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ALLOCHTHONOUS CARBON DELIVERY FROM THE INVASIVE GRASS (*PHALARIS ARUNDIANCEA L.)* TO A LOTIC ECOYSTEM

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ALLOCHTHONOUS CARBON DELIVERY FROM THE INVASIVE GRASS (*PHALARIS ARUNDIANCEA L.)* TO A LOTIC ECOYSTEM

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ABSTRACT

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Reed canarygrass (RCG; *Phalaris arundiancea L.*;) invades wetlands throughout the United States of America, including floodplain forests (FPF) found along the upper Mississippi River (UMR). This study was done to determine how this invasive grass alters the allochthonous material delivered to UMR. A plant litter mass loss study was done to determine the decomposition rate or RCG litter compared to river birch (RB; *Betula nigra L*); silver maple (SM; *Acer saccharinum L.*); swamp white oak (SWO; *Quercus bicolor Willd.*) leaf litter. Screen mesh (inhibit macroinvertebrate activity) and hardware mesh (to allow macroinvertebrate activity) litter packs filled with plant litter from RCG and FPF leaf litter, submerged in a stream and withdrawn nine different days to determine loss of mass. Macroinvertebrates from litter packs were identified, counted and dried to a constant weight. In well water without and with microbial activity, the concentration (mg/L) of dissolved organic carbon (DOC) leached from RCG litter and native FPF leaf litter was compared over time. With microbial activity in well water the decomposition of DOC leached from RCG and native FPF leaf litter was also compared over time. Silver maple leaf litter decomposed at a significantly faster rate than RCG, SWO and RB litter ($P < 0.001$). Macroinvertebrate shredder activity was observed on FPF leaf litter one week prior to RCG litter, and peaked by week 17 compared to week 7 on RCG. Macroinvertebrate biomass and abundance were not significantly different between litter types. With microbial activity DOC (mg/L) leached from SM litter was significantly higher than DOC (mg/L) leached from RCG litter over time ($P < 0.05$). Overall decomposition rate, and rate of the labile portion of DOC leached from RCG litter was significantly higher that DOC leached from native FPF leaf litter ($P < 0.0001$) and ($P < 0.0001$), respectively. These results suggest that RCG litter is of lower quality that FPF leaf litter, which could alter allochthonous carbon made available to secondary consumers in the UMRS.

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

FIGURE PAGE

INTRODUCTION

Phalaris arundinacea L. (reed canargygrass; RCG) is a cool-season, perennial grass, with rhizhomatous growth and is considered a model organism for invasions of wetlands (Lavergne and Molofsky 2004). Throughout North America RCG invades wetlands, wet prairies, sedge meadows, fens, stream banks and seasonally wet areas (USDA Forest Service 2005). Negative effects of RCG on forested wetlands have been observed in the Pacific Northwest (Kim *et al.* 2006; Fierke and Kauffman 2005), Midwest (Knutson 2005; Hovick and Reinartz 2007), southeastern United States (Foster and Wetzel 2005) and on the floodplains of the upper Mississippi River system (UMRS) (Romano 2010).

Floodplain forested wetlands are important to the ecology of the UMRS. These forests provide a source of allochthonous carbon to the UMRS (Grubaugh and Anderson 1989; Junk *et al.* 1989), habitat for wildlife and connect riverine ecosystems with adjacent terrestrial ecosystems (Delong 2010). When inundated during spring flooding these forests provide habitat for fish (Maher 1994). It is estimated that 123,000 ha of floodplain forests occur in the UMRS (Yin 1998). Knutson and Klass (1998), estimated that before European settlement (approx. 1800 CE) forests occupied 50% - 70% of the UMRS floodplain, which has been reduced to less than 22% - 25% of the current land cover.

The current state of UMRS floodplain forests follow the history of anthropogenic modifications to the Mississippi River (Romano 2010). Channel modifications, urban expansion and agriculture reduced forest coverage (Peck and Smart 1986). A large portion of floodplain forests were cut down and then inundated during the Nine-Foot Channel Project (NFCP) (Fremling 2005). The NFCP was started in 1933, and by the end of 1930's the Mississippi River channel was modified to a depth of 2.74 meters (*i.e.*, 9 feet) (Anfinson 2003, Fremling 2005). Today twenty-nine locks and dams maintain a nine-foot channel from Minneapolis to St. Louis. This project significantly altered the hydrology of Mississippi River (Yin *et al.* 1997). Channel modifications to maintain the navigation channel have led to large magnitude flooding events and significantly higher water levels (Lubinski *et al*. 1991), further altering the structure of floodplain forests.

Today the structure of the floodplain forest communities in the UMRS is shaped by several hydrological and geomorphological factors. Alteration to the natural UMRS flow regime disrupts the timing of seed dispersal. Many hardwood species require flooding for seed dispersal (Romano 2010). Dispersal phenology and the ecological requirements of seedlings are factors important to the maintenance of floodplain forest communities (Romano 2006). Phenology of seed dispersal is critical for native hardwood species requiring adequate growth to survive large flood disturbances (Middleton 1995). This was observed after a large flood in 1993, where approximately 32% of trees ≥ 10 - cm DBH (*i.e*., tree diameter at breast height) and 77% of saplings 2.5 - 9.9 cm DBH were lost to mortality (Yin 1998). Increased sedimentation combined with prolonged inundation most likely contributed to sapling mortality (Walls *et al*. 2005).

Overall, plant diversity in the UMRS is high, although the diversity in hardwood communities has decreased since European settlement (Knutson and Klass 1998; Knutson 2005). Flooding is the primary abiotic disturbance reducing UMRS floodplain forests hardwood species diversity (Middleton 1999), but there are also several biotic disturbances operating to reduce tree diversity including the invasion of RCG, Dutch elm disease and emerald ash borer (*Agrilus planipennis*) (Romano 2010). Hardwood communities of the UMRS are now dominated by a more homogenous silver maple (*Acer saccharinum L.*; SM) community (Romano 2010).

Yin (1998) estimated that 80 % of UMRS floodplain forest was comprised of SM cover. It has been hypothesized that SM can tolerate high water levels and static flooding better than other native floodplain forests hardwood species (Spink and Rogers 1996, Knutson 2005, Cosgriff *et al.* 2007). The majority of the large SM trees on the UMRS floodplain are between 50 and 70 years old, and are reaching senescence; the maximum SM tree life span is typically 125 years (Urich *et al.* 2002). Little regeneration of SM or other native trees in UMRS floodplain forests is occurring (Yin *et al.* 1997), and large areas of floodplain forested are predicted to go through successional change.

One prediction assumes that if current hydrologic and climatic conditions continue, older trees will die-off creating fragmentation in floodplain forest canopy. These gaps will then be invaded by herbaceous vegetation, such as reed canarygrass (RCG) (Urich *et al.* 2002). Reed canarygrass germinates and grows at high rates in conditions typically found in disturbed floodplain forests, such as fragmented canopy cover (Lindig-Cisneros and Zedler 2002), waterlogged soil (Kellog *et al.* 2003), and repeated hydrologic stress (Kercher and Zedler 2004). This is of particular concern for

floodplain forests in the UMRS because when RCG invades a disturbed ecosystem, the abundance of native plants declines, thereby increasing the probability of their extirpation (Schooler et al. 2006).

Monotypic RCG stands have detrimental effects on invaded ecosystems (Lavergne and Molfsky 2004), including floodplain forests (Romano 2010), creating persistent challenges for wildlife communities **(**Barnes 1999; Urich *et al.* 2002; Werner and Zedler 2002). In particular, RCG displaces native plant vegetation through competitive exclusion (Barnes 1999, Werner and Zedler 2002, Green and Galatowitsch 2002). Seedlings grow better without RCG litter present (Thomsen *et al.* 2011). When present, RCG stands out-compete seedlings for resources (*e.g.*, light or nutrients), often leading to a reduction in growth and eventually loss of seedling (Fierke and Kauffman2005). This loss of native hardwood species alters the succession trajectory (Knutson and Klass 1998) and is contributing, for example, to a decline of avian species (Knutson 2005). These are well known effects on floodplain forest ecosystems after RCG invades the UMRS floodplain, but the effects on ecosystem function are still poorly understood.

In particular, little is known about how the invasion of floodplain forests by RCG will affect allochthonous carbon (C) made available to riverine ecosystems. Detritus (*i.e.*, non-living organic C), produced in floodplains provides energy that supports food webs in many riverine ecosystems (Allan and Castillo 2007), including the UMRS. Floodplain forests contribute a significant input of allochthonous C to the UMRS in the form of course particulate organic material (CPOM), like fallen litter, and as dissolved organic carbon (DOC) (Grubaugh and Anderson 1989). A study done by Griffiths and colleagues

(2012) found that RCG litter decomposed at a slower rate than maize litter, because there was a differences in lignin and nitrogen (N) content between the two litter types. Plant litter with high initial lignin content decomposes at slower rates (Gessner and Chauvet 1994), and leaches a lower concentration of labile DOC (McArthur and Richardson 2002). Also, plant litter with higher % lignin:%N ratio decompose at slower a rate than plant litter with low %lignin:%N ratio (Ostrofsky 1997, Griffiths *et al.* 2012).

Lignin in less vulnerable to enzymatic degradation from microbial communities (Schubert 1965). This is demonstrated by the fact that the % of lignin component of plant litter increases over time compared to more soluble components (e.g. polyphenols and carbohydrates) (Suberkropp *et al.* 1976). Aquatic fungi produce hydrolyzing enzymes including pectinases, hemicullulases, and cellulases (Sarkanen and Ludwig 1971, Chamier and Dixon 1982a&b, Chamier *et al.* 1984) that contribute to the decomposition process. Overtime microorganism "condition" plant litter, which makes plant litter more palatable to detritivorous macroinvertebrates (Bärlocher 1985). These macroinvertebrates then influence the decomposition rate of plant litter (Hieber and Gessner 2002). Quality of plant can be reflected in rate of activity of microorganisms (Canhoto and Graça 1996; Royer and Minshall 2001, Stelzer *et al.* 2003, Ardón *et al.*2006) and macroinvertebrates (Canhoto and Graça; 1995, Richardson 2004; Flores *et al.* 2013). Microbial respiration was lower on RCG liter, when the lignin content of RCG was higher than other litter types (Griffiths *et al*. 2012). In this same study they found that macroinvertebrates shredder functional feed guild (FFG) density was lower on RCG litter than other litter types. If RCG continues to invade floodplain forests, then there

could be a potential reduction in the quantity and quality of allochthonous C that support UMRS riverine food webs.

