Mactaquac Aquatic Ecosystem Study
Report Series 2015-010

METHODS PAPER: Leaf Decomposition Rates and Hypomycete Characteristics in the Saint John River and Preliminary Analyses

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DISCLAIMER

Intended use and technical limitations of the report, "Leaf Decomposition Rates and Hypomycete Characteristics in the Saint John River and Preliminary Analyses". This report describes the ongoing study of decomposition rates and Hypomycete community composition in the Saint John River. The CRI does not assume liability for any use of the included information outside the stated scope.

1A.2.7 Metrics of ecosystem function-Decomposition rates and Hypomycete characteristics

Introduction

A metric of decomposition rates are a required consideration in present-day, environmental impact assessments (e.g., Woodward et al. 2012, Tank et al. 2010). In the Saint John River (SJR), a cotton strip degradation method to assess decomposition rates in rivers is being develop (J. Culp, unpublished data; Young et al. 2008) and we are continuing the testing versus standardized, leaf litter packs as an appropriate decomposition metric for the SJR. The leaf litter packs are also providing an assessment of the fungal abundance, diversity, and biomass (Baldy et al. 2002, Gessner et al. 2010). The goal is examine the hypomycete structure and function in the SJR and explore the development of metrics for river environment / health assessment.

Methods

Study Design

We selected the use of leaf packs to measure change in ecosystem function through the calculation of leaf decomposition rates and decay coefficients, and b), to measure change in aquatic fungal community structure through the measurement of biomass (ergosterol) and DNA extraction. There are three main deployment sites on the SJR (Figure 1 and Table 1; 2014 and 2015). Site 1 (McKinley Ferry) is located on the main stem of the SJR ~4 km downstream of Mactaquac Dam, Site 2 (Keswick) is located on a lateral channel ~1 km downstream of the Keswick River, and Site 3 (Delta) is located on the main stem of the SJR just upstream of Fredericton (~16 km downstream of Mactaquac Dam). We target 60-100 leaf packs to be deployed at each site. In 2014, 95 were deployed at Sites 1 and 3 and at Site 2, 60 leaf packs were deployed. Five leaf packs are retrieved immediately after being deployed to serve as a handling control. Thirty (30) leaf packs are retrieved at three (3) weeks, six (6) weeks, and nine (9) weeks after being deployed. In 2014, leaf packs were deployed on August 11, 2014 and retrieved on September 3, September 22, and October 15 and 16 (Table 1).

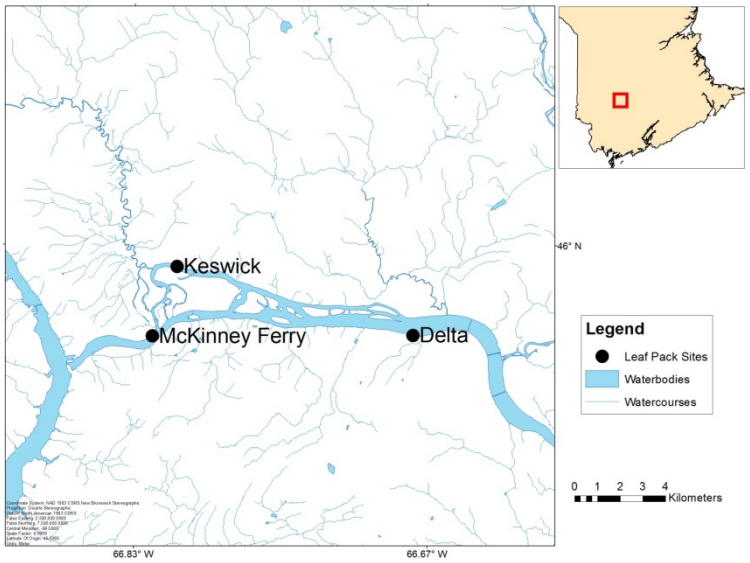

Figure 1. Leaf Pack deployment sites.

Table 1. Leaf decomposition sites for the Mactaquac Aquatic Ecosystem Study – 2014 sites and deploy/retrieve schedule.

		Coordinates[1]		Leaf Packs Retrieved			
Site	**Site Name**	**Latitude**	**Longitude**	**Week 0**	**Week 3**	**Week 6**	**Week 9**
1	McKinney Ferry	N45.96401	W66.82312	5	30	30	30
2	Keswick	N45.99160	W66.80925	-	-	30	30
3	Delta	N45.96450	W66.67481	5	30	30	30

1: Upstream end of deployed leaf packs

Leaf Pack Construction

Leaf Collection and Preparation: Red maple (*Acer rubrum*) leaves were collected from the UNB campus and woodlot. Only leaves that were not infected with black fungal spots were selected (Figure 2). Selected leaves were immersed in distilled water in a medium sized plastic tub until they were pliable. To standardize the size and surface area of leaf pieces, pliable leaves were placed on a black wax dissecting tray and a cork borer was used to cut discs from leaves, avoiding main leaf veins. Leaf discs were air dried before making the leaf packs.

Figure 2. Leaf Pack construction.

