The importance of crayfish in the breakdown of rhododendron leaf litter

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SUMMARY

1. Rhododendron (Rhododendron maximum) is a common evergreen shrub in riparian areas of the southern Appalachians, where its leaves can comprise a large proportion of leaf litter in streams. However, they are relatively refractory and generally considered a low quality food resource for detritivores.

2. Our objective was to assess whether macroconsumers [primarily crayfish (Cambarus bartonii)] influence rhododendron leaf breakdown in a forested southern Appalachian stream in both summer (when leaves other than rhododendron are relatively scarce) and autumn (when other leaves are relatively abundant). We conducted two leaf decay experiments, one in summer and one in autumn, using pre-conditioned leaves. Macroconsumers were excluded from the benthos of a fourth-order stream using electric 'fences'; we predicted that excluding macroconsumers would reduce the decay rate of rhododendron leaves in both summer and autumn.

3. In both experiments, breakdown rate was lower in exclusion treatments. Macroconsumers accounted for approximately 33 and 54% of rhododendron decay in summer and autumn, respectively. We attribute this effect to direct shredding of rhododendron by crayfish. Biomass of insect shredders, insect predators and fungi did not differ between control and exclusion treatments, indicating that insectivorous sculpins (Cottus bairdi) had no effect on rhododendron decay and that omnivorous crayfish did not exert an indirect effect via alteration of insect or fungal biomass.

4. The influence of shredding insects varied between summer and autumn. In summer, when other, more palatable leaf types were not available, rhododendron leaf packs appeared to provide 'resource islands' for insect shredders. There was a significant inverse relationship between insect shredders and leaf pack mass in the summer exclusion treatment: insects were the only organisms eating leaves in this treatment and, as shredder biomass increased, remaining leaf pack mass decreased. In the control treatment, however, we did not see this relationship; here, the effect of insect shredders was presumably swamped by the impact of crayfish. In autumn, when other leaves were abundant, insect shredder biomass in rhododendron leaf packs was less than one-third of summer values.

5. Even at low density (approximately 2 m\(^{-2}\)) crayfish were able to influence an ecosystem process, such as leaf decay, in both summer and autumn. Given the threatened status of many crayfish species in the United States, this finding is especially relevant. Even small alterations in crayfish assemblages, whether via loss of native species and/or introduction of exotic species, may have significant repercussions for ecosystem function.

Keywords: crayfish, fungi, leaf decay, rhododendron, stream
data were collected by Dr J.B. Wallace (University of Georgia, U.S.A.).

The Lower Ball Creek catchment is forested (approximately 100%) by mixed hardwood species such as red maple [Acer rubrum (L.)] and tulip-poplar [Liriodendron tulipifera (L.)]. Riparian areas are densely vegetated by rhododendron (R. maximum), mountain laurel [Kalmia latifolia (L.)] and dogwood [Cornus florida (L.)]. Altitude at our study site is about 700 m, with a stream gradient of approximately 4 cm m⁻¹. Boulder, cobble and gravel comprise the stream substratum. Macroconsumer assemblages in Lower Ball Creek are dominated by crayfish (C. bartonii) and mottled sculpin [Cottus bairdi (Girard)], but longnose dace [Rhinichthys cataractae (Valenciennes)] and rosace dace [Clinostomus funduloides (Girard)] are also present.

Experimental design

Rhododendron decay experiments were conducted in summer and autumn 1999. Freshly fallen rhododendron leaves (i.e. brown but not buried or decomposed) were collected near Lower Ball Creek on 17 March and 18 June for summer and autumn experiments, respectively. Previous research indicated that initial rhododendron decay is very slow (e.g. Webster & Waide, 1982; Benfield et al., 1991). Because we wanted to be able to detect a change in leaf mass over a limited experimental period, we used pre-conditioned leaves to accelerate the decay process. Leaves were placed in plastic mesh (5 mm) bags and secured in the stream with aluminium gutter nails for preconditioning. Summer leaves remained in the stream from 17 March to 15 July, for a preconditioning period of 1574 degree days (mean daily water temperature, 12.9 °C). Autumn leaves remained in the stream from 18 June to 25 August (mean daily water temperature, 17.2 °C). On 25 August, leaves were removed from the stream and rinsed to remove macro-invertebrates; they were then refrigerated at 4 °C from 26 August to 2 October to slow decomposition and compensate for higher preconditioning temperatures (i.e. relative to summer leaves). Pre-conditioning period for the autumn leaves was 1302 degree days.