The objective of this study was to determine how the invasion of UMRS floodplain forests ecosystems by RCG alters the quality and quantity of the allochthonous C made available to UMRS riverine food webs. A field experiment and several laboratory experiments were performed to determine this objective. First, in a stream a plant litter decomposition study was done to determine quality of RCG litter compared to native leaf litter. I compared the loss of mass from RCG litter with native leaf litter, along with, measured the biomass and abundance of macroinvertebrates and shredder communities colonizing RCG litter and native leaf litter. Second, in the laboratory the quantity of DOC that was leached from RCG litter and native leaf litter was determined. With and without microbial communities being inhibited, I measured % dry mass (DM) loss from RCG and native litter overtime, as well as determined the concentration of DOC leached from RCG and native leaf litter overtime. Third, in the laboratory the bioavailability of DOC leached from RCG litter was compared with DOC leached from native leaf litter. This was determined by measuring microbial decomposition of DOC leached from RCG litter and native floodplain forest litter.

METHODS

Field Studies

Study Site

Field experiments were conducted in a fourth-order reach of Coon Creek, a cold-water stream located in the Driftless Area of WI, U.S.A (N 43°42'49.27'', W 91°0'3.90''). Coon Creek is a tributary to the Mississippi River and drains a watershed that is mostly comprised of sandstone, dolomite and shale bedrock (Mudrey *et al.* 1982). This site was located at a riffle-pool sequence of the stream reach, approximately 12-13 m wide with and slope 7.23%. The streambed was primarily comprised of cobble, pebble and coarse sand substrates. At base flow the mean depth of study reach was 17.8 ± 7.1 cm (mean ± SD). Historically, vegetation cover in this area would have been characterized as oak savannah (Curtis 1959). Currently, grassland vegetation dominates the land cover along the stream bank where the study was conducted, leading to an open canopy over this portion the stream reach. Table 1 summarizes the physical and chemical characteristics of the stream reach during the study. A HOBO Water Temperature Pro v2 Data Logger (Onset Computer Corporation, Bourne, MA, USA) was secured in stream to record continuous temperature measurements. Stream water and sediment was collected for use in laboratory experiment to determine microbial decomposition of DOC. Stream water was collected mid channel and was not filtered. Stream sediment used as inoculum was collected from the streambed, and consisted of a coarse sand and silt mixture that

passed through a No.10 (2.00 mm) sieve. Stream water and sediment was collected ~24 hours before use and was stored under aerobic conditions at 19.0 C (*c.*).

Table 1. Physical and chemical characteristics of the study reach in Coon Creek, from 11 November, 2013 to 16 May, 2014

Parameter	*N*	Mean	SD	Range
Water temperature (˚C)	*	3.65	4.24	-0.06 - 20.27
Discharge (m^3/s)	7	1.26	0.320	0.91-1.96
Conductivity (mS/cm)	6	0.451	0.0281	0.391 - 0.446
pH	6	7.45	0.340	6.99 -7.90
DO_2 (mg/L)	6	25.40	6.60	12.46 - 32.05
NH_4-N (mg N L^{-1})	5	0.157	0.210	0.046 - 0.389
NO_3^--N + NO_2^--N (mg N L^{-1})	5	1.57	0.189	1.19 - 1.78
SRP (mg P L^{-1})	5	> 0.01	0.0032	0.01 - $4.89x10^{-3}$

SRP = soluble reactive phosphorus, *N* = number of measurements.
*Continuous record from 11 November, 2013 to 16, May 2014

Field Methods

Senescing RCG litter was collected on October 26, 2013 from a streambank of La Crosse River, WI (N 43°59'20.22'', W 90°44'23.16''). Recently senescing river birch (*Betula nigra L*; RB), SM, and swamp white oak (*Quercus bicolor Willd.*; SWO) litter were picked from a tree or off the ground from a floodplain forested area in Goose Island Conservation Area, WI (N 43°43'25.72'', W 91°12'52.62'') from October 23-25, 2013. The plant litter was collected before exposure of a major precipitation event, air dried in the laboratory and stored under a polyurethane sheet. In the laboratory, a portion RCG litter and SWO, SM and RB litter was ground using an 8000D Mixer/Mill® (Spex Sample Prep, Metuchen, NJ, U.S.A.), and carbon to nitrogen atomic ratio (C:N ratio) of ground plant litter was determined using a ECS 4010 CHNSO Analyzer elemental combustion system (Costech Analytical Technologies, Inc., Velencia, CA, USA).

Another portion of dried plant litter from RCG, and native RB, SM, and SWO litter were separately homogenized to test in-stream decomposition rates. Plant litter, 5.47 ± 0.03 g (mean each species ± SD), was placed in plastic hardware mesh packs (152.4 x 127 mm; 12.7 mm mesh size) and screen mesh packs (152.4 x 127 mm; 1 mm mesh size), correspondingly. Two different mesh sizes were used to either allow for macroinvertebrate colonization of plant litter or not. Hardware mesh packs were used to allow macroinvertebrate colonization and fiberglass screen mesh packs were used to inhibit macroinvertebrates and insure plant litter would not completely be lost to fragmentation. There were a total of 216 litter packs assembled (i.e., 108 hardware mesh and 108 fiberglass mesh packs). One hardware and screen mesh pack were secured to a brick with zip-ties.

The bricks were then randomly placed in stream and secured to stream bottom with rebar. Three bricks with two litter packs, hardware and screen mesh pack filled with native litter and RCG litter were randomly chosen, and retrieved on day 0. Six litter packs, three hardware and three screen mesh packs filled with each type of native and RCG litter were returned to the laboratory. Plant litter was removed from mesh packs, rinsed, then dried to a constant weight, and weighed to determine "handling loss," (*i.e.*, how much plant material was lost from litter packs while transporting to stream) (Benfield 2007). After accounting for "handling loss" the initial dry mass of RCG, RB, SM and SWO litter in hardware mesh and screen mesh packs were determined (Table 2). The remaining bricks were randomly retrieved after submersion on day 2, 4, 6, 14, 24, 48, 124 and 188. Three bricks with two litter packs, hardware and screen mesh, from each plant species were retrieved on those days. Every litter pack was placed in separate Ziploc® bags and placed on ice or refrigerated until processing occurred in the laboratory.

Table 2. Initial dry mass (g) of Reed canarygrass (RCG) litter, and Swamp white oak (SWO), Silver maple (SM) and River birch (RB) leaves in hardware mesh and screen mesh litter packs on day 0. Litter packs placed in Coon Creek, Coon Valley, Wisconsin

	Hardware mesh initial dry mass (g)		Screen mesh initial dry mass (g)	
Plant species	Mean	SD	Mean	SD
SM	4.69	0.09	4.90	0.03
SWO	4.80	0.04	4.96	0.06
RB	4.86	0.18	4.98	0.05
RCG	4.80	0.04	4.84	0.04

Litter Mass Loss

In the laboratory plant litter from each species was carefully removed from the mesh packs and gently rinsed with water over a sieve to remove debris and invertebrates. The invertebrates that were gathered on sieve from washing off plant litter were collected and stored in 70 % ethanol. Rinsed plant litter from hardware and screen mesh litter packs were placed in Ziploc® bags and dried in an oven (60° C) to a constant dry weight. Dried plant litter was ground as described above, and one 250 mg subsample was used to a determine % ash free dry mass (% AFDM) according to Benfield (2007).

Macroinvertebrates

Preserved invertebrates were sorted, identified to the lowest possible taxon and counted. Macroinvertebrates taxa were assigned to functional feeding groups following Merrit and Cummins (1995) and Thorp and Corvich (2010). Macroinvertebrates were then dried in an oven at 70° C to a constant weight to determine biomass.

Laboratory Experiments

Quantity of DOC Leached

In the lab dried plant litter from each species was separately added to a Ziploc® bag and broken apart into smaller pieces, and 0.18 ± 0.01 g (mean ± SD) of RCG, SM, RB, SWO plant litter was placed into a sterilized fiberglass screen mesh pack (24 X 61 mm sides; 1-mm mesh size). Forty-six total screen mesh packs were constructed, 10 mesh packs were made from each plant species and six control mesh packs filled with acid washed glass marbles. Well water was collected ~24 hours before use, and well water was used because the microbial communities present would be similar to study site. Five mesh packs from each plant species and three control packs were placed in separate

125-mL Erlenmeyer flask, and flasks were filled with 125.0 mL of unfiltered well water and 0.125 mL of 3000 mM sodium azide (NaN_3). Sodium azide was added to flasks to inhibit respiration by microbial communities found in well water. The other five mesh packs from each plant species and three control packs were placed in separate 125-mL Erlenmeyer flasks and filled with 125.125 mL of unfiltered well water. To maintain aerobic conditions the flasks contents were agitated at 175 rpm on an Excella E10 Platform shaker table (New Brunswick Scientific, Enfield, CT, U.S.A.). Flasks were kept in the dark, and at room temperature 24.50 ± 3.30 (ºC) (mean ± SD). On days 0, 2, 4, 6, 14 and 21, a 6-mL subsample was removed from flask, filtered through a 0.45 µm Whatman™ Nylon Membrane Filters (GE Healthcare Life Science, Pittsburg, PA, U.S.A) and analyzed for DOC concentration with Shimadzu TOC-Vcsh (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.). On day 21 native litter and RCG litter was dried to constant weight at 70°C and weighed.

Microbial Decomposition of DOC

Leachates from each plant species was exuded from 3.0 g of homogenized native leaf litter and RCG litter for 24 hours in reverse osmosis-treated (RO) water at 19.0 C (*c.*). Particulate matter and bacteria were removed by passing leachate through sieves and filters, until the leachate finally passed through a 0.22 µm Millipore® Stericup. Leachates were stored in Stericup at 4 C. Dissolved organic carbon concentration of filtered leachates were analyzed as described before.

Dissolved organic carbon quality for each plant species was determined by quantifying the microbial decomposition of DOC in leachates. An aliquot of each leachate was added to a 250-mL Erlenmeyer flask filled with unfiltered stream water and

1.0 g stream sediment inoculum collected from Coon Creek streambed. The initial liquid volume in each flask was 200 mL with a final carbon amendment concentration of 43.3 ± 3.2 (mean ± S.D.) mg C L^{-1}. Flasks were loosely covered with aluminum foil and stored in a dark at room temperature 18.9 ± 0.12 C (mean ± S.E.). On days 0, 2, 4, 6, 14, and 24, a 20 mL subsample was removed from the flasks and filtered through 0.7 µm glass fiber filter (Pall Corporation, Type A/E). Subsamples were analyzed for DOC concentration as described above.

