. 2014). As Vyšná et al. (2014) mentioned in their study, the decomposition rate appears to change with changing temperature, and therefore, using only average daily temperatures does not reflect the effect of temperature.

Many of the sites had a similar average daily temperature but they did not share the similar decay rate. For example, in site-1, watershed NBE, cotton strips had their lowest PMLAD value but not for leaf packs. Therefore, its average daily water temperature may not have been the most important driver of differences between methods for our study.

Flow velocity

Flow has the potential to affect decomposition rates in several ways. Flowing waters scour and wash the leaves, twigs (organic matter) in the streams, which could have a physical impact on the distribution of microbial organisms in aquatic systems(dos Santos Fonseca et al. 2013) or could contribute to the physical process of organic matter breaking(Weiss 2007). Faster flows could affect the decay rate of organic matter by carrying and washing away the bacteria and fungi, which are key to decomposition (Belancić et al. 2009). However, due to the lack of flow velocity data, we could not test whether flow velocity was an important variable contributing to method differences.

Flow velocity may have a positive effect on the decay rate of organic matter, up to a maximum flow rate, after which the decay rate begins to drop (dos Santos Fonseca et al. 2013). High velocities would be expected to wash away the organic matter and remove

substrate on which bacteria and fungi communities can develop (Benkf and Bruce Wallace 1997; Weiss 2007). If flow differed between leaf packs and cotton strips at a site, this could have contributed to differences between methods in the sites with maximum or minimum PMLAD. Although leaf packs and cotton strips were placed at the same site, river flow could have differed within the site. For example, the velocity at the middle of the river was different from that of both sides. Also, rocks and tree trunks may have changed the velocity near some samples. These could have caused the difference within one site and contributed to differences among methods. By adding the flow velocity in future sampling, we may do further research on the relationship between leaf packs and cotton strips.

Caged and uncaged cotton strips

We found little evidence of a difference between caged and uncaged cotton strip treatments, which suggests the cage that used in this study had no significant impact on the decomposition of cotton strips. Thus, both caged and uncaged cotton strips can offer the same results to measure the decay rate. The use of a cage was originally from the leaf packs, since leaf litter needed bags to keep them not being disturbed by bugs and washed away by flows (Belancić et al. 2009). Similarly, the cage for cotton strips has been used for preventing access from invertebrates and other animals, which may damage the samples (Benkf and Bruce Wallace 1997). However, our results indicated that the use of a cage did not affect the decay rate. The preparations for uncaged cotton strips were easier than leaf packs and caged strips, as there was no need to collect the leaf litter in the

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study area, all procedures can be done in the laboratory. Also, the measurement of tensile strength reflected the mass loss faster than leaf litter, because even coton strips lost less mass than leaf packs, the tension for cotton strips still changed in a large range (A. S. D. Tiegs et al. 2017). Overall, the results of our study indicated the uncaged cotton strips may offer the most convenient way to measure the decomposition of organic matter in aquatic systems with the most reliable results.

In conclusion, this study did not find similar mass loss values for leaf packs and cotton strips, but patterns among sites were similar. More variables should be measured in order to find the reason for method differences among sites. Also, uncaged cotton strips would be the best choice when estimating the decay rate of organic matter in streams, which saved more time and labour with reliable results. Measuring the decomposition of organic matter by leaf packs is the most natural way in stream system assessment, offering the baseline for other methods. However, the uncaged cotton strips can be widely used in other regions due to its standard level and fast results, which can be a great help for unassessed areas or even can develop into a quick test for decay rate.

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