Leaf Pack Construction:
Nitex mesh was cut into 10 cm × 20 cm strips, folded in half, and sewn or stapled on two sides create a 10 cm × 10 cm bag (Figure 3). A steel washer was placed in the corner of each mesh bag and held in place with a zip-tie. Twenty five (25) leaf discs were selected, weighed, and placed in the mesh bag. The bag was labelled and then stapled or sewn shut, and the bag number was recorded with the leaf disc weight (Figure 3). Two (2) and four (4) foot lengths of cord were cut and tied to the mesh bags via the zip-tie with a bowline knot. Completed bags were stored in a plastic tote.

Figure 3. Leak Pack.

Leaf Pack Deployment

Before leaving the laboratory, leaf packs are divided into three (3) plastic totes by site and each tote was filled with distilled water to avoid leaf fragmentation. At each site, leaf packs are tied to a 6 × 8 × 16 concrete block such that each block had two (2) leaf packs, one with a short (2 ft) and long (4 ft) cord. Wading upstream to downstream, the blocks with leaf packs attached are placed along the river margins at a target depth of 50 cm (Figure 4). A sketch of the shoreline and leaf pack locations is made and GPS coordinates of the upstream and downstream limits recorded. Five (5) leaf packs are retrieved immediately after being deployed to assess the amount of leaf litter lost due to handling. Leaf packs are deployed at all three (3) sites on the same day.

Figure 4. Leak Pack deployment.

Leaf Pack Retrieval

Leaf packs are retrieved at 3, 6, and 9 week time periods (See Table 1 for 2014 schedule). Low water conditions can threaten to expose leaf packs (e.g., Delta Hotel and Keswick sites) and in these cases the shallowest leaf packs are collected first. Otherwise, leaf packs are collected systematically from downstream to upstream. Concrete blocks, with leaf packs attached are removed from the river. On shore, the zip-ties are cut and leaf pack placed in a new Ziplock bag labelled with the site number and name, date, and retrieval week. Leaf packs are stored in a cooler on ice in the field and frozen upon return to the laboratory.

Cotton Strip Studies
(adapted from Ritchie (2008): Environmental Drivers of Stream Ecosystem Structure and Function in Subarctic Labrador, Canada. MSc Thesis, UNB Fredericton)

Cotton Strip Assay: Preparation, Deployment and Retrieval of Cotton Strips

Standardized strips of 100% unbleached cotton (5 x 10 cm) are used in a cellulose bioassay following the general approach of Tiegs et al. (2007).[1] Cotton strips are placed individually in coarse-mesh bags constructed of 1-cm plastic poultry fencing sewn with nylon thread. Groups of 10 mesh bags are wrapped in aluminum foil, autoclaved for 30 minutes at 121ºC and kept sealed until deployment. Ten replicate strips are individually fastened to 12-inch rods and anchored flush with the substrate in each stream along a 5 – 10 metre run. Five replicate strips are recovered after 21 (± 2) and 31 (± 3) days of incubation, since previous studies have shown this incubation time to be adequate for cotton strip decomposition in temperate regions (Tiegs et al. 2007). Upon removal, strips are gently removed from the mesh bags, rinsed with stream water to remove debris and are placed in individual plastic bags. Within approximately 2 h after removal from the stream, microbial respiration associated with the fabric

[1] Tiegs et al. 2007. "Cotton strips as a leaf surrogate to measure decomposition in river floodplain habitats." Journal of the North American Benthological Society 26: 70-77.

was measured in the field laboratory and the strips are soaked in 95% ethanol for 24 h to arrest further microbial decay. The strips are subsequently removed from the ethanol, air dried, wrapped in aluminum foil and stored in sealed plastic bags until tensile strength could be determined.

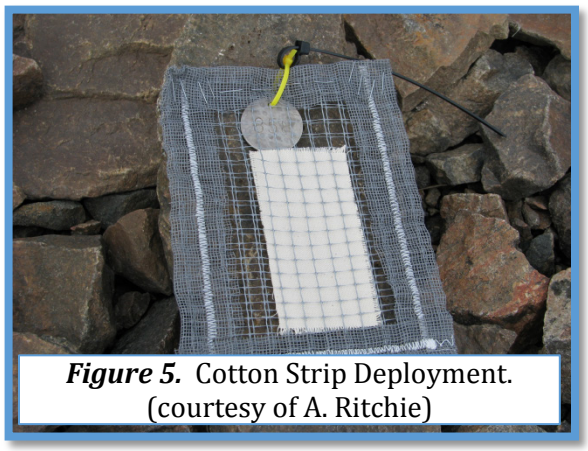

Figure 5. Cotton Strip Deployment. (courtesy of A. Ritchie)

Microbial Respiration Rates Associated with Cotton Strips

Respiration rates are used as a measure of microbial activity and are determined as the amount of oxygen consumed in the absence of light. A soft-bristled paintbrush was used to remove small debris and invertebrates from the cotton strips before they are individually folded into 120-ml vials containing filtered stream water with conductivity, pH and temperature similar to study sites. After initial dissolved oxygen was measured, vials containing the cotton strips are wrapped in aluminum foil to eliminate light exposure, thereby preventing the production of oxygen by primary producers. Incubation occurred for 18 – 24 h (mean water temperature 11 ± 3°C) after which final dissolved oxygen concentrations are measured. As a control, oxygen consumption rates are measured in 5 additional vials containing only filtered stream water.