Statistical Methods

According to Benfield (2007), the breakdown coefficient (*k*) for RCG litter, and native litter was determined by taking the regression of natural log (Ln) of the mean % AFDM remaining from plant litter (*y-axis)* over days of exposure (*x*–axis). Using the AFDM of the "handling loss" native litter and RCG litter packs as 100% AFDM remaining on day 0. The negative slope of regression line equals *k*. The labile portion and overall decomposition of plant litter was calculated using the exponential decay model

$$W_t = W_i e^{-kt}$$

(Webster and Benfield 1986). Data from litter mass loss was fit with the simple exponential model, where W_t is the %AFDM remaining at time *t*, W_i is the estimated initial %AFDM and *k* breakdown coefficient. This breakdown model assumes mass loss from litter is directly related to litter mass present. Overall decomposition and decomposition of the labile portion of leachate were calculated using the exponential decay model

$$C_t = C_o e^{-k}$$

(Strauss and Lamberti 2002); where C_t is the DOC concentration of leachate at the time *t*, C_o is the initial DOC concentration of leachate and *k* the breakdown coefficient. One-way analysis of variance (ANOVA) with native litter and RCG litter as the single factor was used to test the differences in the natural log (ln) of decomposition rates of native litter and RCG litter, maximum abundance and biomass of macroinvertebrate and shredders on native litter and RCG litter, % DM from reaming from native leaf and RCG litter with and without microbial communities being inhibited, and ln of decomposition rates of DOC leachates from native leaf and RCG litter. One-way ANOVA with functional feeding groups (FFG) as single factor was used to determine the difference of biomass and abundance on native litter and RCG litter. Tukey's multiple comparison test was used to determine if any significant differences occurred between pairs of native litter and RCG litter, and pairs of FFG. Dissolved organic carbon concentrations leached from RCG litter and native litter were not normally distributed, so a non-parametric test (Kuskal-Wallis ANOVA) was used. A repeated measures design based on a non-parametric pairwise comparison procedure was used to compare mean DOC concentration leached from RCG litter and native litter with and without inhibition of heterotrophic microbes. A Spearman rank correlations on a species-specific basis was used to compare both total decomposition of DOC leachate and decomposition of the labile portion of DOC Leachate with processing rates from RCG litter and native litter. All statistical analyses were performed using IBM SPSS Statistics for Windows, Versions 22.0 (Armonk, NY: IBM Corp., 2013), and differences were considered significant when the P-value was less than 0.05.

RESULTS

Litter Mass Loss

Breakdown of the RCG litter and native litter occurred rapidly. Within three weeks <76%, <87%, and <97% AFDM remained from SM litter, RB and SWO litter, and RCG litter, respectively (Figure 1). On day 24 there was an increase in %AFDM among all the liter types. After day 24 %AFDM continue to decrease until final time interval. The ninety-five % confidence intervals associated with predicted breakdown coefficients, after 188 days, indicate that there was a significant differences in breakdown rates between SM litter and the other litter types (Table 3). Silver maple litter lost 61% of initial AFDM, and RB litter, SWO litter, and RCG litter lost, 30%, 39% and 31% of initial AFDM, respectively. After 188 days breakdown rate for SM litter was significantly greater ($F_{(3,14)} = 13.361$, $P < 0.001$) than other native litter and RCG litter. Silver maple litter had the greatest C:N ratio and RB leaf litter had the lowest (Table 3), but there was no significant difference in the C:N ratio of native leaf litter and RCG litt

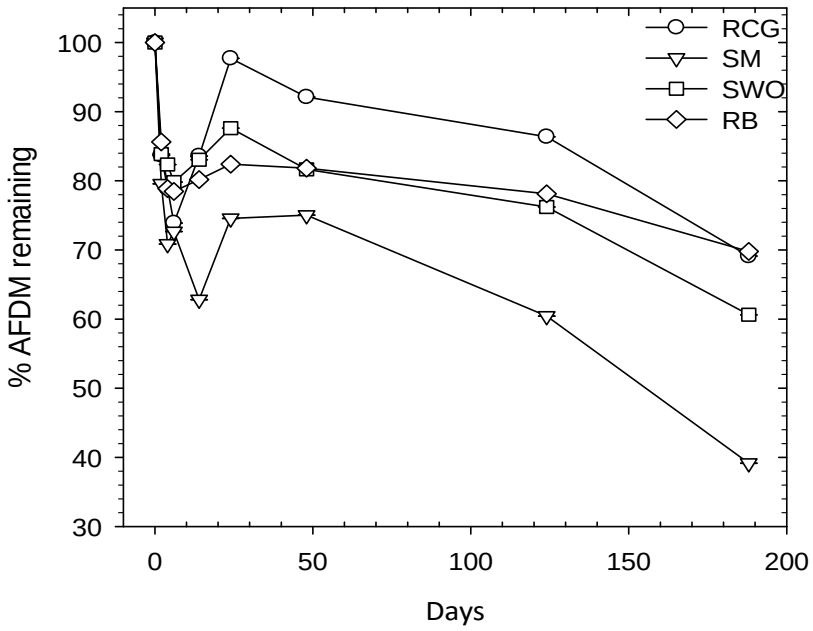

Figure 1. % ash-free dry mass (AFDM) remaining from Reed canarygrass (RCG) litter packs, and Silver maple (SM), Swamp white oak (SWO) and River birch (RB) leaf packs in a fourth-order reach of Coon Creek, WI. Error bars contained within shapes indicate standard error

Table 3. Summary of mean breakdown coefficients (*k*), and mean atomic carbon to nitrogen ratio (C:N ratio) (n=3) of Reed canarygrass (RCG) litter, and Swamp white oak (SWO), Silver maple (SM) and River birch (RB) leaves that decomposed in Coon Creek, Coon Valley, Wisconsin. Breakdown coefficients were estimated by nonlinear regression equation $m_o/m_t=e^{-kt}$; where *k* equals negative slope

Plant species	$-k \pm 95\%$ CL (d^{-1})	r^2	*N*	C:N ratio	SE
RCG	$9.450 \times 10^{-4} \pm 5.320 \times 10^{-4}$	0.199	53	42.3	1.84
SWO	$1.679 \times 10^{-3} \pm 3.890 \times 10^{-4}$	0.595	53	35.8	0.08
SM	$3.049 \times 10^{-3} \pm 9.250 \times 10^{-4}$	0.480	50	60.3	3.00
RB	$9.600 \times 10^{-4} \pm 4.400 \times 10^{-4}$	0.274	53	32.2	2.14

CL = 95% confidence limit; *N* = number of plant litter packs

Macroinvertebrates

Macroinvertebrates colonized native litter and RCG litter packs rapidly (Figure 2a,b). On week 7 the greatest mean biomass of macroinvertebrates was observed on RCG, with a mean macroinvertebrate biomass of 80 mg per 3.43 ± 0.10 g (mean ± S.E.) of RCG litter (Figure 2b). On week 7 and 26 the greatest mean abundance of macroinvertebrates observed on RCG litter, 10 animals per 3.43 ± 0.10 g (mean ± S.E.) of RCG litter (Figure 2a) On RCG litter the greatest biomass and abundance only corresponded on week 7 (Figure 2a,b). Different trends where observed on native litter.

During week 2 the greatest mean macroinvertebrate biomass and abundance were observed on SM leaf litter, 79mg per 4.80 ± 0.24 g (mean ± S.E.) of SM litter and 18 animals per 4.80 ± 0.24 g (mean ± S.E.) of SM litter, respectively (Figure 2 a,b). On week 17, the maximum abundance of macroinvertebrates was observed on other native litter, with an mean 13 animals per 4.26 ± 0.07 g (mean ± S.E.), and 9.5 animals per 3.17 ± 0.14 g (mean ± S.E.) of SWO and RB litter, respectively (Figure 2a). At week 17, maximum abundance of macroinvertebrates coincided with the peak for biomass of macroinvertebrates colonizing SWO and RB litter, 48 mg per 4.26 ± 0.07 g (mean ± S.E.) and 56 mg per 3.17 ± 0.14 g (mean ± S.E.) SWO and RB litter, respectively (Figure 2a,b). Maximum abundance and biomass of macroinvertebrates communities, including shredders found on native litter were not significantly different than macroinvertebrate communities, including shredders abundance and biomass found on RCG litter for the same time points ($P > 0.05$). Shredders colonized native leaf types faster than RCG litter (Figure 2 c,d). During the second week filterer-collectors were observed, and then on the third week shredders were observed on RCG litter. Whereas, by the second week both

filterer-collectors and shredders were observed on the native leaf types. Dominant shredders included *Prostia* sp. (Plecoptera), *and Pteronarcys* sp. (Plecoptera), *Tipula* sp. (Diptera), and *Gammarus* sp. (Amphipoda). Filterer-collector taxa were dominated by the *Hydrosyche* sp. (Trichoptera), *Simulium* sp.(Diptera). Grazers, predators and collector-gatherer FFG contributed to a small portion in the abundance and biomass of total macroinvertebrates found on native litter and RCG litter.

Shredders and filterer-collectors were the dominant functional feeding groups (FFG) encountered on both native litter and RCG litter packs (Figure 3a). On RCG litter, filterer-collectors accounted for a higher total macroinvertebrate abundances than other FFGs, including of shredders (Figure 3a). The abundance of filterer-collectors FFG were not significantly different than other FFG found on RCG litter. Among native litter types shredders accounted for the greatest number of macroinvertebrates (Figure 3b). Shredder abundance on RB litter was significantly greater than abundance of a other FFG ($F_{(2,34)}$=3.207, P = 0.053). Shredder abundances on SWO and SM litter were not significantly greater than all other FFGs. Shredders accounted for the highest total biomass of macroinvertebrates on native leaf and RCG litter packs (Figure 3b). The biomass of shredders on RB litter was significantly greater than all other FFGs ($F_{(2,33)}$=7.670, P > 0.016). Biomass of shredders on SM and SWO litter, and RCG litter was not significantly different than other FFGs.

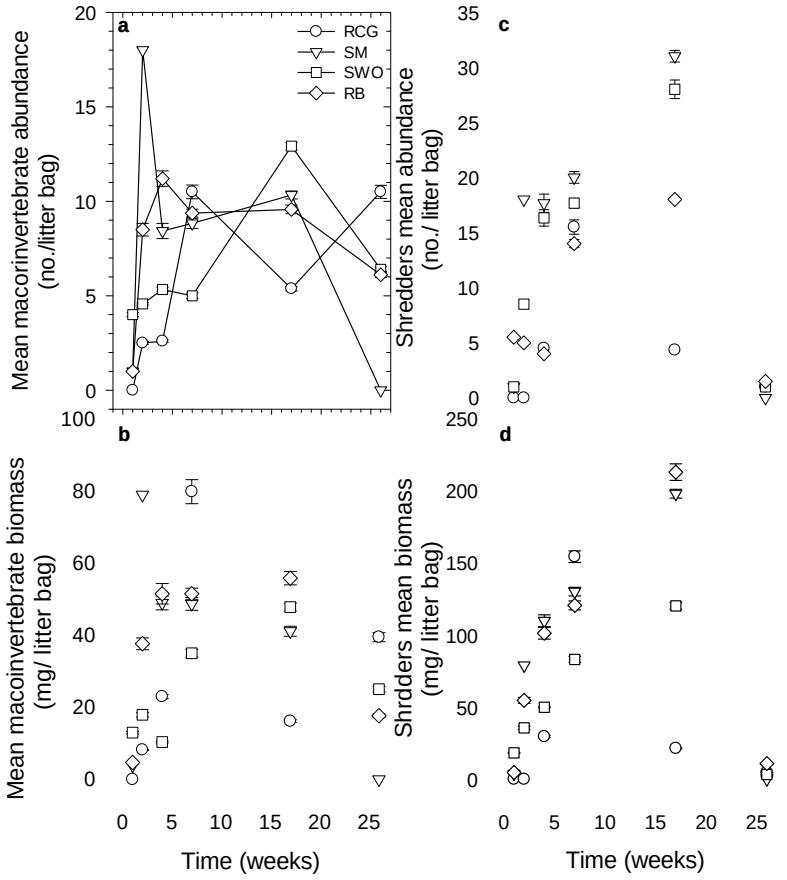

Figure 1. Mean (a) abundance and (b) biomass of total macroinvertebrates, and average (c) abundance and (d) biomass of shredders found on Reed Canarygrass (RCG) litter packs, and Silver Maple (SM), Swamp White Oak (SWO), and River Birch (RB) leaf litter packs in a fourth-order reach of Coon Creek, WI. Error bars indicate 95% confidence intervals

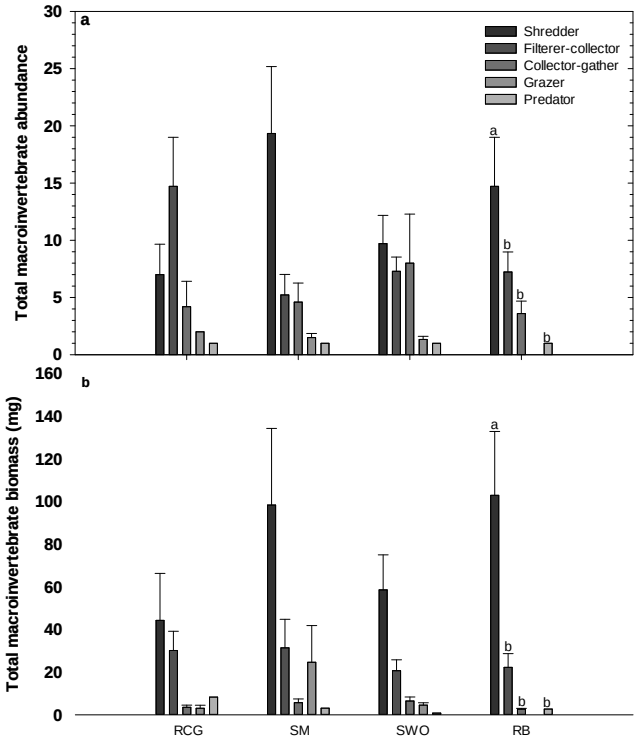
a
Shredder
Filterer-collector
Collector-gather
Grazer
Predator
Total macroinvertebrate abundance
30
25
20
15
10
5
0
a
b
b
b
b
Total macroinvertebrate biomass (mg)
160
140
120
100
80
60
40
20
0
a
b
b
b
RCG
SM
SWO
RB

Figure 3. Abundance (a), and biomass (b) of macroinvertebrate functional feeding groups on reed Canarygrass (RCG) litter, and silver maple (SM), swamp white oak (SWO) and river birch (RB) leaf litter. Error bars indicate standard error. Letters denote significant differences in macroinvertebrate functional feeding groups per litter type during sampling period (One-way ANOVA, Tukey's HSD, $P < 0.05$). ($F_{(2,34)}=3.207$, $P = 0.053$) (a) ($F_{(2,33)}=7.670$, $P > 0.016$) (b)

Quantity of DOC Leached

Percent Dry Mass Loss With and Without Microbial Activity

After 21 days the % DM remaining from RCG litter was less in flasks without inhibition of microbes from sodium azide than with inhibition of microbes from sodium azide (NaN_3) (Figure 4). When microbes were not inhibited, the % DM remaining from RCG litter and SM litter was significantly less than % DM remaining from RB and SWO litter ($F_{(3,16)}= 23.681$, $P > 0.001$), respectively (Figure 4). After 21 days, native litter types had lost a greater % DM in flasks when microbes were inhibited than without inhibition (Figure 4). The % of DM remaining from SM litter was significantly less than % of DM remaining for SWO and RB litter, and RCG litter ($F_{(3,16)}= 85.496$, $P > 0.001$). The % of DM remaining from RB litter was similar to RCG and SWO litter, but the % of DM remaining from RCG litter was not similar to SWO litter.

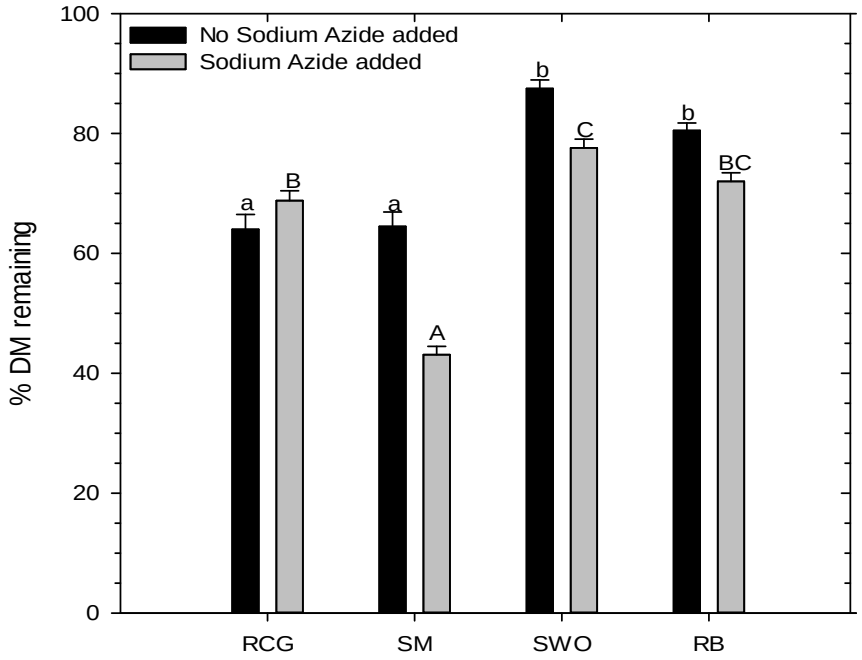

Figure 4.The % of plant litter dry mass (DM) remaining from Reed Canarygrass (RCG) litter, and Silver Maple (SM) Swamp White Oak (SWO), and River Birch (RB) leaf litter. Black bars indicate treatment without sodium azide (NaN_3) added to inhibit microbes and gray bars indicate treatment with sodium azide (NaN_3) added to inhibit microbes. Error bars indicate standard error. Lower case letters (No NaN_3 added) and upper case letters (NaN_3 added). Letters denote significant differences in % DM remaining among litter type during sampling period (One-way ANOVA, Tukey's HSD, $P < 0.05$). ($F_{(3,16)}= 23.681$, $P > 0.001$) (No NaN_3 added), ($F_{(3,16)}= 85.496$, $P > 0.001$) (NaN_3 added)

DOC Concentration Overtime With Microbial Activity

When microbes were not inhibited the DOC (mg/L) leached over time from RCG litter and among native litter types declined in a linear pattern (Figure 5a). On day 2 the highest concentration of DOC was leached from both native litter and RCG litter (Figure 5a). The greatest concentration of DOC was leached from SM litter and the lowest concentration of DOC was leached from RCG litter. On days 2, 4, 6 and 21, when microbes were not inhibited, the mean DOC leached from RCG litter was significantly less than the mean DOC concentration leached from SM litter ($P < 0.05$). On day 14 the mean concentration of DOC leached from SWO litter was significantly less than mean concentration of DOC leached from SM litter ($P = 0.009$). No other significance of DOC (mg/L) leached from native litter and RCG litter occurred.

DOC Concentration Overtime Without Microbial Activity

When microbes were inhibited the DOC (mg/L) leached from RCG litter and native litter both increased from day 0 in a linear pattern (Figure 5b). On day 21 the highest concentration of DOC was leached from both native litter and RCG litter (Figure 5b). On day 21 the highest concentration of DOC was leached from SM litter and the lowest concentration was leached from SWO litter. When microbes were inhibited, on days 2, 4, and 6, the mean concentration of DOC leached from SWO litter was significantly lower than mean DOC leached from SM litter ($P < 0.05$). No significant differences occurred on days 14 or 21 ($P \geq 0.079$). No other differences in the concentration of DOC leached from native litter or RCG litter were observed.