Cotton Strip Mass Loss

Replicate strips are numbered with permanent archival ink and individual dry masses are recorded before strips are placed into mesh bags and autoclaved. Dry masses of individual strips are measured again post-incubation before they underwent tensile strength testing. Mass losses are calculated as the original dry mass of individual strips less their corresponding post-incubation dry masses.

Cotton Strip Tensile Strength Loss

Preliminary tests on 15 non-deployed strips revealed that more than 60% broke within the area gripped by the tensometer (Instron 5500R) clamps. Tensile strength measurements associated with these 'jaw breaks' are considered invalid. A subsequent study of 15 non-deployed strips was initiated to examine whether a tapered design cut into the strips using a scalpel and template (leaving 60% of the original area; Fig. 2) would effectively reduce the variance and invalidity of tensile strength measurements that can arise due to 'jaw' breaks. A tapered design increased the stress within the reduced cross-sectional area under load thereby preventing breakage at the clamps. This is the common method for testing the tensile strength of various materials such as steel and plastic. All but one of the newly-designed tapered strips ripped at a distance from the clamps and the new procedure greatly reduced variance in tensile strength measurements (s^2 = 6.76) compared to rectangular strips (s^2 = 78.58).

Tensile strength loss of incubated strips was determined as the difference in tensile strength between tapered control strips (n = 15) and deployed strips cut with the same tapered design post-incubation. As a procedural control, non-deployed strips are autoclaved for 30 minutes at 121ºC, soaked in 95% ethanol for 24 hours and air dried before testing. Small pieces of cork rubber are glued to the top and bottom tabs of the strips to minimize slippage in the tensometer clamps and strips are conditioned to 65% humidity in a humidor for 24 hours (at 20 ± 2 °C; Tiegs et al. 2007). Strips are removed from the humidor one at a time immediately before tensile strength testing which occurred at an elongation rate of 4 mm/min.

Appendix 1: Summary of the 2015 Sampling Season

Table 1: 2015 study schedule with sample size indicated. Green cells indicate the samples that have been retrieved.

		Leaf Litter – Ergosterol				*Leaf Litter -Sporulation*				*Leaf Litter – Decomp*				*Shirley Cotton*				*Artists Canvas*			
		Sites				*Sites*				*Sites*				*Sites*				*Sites*			
Week	*Date*	*Hartland*	*McKinley*	*Keswick*	*Hartt Island*	*Hartland*	*McKinley*	*Keswick*	*Hartt Island*	*Hartland*	*McKinley*	*Keswick*	*Hartt Island*	*Hartland*	*McKinley*	*Keswick*	*Hartt Island*	*Hartland*	*McKinley*	*Keswick*	*Hartt Island*
0	*Aug 12-14*									*5*	*5*	*5*	*5*			*5*		*5*		*5*	
1	*Aug 19-21*									*30*	*30*	*30*	*30*		*5*	*5*	*5*	*5*	*5*	*5*	*5*
2	*Aug 26-28*														*5*	*5*	*5*	*5*	*5*	*5*	*5*
3	*Sept 1-3*	*7*	*7*	*7*	*7*	*12*	*12*	*12*	*12*	*30*	*30*	*30*	*30*	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*
4	*Sept 9-11*														*5*	*5*	*5*	*5*	*5*	*5*	*5*
5																					
6	*Sept 23-25*									*30*	*30*	*30*	*30*	*5*	*5*	*5*	*5*	*5*	*5*	*5*	*5*
7																					
8																					
9	*Oct 14-16*									*30*	*30*	*30*	*30*		*5*	*5*	*5*	*5*	*5*	*5*	*5*
10																					
11																					
12	*Nov 4-6*														*5*	*5*	*5*	*5*	*5*	*5*	*5*

Site Locations:

a) Hartland (Picnic Table Site); Upstream of the Mactaquac reservoir.
b) McKinley Ferry; Mainstem just downstream of the dam (McKinley Ferry - SJR-MKF).
c) Keswick; Downstream of Keswick River, upstream of the ferry (SJR-KWF).
d) Hartt Island; In the braided island area (SJR-ISL).

Table 2: Preliminary leaf litter decomposition analyses: Exponential Decay Coefficient (k) ± 1SE, Y0± 1SE, and R2, values for leaf litter at each site during August-November, 2015

Metric	Site			
	Hartland	**Hartt Island**	**Keswick Ferry**	**McKinley Ferry**
K	0.172 ± 0.0061	0.192 ± 0.008265	0.1848 ± 0.007288	0.1663 ± 0.005577
YO	77.67 ± 1.197	76.94 ± 1.708	78.23 ± 1.573	74.52 ± 1.222
R2	0.9175	0.8887	0.9028	0.9223

Figure 1. Preliminary decomposition rates as dry mass loss, August-November, 2015