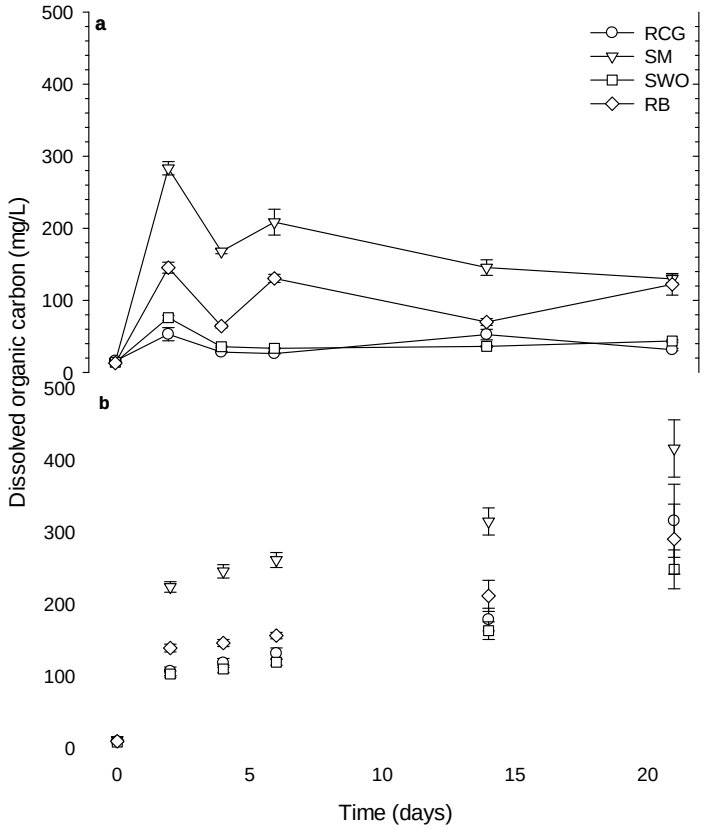

Figure 5. Concentration of dissolved organic carbon (DOC) leached from Reed Canarygrass (RCG) litter, and Silver Maple (SM), Swamp White Oak (SWO) and River Birch (RB) leaf litter. (a) Without sodium azide (NaN_3) added to inhibit microbes; (b) with sodium azide (NaN_3) added to inhibit microbes. Error bars indicate standard error

Microbial Decomposition of DOC

Litter from SM and RB leached highly colored leachates and, SWO litter and RCG litter produce lightly colored leachates. These results were observed and not measured. Color of DOC leachate from native litter types and RCG litter was similar to the color of water observed in the experiment to determine the quantity of DOC leached from native litter and RCG litter.

Dissolved organic carbon decomposition in leachates from native litter and RCG litter followed an exponential or logarithmic decay pattern (Figure 6). The decomposition of DOC in leachates declined rapidly during the first 6 days, and then remained stable through day 24. On average, 83% of the DOC in each leachate was metabolized from all litter types. During the first 6 days decomposition rates of DOC in the different leachates ranged from 0.22 to 0.38 (mg C L^{-1} $days^{-1}$). The greatest decomposition rate during the first 6 days was observed for RCG litter and the lowest rate were seen from SM and SWO litter, 0.38 mg C $L^{-1}days^{-1}$, and 0.22 and 0.23 mg C $L^{-1}days^{-1}$, respectively (Figure 7a). Through day 6 the differences in decomposition rate of DOC among leachates from native litter and RCG litter were significantly different ($F_{(3,16)} = 473.10$, $P < 0.0001$). Through day 24 the overall microbial decomposition rates of different DOC leachates ranged from 0.056 to 0.14 (mg C L^{-1} $days^{-1}$) (Figure 7b). The greatest rate of decomposition of DOC in leachates was observed from RCG litter and the lowest rate from SM and SWO litter, 0.14 (mg C L^{-1} $days^{-1}$), and 0.056 and 0.070 (mg C L^{-1} $days^{-1}$), respectively. There was significant differences in overall decomposition of DOC leachates from native litter and RCG litter ($F_{(3,16)} = 705.10$, $P < 0.0001$). Spearman rank correlations on species-specific processing rates did not significantly correlate with

the overall (r = -0.80, P = 0.20, n = 4) or labile (r = -0.40, P = 0.60, n = 4) DOC decomposition rates from DOC leached from native litter or RCG litter.

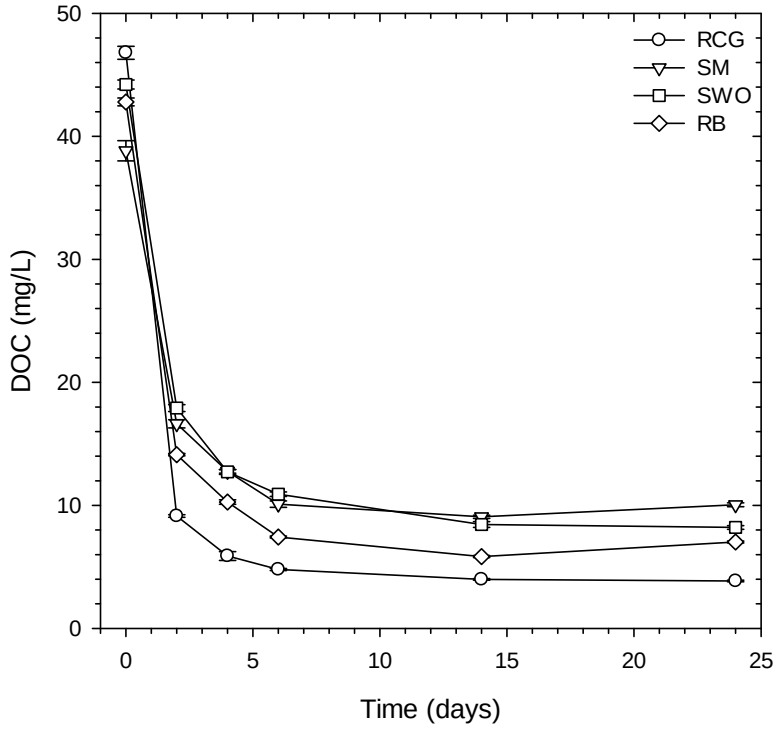

Figure 6. Dissolved organic carbon (DOC) concentration over time from Reed Canarygrass (RCG) litter, and Silver Maple (SM), Swamp White Oak (SWO), and River Birch (RB) leaf leachates incubated in water and sediment inoculum from Coon Creek, WI. Incubations (n = 5 per leachate) were conducted in darkness at room temperature (c. 19.0 ˚C). Error bars indicate standard error

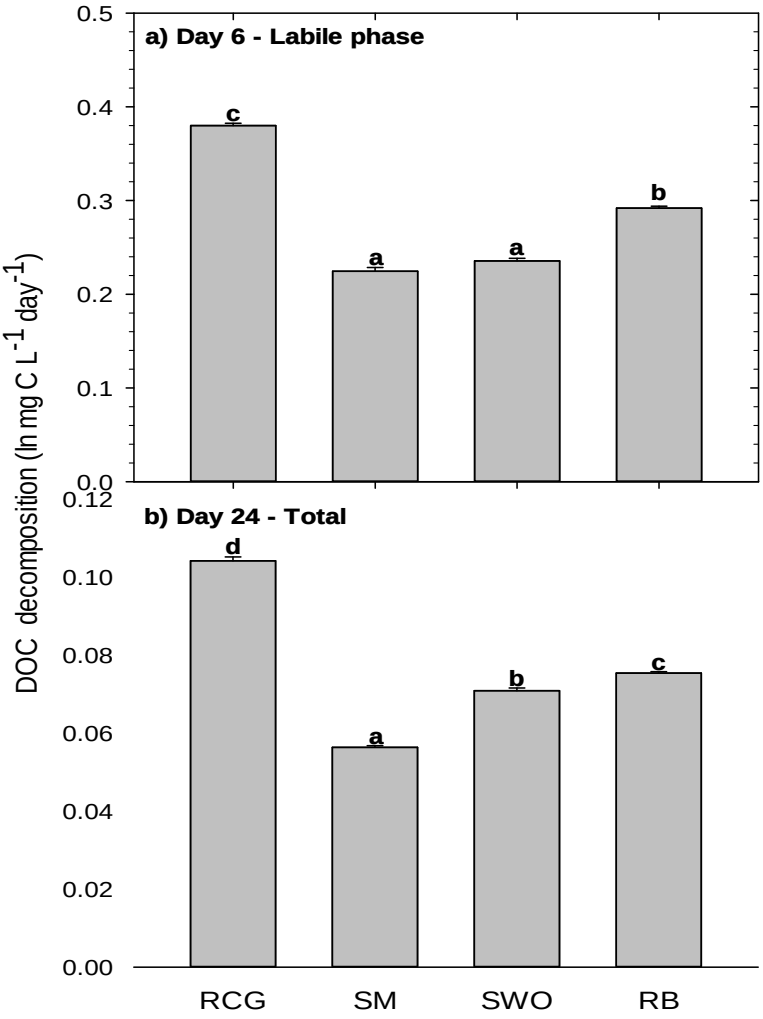

Figure 7. Decomposition of dissolved organic matter (DOC) in Reed Canarygrass (RCG) litter leachate, and Silver Maple (SM), Swamp White Oak (SWO) and River Birch (RB) leaf leachates. (a) Decomposition rate of labile portion of DOC leachate; (b) overall decomposition of DOC leachate.
Error bars indicate standard error**.** Letters denote significant differences in DOC decomposition among litter type during sampling period (One-way ANOVA, Tukey's HSD, $P < 0.05$). ($F_{(3,16)} = 473.10$, $P < 0.0001$) (a) ($F_{(3,16)} = 705.10$, $P < 0.0001$) (b)

DISCUSSION

Leaf Litter Breakdown and Macroinvertebrate Colonization

The rapid breakdown rates observed for RCG litter and native tree litter in Coon Creek were similar to other studies conducted in temperate stream ecosystems (Griffiths *et al.* 2012; Bruder *et al.* 2014). However; SM litter was processed at a faster rate than RCG, RB and SWO litter. According to the classification scheme from Petersen and Cummins (1974; fast: $k > 0.01$ / d, medium: $0.005 < k < 0.01$ / d, slow: $k < 0.005$ / d) for decomposing plant litter in aquatic ecosystems. Silver maple and SWO litter decomposed at medium rate and RB and RCG litter decomposed at slow rate. Intrinsic factors related to the chemical and structural composition of the plant litter influence decomposition rates of plant litter (Kaushik and Hynes 1971, Ostrofsky 1997). So, differences in decomposition rate between SM litter, and RCG litter could be due to differences in the chemical and structural composition between the litter types.

Reed Canarygrass litter decomposed slower than maize (*Zea mays*) litter and cutgrass (*Leersia oryzoides*) litter (Griffiths *et al.*2012). Reed canarygrass litter had a higher % of initial lignin, but lower % N than maize. Difference of decomposition rates were due to differences in % lignin and % N content. This relationship between % initial lignin and % N was also observed in deciduous leaves (Ostrofsky 1997). But, RCG litter % initial lignin content is lower than SM litter (Ostrofsky 1997; Griffiths *et al.*2012). For example, the initial lignin content for SM litter was 21.49% (Ostrofsky 1997) and initial

lignin content for RCG litter was 9.1% (Griffiths *et al.* 2012). A variety methods used totest the lignin concentration support the fact that SM litter had a higher concentration of lignin than RCG litter (Goff *et al.* 2011). So differences in lignincontent between RCG and SM litter cannot help explain why this grass is processed at a slower rate than SM litter.

The difference in decomposition rates could be explained by abundance of essential nutrient elements. Typically plant litter with higher C:N ratio decompose at slower rates (Ostrofsky 1997; Griffiths 2012). My measured C:N ratio for native and RCG litter would suggest that SM litter would decompose at a faster rate than RCG litter. Which didn't occur in this experiment, as RCG litter decomposed at a slower rate than SM. In other experiments the C:N ratio for RCG litter and SM litter our different than the C:N ratio reported in this experiment**.** According to Ostrofsky (1997), SM litter had a C:N ratio of 30.4, which was lower than C:N ratio (60.3) of SM litter reported in this experiment. Reed canarygrass litter in this experiment had a C:N ratio of 42.3, which is higher than C:N ratio for SM litter as reported by Ostrofsky (1997). But, RCG litter C:N ratio is different for European and native genotypes (*e.g.*, C:N ratio ranged from 8.8-19.3) (Kaproth *et al.* 2013). These resuslts suggests that RCG litter would have a lower C:N ratio than SM litter as reported in this study or by Ostrofsky (1997). Obviously, the measured C:N ratio for RCG and SM litter vary throughout the year and geographical region. So, differences in the C:N ratio content of RCG and SM litter cannot be used to predict the differences in decomposition rates between the two litter types.

Total initial fiber content of plant litter is another intrinsic factor that can be used to predict the decomposition rate of the plant (Webster and Benefield 1986). The total

fiber content (*i.e.*, hemicellulose, cellulose and lignin) correlate with breakdown rates of aquatic macrophytes better than individual fiber components (Godshalk and Wetzel 1978). In a two year experiment, the initial acid detergent fiber (ADF) % was a better predictor of decomposition of plant litter in an aquatic system than lignin (Sariyildiz and Anderson 2003). So, the difference in fiber content of native and RCG litter could explain why RCG litter decomposes at a slower rate than SM litter. But, there is little data to compare the fiber content of RCG liter with native litter.

Some leaf litter has a lower fiber content than grass litter. *Millettia ferruginea* is a hardwood tree that is native to Ethiopia, and leaf litter from this tree had a lower fiber content than pasture grasses (Alemu *et al.* 2014). Also, grasses typically have higher total fiber concentrations than forbs and shrubs (Holechek 1984). The fiber content of RCG litter is similar to other grasses (Casler and Jung 2006). So, RCG litter could have a higher fiber content than native litter, which would explain why RCG litter decomposes at a slower rate than SM litter. The aromatic, polymeric and cross-linked structures in compounds of fiber constituents are not easily broken down during decomposition process.

Some individual fiber constituents (*i.e.*, lignin, cellulose or hemicellulose) affect the decomposition of other (Cowling and Brown 1969). Lignin is often the limiting factor in the enzymatic degradation of cellulose, other carbohydrates and plant proteins (Alexander 1977). Cellulose and hemicellulose, polysaccharides in plant cell walls, are physically bound to lignin, forming lignocellulose, which makes cellulose and hemicellulose less susceptible to enzymatic breakdown (Sarkanen and Ludwig 1971, Kirk *et al.* 1977, Crawford 1981). If RCG litter is higher in fiber content than native

litter, then this could explain why RCG litter decomposed at a slow rate compared to SM litter. However, there are factors other than intrinsic characteristics of plant litter that influence the decomposition of plant litter.

Decomposition rates are influenced by several extrinsic factors (*e.g.*, water temperature, fragmentation, photodegradation, and microbial or macroinvertebrate detritivores) (Petersen and Cummins 1974, Webster and Benfield 1986; Allan and Castillo 2007). In aquatic systems variation of plant litter processing rates is strongly influenced by microbial selection of plant litter as substrate and the level of macroinvertebrate colonization (Hieber and Gessner 2002, Allan and Castillo 2007). Shredders select coarse particulate organic matter covered by biofilms with high microbial biomass and cell counts (Kaushik and Hynes 1971); these biofilms contributing to macroinvertebrate processing of plant litter (Hieber and Gessner 2002). Shredders breakdown plant by fragmentation while they consume nutrition rich microbial communities on plant litter (Allen and Castillo 2007).

In this study, total macroinvertebrate and shredders numbers and biomass observed on RCG litter was no different from what was observed on native litter. Thus, RCG litter may be similar in substrate quality as native leaf litter, and macroinvertebrate activity on SM and SWO litter didn't increase decomposition compared to RCG litter. However, my results suggest RCG litter takes longer to condition than native litter. Fungal biomass and sporulation rates were seen earlier on leaf litter with lower tannin and higher N content (Mathuriau and Chauvet 2002). In this same study macroinvertebrate colonization occurred on leaf litter with higher fungal activity. Shredders select more conditioned leaf litter over less conditioned leaf litter **(**Kaushik and

Hynes 1971; Golladay *et al.* 1983; Graca *et al.* 1993), and begin feeding when microbial communities have altered the structural and chemical components of plant litter (Cummins *et al.* 1989). Macroinvertebrate shredders colonized native litter one week before RCG litter. This suggests that RCG litter took one week longer to be conditioned than native litter, evident by shredder activity. But, by week 3 shredders were seen on all four litter types. So, RCG litter became as palatable for shredders as native litter.

This was also observed in the initial decrease in %AFDM in all litter types, and then an increase % AFDM after 14 days. But, by Day 21 % AFDM began to decrease again. I used %AFDM to measure mass in litter processing experiment. This dry mass measuring method measures the organic fraction of sample (Benfield 2007). The biofilm from microbial communities conditioning plant litter or organic matter (OM) in streambank soils could have added to the % AFDM seen after day 14. Soils in Wisconsin typically contain 1.5 -4.0 % organic matter (OM), and can be as high as 20% (Schulte *et al.* 2005). The amount of OM in soil depends on location, plant cover type, hydrology and anthropogenic factors. Soil OM probably didn't contribute to this extreme increase of %AFDM, because sedimentation was rinsed off of plant litter, and didn't continue to increase throughout the experiment.

The increase in %AFDM could have occurred as the biofilm matrix from microbial communities increased on plant litter. During a laboratory experiment, microbial respiration increased (15-20 days) on 4 types deciduous leaf litter (Golladay *et al.* 1983). Similar results were seen in a black water stream in Germany. In this experiment fungal and bacterial biomass on two different leaf types increased rapidly with 4 week and then leveled off (Hieber and Gessner 2002). It has been predicted that

2-10% of weight of conditioned leaves is from microbial cells (Bärlocher and Kendrick 1981; Rosset *et al.* 1982), and with nutrient-enrichment 10.8-17.4% of detrital mass was from microbial cells (Gulis and Suberkropp 2003). So, the increase in % AFDM after day 14 could further indicate that native and RCG litter was being conditioned by microbial communities, thus contributing to an increase in %AFDM. This increase in % AFDM could indicate the beginning of condition phase, because shredder biomass and abundance increased after 24 days on both native and RCG litter.

However, RCG litter may have a shorter conditioning time than native litter, as was evident by shredder activity on native and RCG litter types. When plant detritus enters an aquatic ecosystem there is a definite peak in microbial activity on litter and any additional conditioning doesn't make litter more palatable for shredder macroinvertebrates (Bälorcher and Kendrick 1981, Bälorcher 1985). Thus there is a pre-conditioned, fully conditioned and post conditioned phase on plant litter. When there is a peak in microbial activity on plant litter, this plant litter is "fully conditioned", and when plant litter is fully conditioned shredder biomass and abundance is highest (Bälorcher and Kendrick 1981, Hieber and Gessner 2002). There was a peak in the biomass and abundance of shredders by week 7 on RCG litter, and by week 17 on native litter. This suggests native litter remains palatable for shredders longer than RCG litter.

Typically, plant litter that has a slow decomposition rate (*i.e.*, RCG litter) remains conditioned longer than plant litter that decompose at a faster rate (Cummins *et al.* 1989), like SM litter. This didn't not occur in this experiment. Grass stems have greater lignin percentage than grass leaves (Mann *et al.* 2009). Plant litter with high lignin content decomposes at a slower rate (Melillo *et al.* 1982, Ostrofsky 1997). The leaves on RCG

could have been consumed quickly and stems could have been decomposed more slowly. Which, could be why there was a peak in shredder biomass and abundance by week 7. When leaves were consumed, the stems could have made RCG litter tougher to condition, and less palatable for shredders. Although, SM litter has a higher initial lignin content than RCG, the stems of RCG may have a high fiber content that may inhibit microbial conditioning. Which, could explain why there was decline in shredder biomass and abundance after week seven on RCG litter.

Differences in the Leaching and Decomposition of DOC Released by Native Leaf & RCG Litter

Native litter and RCG litter showed species-specific leaching rates for dissolved organic carbon (DOC). When heterotrophic microbes were not experimentally inhibited, only SM litter released a greater concentration of DOC than RCG litter. Species-specific leaching rates have been observed in others studies (Kaushik and Hynes 1971; Sun *et al.* 1997; McArthur and Richardson 2002), and have been attributed to the difference of interspecific variation in the chemical composition among plant taxa (*i.e.,* nutrient and polyphenolic content). Only the difference in C and N content were examined among the plant species in this study, thus contribution of other chemical factors that might affect the rate of DOC leaching cannot be directly assessed. However, observed differences in the color of leachates may provide insights.

Leachate color was not measured, but the darker colored leachate observed from SM litter suggests that litter from this species produces greater concentration of DOC than does RCG litter. Darker colored leachates tend to have greater DOC concentrations than lighter colored leachates (McArthur and Richardson 2002; Helms *et al.* 2008;

Spencer *et al.* 2008; Fasching *et al.* 2014). Darker leachates generally possess a greater portion of higher molecular weight (HMW) DOC, than do lighter colored leachates (McArthur and Richardson 2002; Aitkenhead-Peterson *et al.* 2003; Helms *et al*. 2008). Native litter, especially DOC leached form SM litter could be comprised of HMW constituents, evident by observed dark colored leachate. Furthermore, leachate from plant litter higher in polyphenolic content (*i.e.*, lignin content) were comprised of lower molecular weight (LMW) DOC (McArthur and Richardson 2002). Thus, the fiber content of RCG litter could indicted that the DOC leached from RCG litter is comprised of LMW DOC. Which would be utilized by microbial communities as labile carbon source.

When microbes were not experimentally inhibited RCG litter lost similar % dry mass (DM) as SM, but released less DOC than SM litter. But, when microbial communities were experimentally inhibited RCG litter lost less DM than SM litter, however similar DOC concentrations were leached from both litter types. Heterotrophic microbial communities contribute to loss of DM from plant litter (Suberkropp 1991). Microbial assimilation and later mineralization (*i.e.*, conversion of DOC to CO_2) are the process involved in the decomposition of DOC leached from plant litter (Aitkenhead-Peterson *et al.* 2003). This would suggest that microbes are contributing to the weight loss and are utilizing DOC leached from RCG litter and native litter. Unfortunately, microbial respiration rates were not measured, which could have provided insight to how much microbial activity was occurring. I did however, measure the decomposition of DOC leached from both RCG and native litter. Both the total DOC and the labile portion of DOC leached from RCG litter decomposed at faster rate than DOC leached from

native litter. Which suggests that DOC leached from RCG litter is more labile than DOC leached from native litter.

I examined the correlation between species-specific leachate decomposition rates and *in situ* litter breakdown rates. Native litter and RCG litter processing rates did not significantly correlate with overall or labile DOC decomposition rates from DOC leached from native litter or RCG litter. Low correlations suggest DOC leached from native litter and RCG litter may not decompose at rates similar to processing rates of intact native litter and RCG litter. Strauss and Lamberti (2002) reported similar results with leaf litter from different hardwood species. My results suggest that faster rates microbial assimilation of DOC leached from RCG litter relative to native leaf litter, does not increase RCG litter breakdown rates relative to the native litter examined. Again I contribute the slow decomposition of RCG litter to the fiber content of the stem of this grass. This finding should be taken in to consideration when evaluating the implications of RCG invasion of floodplain forest on the carbon dynamics in UMRS and other aquatic ecosystems.

Ecological Implications for UMRS Riverine Ecosystems

First, my research suggests that RCG litter is of lower quality than native litter. Again, only the C:N ratio of RCG litter was measured, but no other chemical and physical characteristics were measured that could explain why RCG litter would be a low quality plant litter source. But, this is evident by low rates of decomposition compared to SM litter, and RCG litter was colonized by shredders for a shorter period of time than native litter. Also my results suggest RCG litter was conditioned at a slow rate, and remained palatable for shredders for a short period of time. This implies further that

RCG litter is a lower quality substrate. If RCG monocultures outcompete old growth floodplain forest on the UMRS, then my research would suggest that there would be a loss of a higher quality carbon source, and succession towards lower quality carbon source.

Lower quality plant litter affects microbial and macroinvertebrate communities in riverine ecosystems. Sporulation and growth rates of aquatic fungi are reduced on lower quality plant with high lignin content (Gessner 1997). Macroinvertebrates select plant litter based on quality (Canhoto and Graça 1995; Richardson 2004; Flores *et al.* 2013) and show faster growth rates on higher quality plant litter (Canhoto and Graça 1995; Flores *et al.* 2013). Shredder communities could also be effected by how short RCG litter is palatable. Species in the shredder FFG show different growth periods throughout the year, and depend on diversity tree species and differences in processing rates (Grubbs and Cummins 1996). Plant litter that decompose at a fast or medium rate provided litter to autumn-winter shredder communities (Cummins *et a*l. 1989; Grubbs and Cummins 1996). Plant later that decompose at medium rate (*i.e.*, end of decomposition process) or at a slow rate provided litter to spring-summer shredder communities. (Cummins *et al.* 1989; Grubbs and Cummins 1996). Some shredders consume detritus for growth year round (Grubbs and Cummins 1996).

Leaf litter from SM and SWO hardwood trees decomposed at a medium rate. So, SM and SWO litter would be available for autumn-winter and spring-summer shredder communities. But, SM litter was not found on day 188, so this litter type maybe available for only autumn-winter shredder communities. Litter from RCG and RB decomposed at slow rates, so these litter types would be available for spring-summer

shredder communities. However, as I have already mentioned litter from RCG took longer to condition than native litter, and remained palatable for shredders for a shorter period of time than native litter. Which suggests that RCG litter would be palatable for autumn-winter shredder communities and not spring-summer communities, or shredders that consume detritus year round. If RCG monocultures continue to invaded floodplain forest wetlands then there could be a loss of detritus for shredders throughout the year, which would be provided by floodplain forest leaf litter.

There is little known how the invasion of RCG alters growth of microbial and macroinvertebrate communities in UMRS riverine ecosystems. Experiments that determine the chemical and physical characteristics of RCG, along with, experiments that determine how RCG litter alters the biomass and abundance of UMRS microbial and macroinvertebrate communities, could provide some insight into how the invasion of RCG will alter microbial and macroinvertebrate communities in UMRS. Differences in fungal and bacterial biomass on RCG litter and native litter could be measured with egestrol assays and [^{3}H] leucine or thymidine incorporation into protein of bacteria, respectively. Fungal sporulation counts and enumeration of bacteria (*e.g.* most probable number technique, or acridine orange direct counts) could be used to measure microbial community abundance on RCG litter and native litter. These experiments, in conjunction with experiments comparing macroinvertebrate colonization, consumption and growth rates between RCG and native litter, could provide a better assessment of the costs to ecosystem services by the replacement of native vegetation with RCG in UMR floodplains and its connected ecosystems.

Secondly, my research suggests that DOC leached from RCG litter is more labile than DOC leached by the litter of the native taxa I examined. Microbial ecologists refer to DOC as labile when it DOC is biodegraded within hours or days (Benner 2003). The labile fraction of DOC is hydrophilic or easily dissolved in water. Approximately, 20% of hydrophilic DOC is comprised of LMW constituents, such as carbohydrates (*e.g.*, glucose, fructose and meliitoze), carboxylic acids and amino acids (Herbert and Bertsch 1995), and greatest response of bacterial activity was to LMW form of DOC (Findlay *et al.* 2003, Olapade and Leff 2005). Within 6 days the DOC leached from RCG litter was biodegraded at a faster rate than DOC leached from native litter. This suggests that the DOC leached from RCG has a higher proportion of LMW DOC constituents than native litter. So, there could be a loss of higher molecular weight (HMW) DOC leached from native litter to UMRS riverine ecosystems. High molecular weight DOC is utilized by bacterial communities as a source of carbon (Meyer *et al.* 1998, Tranvik 1990, McArthur and Richardson 2002).

DOC that is comprised of LMW substances are more readily bioavailable to microorganisms (Berggren *et al.* 2010 a; Berggren *et al.* 2010 b) and support higher bacterial growth and abundance (Axmanová *et al.* 2006). Dissolved organic carbon that is more labile is often more preferred by microorganism in soil ecosystems (Cleveland *et al.* 2004) and aquatic ecosystems (Olapade and Leff 2005). But, little to nothing is known how soil and aquatic microbes readily utilize RCG and native litter DOC leachate. Chemical properties of DOC leached from RCG litter and native litter could be determined by XAD resin fractionation or by determining molecular weight by ultrafiltration. Along with, batch culture experiments that measure soil fungal and

bacterial communities response to DOC leachate produced by RCG and native litter. All of which could provide insight to how the DOC leached from RCG litter is utilized by microbial communities in UMR floodplains.

Also, the labile DOC leached from RCG litter could potentially not reach riverine ecosystems adjacent to floodplains of the UMRS. Up to 16 to 68 % of DOC in soil solution is labile DOC (Zsolnay and Steindl 1991), and is immobilized or mineralized within 2-3 hours or over a short time span (Qualls and Haines 1992, Yano *et al.* 1998, 2000). This immobilized carbon can be stored within the B horizon for hundreds of years (McDowell and Likens 1988; Moore 1989). Dissolved organic carbon leached from RCG litter could also potentially be lost in organic and mineral soils through adsorption (Qualls 2000; Kaiser *et al.* 2001). Furthermore, the flux of DOC in water solution decreases in concentration as solution passes through the soil profile (Hope *et al.* 1994). These process that control the flux of DOC through soil profiles could alter the flux of DOC leached from RCG to UMRS riverine ecosystems. Little to noting is known about the flux of DOC leached from RCG litter passes through soil profile. This could be achieved with experiments that determine sportive and biodegradability process in floodplain soil profile as DOC leached from RCG litter and native litter percolates through ex-situ floodplain soil samples

More labile DOC leached from RCG could also potentially alter nitrogen cycling rates in floodplain soils and adjacent UMRS riverine ecosystems. Area invades by RCG has the potential to alter nitrification rates in UMRS floodplain wetlands (De Jager *et al.* 2015). Labile DOC reduces nitrification rates in soils (Rice and Pancholy 1973; Paavolainen *et al.* 1998) and aquatic systems (Strauss and Lamberti 2000; Strauss and

Lamberti 2002). Dissolved organic carbon leached from RCG, could reduce nitrification, because labile DOC facilitates completion between nitrifying and heterotrophic bacteria for ammonium (Strauss and Lamberti 2002), or the lignin content of RCG could inhibit nitrifying bacteria via allelopathy. Rice and Pancholy (1973) found that nitrification rates in terrestrial soils declined in allelopathy response to phenolics and tannins in organic carbon leached from plant litter.

There is proof that denitrification may actually occur in areas invaded by RCG. From August through September there was a significant loss of nitrate (NO_3^-) in floodplain wetland areas invaded by RCG (Swanson 2016). Coupled nitrification-denitrification could help explain this loss. Coupling between nitrification and denitrification process results from the transport of nitrite (NO_2^-) and NO_3^- across an oxic/anoxic boundary (Jenkins and Kemp 1984). These nitrification products are denitrified and then lost from system. Labile DOC limits denitrification rates (Zarnetske *et al.* 2011). So labile DOC leached from RCG litter could act as electron donor for denitrification process, thus increase denitrification rates. This could have important implications for the nitrogen cycle in riverine ecosystems and adjacent terrestrial ecosystems.

Lastly, it has been predicted that some geographical areas will either experience sever precipitation, flooding and drought events (Pagano *et al.* 2014). Precipitation in the Midwest is greatest in the east and declines in the west (Pryor *et al.* 2014). Higher precipitation events are projected to continue in the future across the entire Midwest (Schoof *et al.* 2010). But, during summer months precipitation events will decline. During these months droughts severity will increase in the southern portion of Midwest

region, and in the north severity of floods and droughts will vary regionally (Pryor *et al.* 2014). If increased precipitation events occur it has been predicted that terrestrial primary production would increase, which would increase the export of DOC to riverine ecosystems (Evans *et al.* 2002). Whereas, DOC concentrations have been observed to decline during droughts (Freeman *et al.* 1994). Reed canarygrass can survive through saturated or dry conditions (Lavergne and Molofsky 2004), but floodplain forest species are susceptible to changes in saturation. Severe droughts could alter floodplain forest communities, because hardwood species require flooding for seed dispersal (Middleton 1995). Static flooding could also alter floodplain forest communities, because many hardwood species seedlings cannot survive long periods of inundation (Romano 2010).

If these climate patterns occur, RCG may be able to persist better than native hardwood species associated with UMRS floodplain forests. Compounded by climate alterations in precipitation and runoff, a floodplain dominated by RCG could produce and deliver lower amounts of DOC to the upper Mississippi River ecosystem. So, there needs to be a better understanding of how the flowpath of DOC leached from RCG litter reaches riverine ecosystems during periods of drought and high precipitation events. During periods of drought and high precipitation events UMRS floodplain soil pore water DOC age and chemical characteristics can be assed with isotopic (^{13}C and ^{14}C) and spectroscopic (UV and ^{13}C NMR) analytical techniques characteristics, respectively. Along with experiments to determine sportive and biodegradability process in floodplain soils as DOC leached from RCG litter and native litter percolates through ex-situ floodplain soil samples. These experiments could start to explain how the flowpath of DOC leached from RCG litter reaches riverine ecosystems compared to native litter.

Conclusion

It has been predicted that the invasion of UMRS floodplain forest by RCG will expand. This invasion may increase during severe drought and flooding events from climate change, and thus a lower quality substrate may persist. This could lead to an alteration biomass and abundance of microbial and macroinvertebrate communities. Martinez *et al.* (2013), found that there was a higher biomass and abundance of detritivore macroinvertebrates on higher quality food than on poorer quality food. In Australia, invasive willow trees (*salix* spp.) provide a lower quality litter to riparian ecosystems, and litter from willow trees altered fungal, algal and macroinvertebrate communities (McInerney *et al.* 2016b). Medina-Villar and colleagues (2015) found that there was lower biomass on invasive tree species litter than native litter, and the macroinvertebrate activity contributed less to decomposition of invasive litter than native litter. Amur honeysuckle (*Lonicera maackii* (Rupr.) Maxim.) is an invasive shrub to riparian zones in eastern United States. This shrub decomposed two times faster than native sugar maple (*Acer saccharum* Marshall) litter, but sugar maple supported a higher abundance of macroinvertebrates than invasive shrub (Fargen *et al.* 2015).

Similar to the result of my experiment, where RCG litter was palatable for macroinvertebrate shredders for a shorter period of time, the results from these different experiments also suggest if native, higher-quality vegetation is replaced by lower quality vegetation, then there could be effect on the transfer of energy throughout a riverine food web. Microbial production, which makes plant litter palatable for macroinvertebrates, can be consumed by organisms through ingestion of individual cells or biofilms on particulate organic matter (POM). There are questions whether microbial activity role is

to recycle nutrients and remineralizing of C for microbial food web, or transfer an energy flux to higher tropic levels (Allan and Castillo 2007). It is well known fungal cells are consumed by macroinvertebrates (Bärlocher 1999), thus contribute to energy flux to higher trophic levels, but bacterial production in the water column maybe less available to macroinvertebrates in riverine ecosystems (Allan and Castillo 2007).

However, benthic biofilms are an association of microorganisms (*e.g.*, cyanobacteria, fungi and bacteria communities) found on leaf litter and other POM. The presence of biofilm on POM substrates facilitates the consumption of microbial cells by macroconsumers (Lodge et al. 1988, Meyer 1994). This suggests that the C in microbial cells would reach macroinvertebrates in a high enough quantity to support higher trophic levels. This was observed in in a small steam where ^{13}C sodium acetate was added to a whole stream reach to label benthic bacteria (Hall and Meyer 1998). The ^{13}C label was seen throughout stream food web, and bacterial cells provided 0% - 100% of C to macroinvertebrates. Which, significantly correlated with the % of amorphous detritus in macroinvertebrates gut. This suggests that biofilm was consumed and contributed to C made available to macroinvertebrates.

Gross primary production and chlorophyll-*a* of biofilm on leaf litter from *Salix fragilis* L. an invasive tree was lower than biofilm on native leaf litter (McInerney *et al.* 2016a). At stream reaches by this same invasive tree the grazer FFG were replaced by collector-gatherer FFG, and primary trophic pathway was altered from an autotrophic algal-based pathway to detritus-based trophic pathway (McInerney *et al.* 2016b). In a headwater stream there was an increase in macroinvertebrate density when an invasive shrub was removed from riparian zone (McNeish 2015). Gratton and Denno (2005) also

observed an increase in anthropod communities when invasive common reed grass (*Phragmites australis* (Cav.) Trin. ex Steud.) was removed from wetland area. This demonstrates that when an invasive plant takes over a riparian ecosystem and provides a lower quality C source, like RCG, there is reduction of growth of biofilm, and loss of energy to higher trophic levels.

Furthermore, heterotrophic microorganisms and macroinvertebrate secondary production could be altered with the potential loss of DOC from native litter, and loss of labile DOC leached from RCG that may not be exported to UMRS riverine ecosystems. Turnover time for HMW DOC is higher than more LMW DOC as it is exported from source and transported downstream (Kaplan *et al.* 2008). Where, HMW DOC is utilized by bacterial communities as a source of carbon (Meyer *et al.* 1998, Tranvik 1990, McArthur and Richardson 2002). After prolonged exposures less labile DOC molecules are metabolized (Kaplan and Newbold 1995, Riemann and Søndergaard 2004). Less labile DOC degrades slowly as it is transported downstream (Kaplan and Newbold 2003) and provides metabolic stability within lotic ecosystem (Wetzel 2003). If RCG continues to establish monocultures in place of floodplain forest then there could be a loss of C to stream metabolism. But, the DOC leached from RCG is more labile than native litter and could be comprised of a greater concentration of LMW constituents. Which would provide an input of C to stream metabolism. Bacterial communities showed greatest response (*e.g.*, abundance, growth and respiration) to highly labile LMW DOC (Olapade and Leff 2005, Wilcox *et al.* 2005**).** Although this sounds like a positive that the DOC leached from RCG litter would provide C source to riverine ecosystems, as I suggested

before the labile LMW DOC portion of DOC leached from RCG may not reach riverine ecosystem.

RCG litter does senesces in autumn, but doesn't fall off a tree into a lotic ecosystem like leaves from native hardwood tree species. This means that a majority of DOC that is leached from RCG litter would occur as trough fall from precipitation. When precipitation passes through plant litter it becomes enriched with DOC (Aitkenhead-Peterson *et al.* 2003). When referring to "throughfall" in this sense the source of allochthonous DOC to riverine ecosystems would occur as a tree branch extends over a stream. The flux of DOC from throughfall varies between ecosystem types. For example, 10.6 kg C ha^{-1} yr^{-1} and 340 kg C ha^{-1} yr^{-1} in a cool deciduous forest and Manuka scrub, respectively (Moore and Jackson 1989, Leichty *et al.* 1995). But, DOC leached from RCG would follow a different flowpath to reach riverine ecosystems.

After a precipitation event a flux of DOC from RCG litter would occur from throughfall as precipitation passes though grass litter. This flux of DOC would have to passes through or over floodplain soils before it would reach UMRS. Riiegg and colleagues (2015) found the annual export of DOC from a grassland stream ranged 0.00-9.09 kg C ha^{-1} yr^{-1} annually, which was 30 times lower than other grasslands reported in literature. The DOC concentrations where highly correlated with annual runoff after precipitations events, and they attributed the DOC concentration to leaching from terrestrial organic matter is soils. McDowell and Wood (1984), found that DOC concentrations are highest when flowpath of runoff is near the surface or through organic-rich upper soil horizons (*i.e.*, O horizon). This could be explained, because in grassland soils DOC concentration declines with depth (Sanderman and Amundson

2008). In soils labile DOC constituents are utilized as a carbon source by soil microbes (Qualls and Haines 1992, Yano *et al.* 1998), and the hydrophobic constituents (i.e., humic substances) are sorbed by mineral soils (Qualls 2000), were the remaining DOC makes it to riverine ecosystems (Aitkenhead-Peterson *et al.* 2003). The DOC leached from RCG litter is more labile then DOC leached from native litter, which suggest that labile DOC leached from RCG litter could be lost even when flowpath is near surface of soil horizons or through deeper horizons.

Field-collected DOC from O horizon are not dominated by LMW DOC or hydrophilic labile DOC (Kalbitz *et al.* 2003). This is occurring because, in soil horizons near the surface microbial activity is high and microbial communities can metabolize C compounds within hours of being leached into solution (Fierer and Schimel 2002, Fierer and Schimel 2003). In these soil horizons the extracellular activity of hyporheic bacterial communities was highest with labile DOC compared to refractory doc (Findlay *et al.* 2003). Sanderman *et al.* (2008), suggests that in grasslands microbes rapidly utilize C substrate leached from plant litter, which is why the DOC source does not contribute to the soil DOC pool. So, labile DOC leached from RCG could be lost in soil horizons and may not reach riverine ecosystems. If this occurs then there could be a loss of energy throughout UMRS aquatic ecosystems. This is evident during an enrichment of a lotic ecosystem with highly labile DOC. When labile DOC was added to a stream the density, growth and respiration of benthic bacteria increased compared to control stream, and growth rate of chironomid midge larvae increased as well (Wilcox et al. 2005). As stated by Benner (2003), the "microbial loop" is responsible for much of the flow of C and energy through many aquatic ecosystems, which is a key component of ecosystem

function and structure. If there is a loss of allochthonous DOC to aquatic ecosystems due to the invasion of RCG, then there could be alteration in the transfer of carbon and energy to higher trophic levels. Reed Canarygrass could not only be detrimental to the floodplain forest, but to the UMRS riverine ecosystem at large.

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