On 7 July, 10 intact leaves were removed from summer pre-conditioning bags to determine a wet/dry mass conversion factor. Each leaf was weighed immediately upon removal from the stream to obtain a wet mass, then dried at 70 °C for 24 h and reweighed to obtain a dry mass. The wet/dry mass ratio (mean ± 1 SE) was 5.25 ± 0.08; thus, we used 26.2 g wet mass per pack for approximately 5 g dry mass leaf packs. The same ratio was used for autumn leaf packs, and initial dry mass was similar between summer and autumn experiments (mean ± 1 SE, 4.49 ± 0.08 g in summer versus 4.88 ± 0.21 in autumn). All macro-invertebrates were rinsed from leaves prior to leaf pack assembly. Rinsed leaves were distributed into packs of appropriate mass, which were held together by two plastic fasteners placed near leaf midribs. Because the pre-conditioned leaves were relatively fragile, packs also were wrapped in plastic mesh (2 cm) to minimize the loss of large leaf fragments; this mesh was large enough to allow access by macro-invertebrates, including crayfish.

Leaf packs were attached with nylon monofilament to polyvinylchloride frames (0.25 m²) lined with copper wire. Each pack was weighted with a lead weight (85 g) to keep it flush with the substratum. In the summer experiment five leaf packs were secured in each frame. In the autumn experiment fewer intact leaves were available, so four packs were used per frame. During both summer and autumn, 10 frames (five pairs) were placed in run habitats of Lower Ball Creek, along an approximately 0.5 km stream reach. Placement of pairs was determined by preliminary shear stress measurements using calibrated hemispheres (Statzner & Müller, 1989); only sites which provided a suitable area (e.g. without large boulders) with similar shear stresses were used. Water velocity and depth were measured at the four corners of each frame using a Marsh McBirney® current meter and a metre stick. Canopy cover was measured over the centre of each frame using a spherical densitometre.

To exclude macroconsumers, one frame in each pair was chosen by coin toss to be the exclusion treatment. This frame was connected to a 6-V solar-powered fence charger (Parmak Model DP-SP-SS, Parker McCrory Manufacturing Company) that delivered repeated pulses of electricity to the 0.25 m² frame area. These electric pulses prevented the entry of crayfish and fish, but did not adversely affect smaller organisms such as aquatic insect larvae. Many other studies have used this electric exclusion technique (e.g. Pringle & Blake, 1994; Pringle & Hamazaki, 1997), which avoids some artefacts associated with
traditional cage enclosure experiments (e.g. reduced water flow and increased sedimentation). The other frame in each pair served as a control area to which macroconsumers had access. Frames were placed approximately 0.5 m apart to minimize the impact of exclusion treatments on controls; given that macroconsumers were frequently found immediately outside electrified frames, this distance appeared to be more than adequate. Throughout the experiment, fence charger batteries were replaced every 5 days to ensure a consistent 6 V charge. Frames also were cleared of accumulated leaves every 5 days to minimize flow alterations and prevent loss of frames during spates.

**Sampling**

The summer experiment began on 16 July and ended on 29 August. One leaf pack was removed from each frame on days 5, 10, 20, 32 and 44. In addition, six packs were brought back to the lab on day 0 to determine initial leaf weights and fungal biomass. The autumn experiment began on 3 October and ended on 28 November. Leaf packs were sampled on days 8, 20, 35 and 56, and nine packs were used for day 0 assessments. Fence chargers at exclusion treatments were turned off briefly (5–10 min) for sampling. A 210-μm mesh hand net was held downstream of each leaf pack as it was removed from the stream to retrieve any dislodged invertebrates. Leaf packs were placed in plastic bags, put on ice and returned to the laboratory (2 h away) for processing. Prior to removing the leaf packs, all replicates were examined using a clear plastic viewing box to determine whether any macroconsumers were present. In previous experiments we observed replicates for 5 min, but limited visibility in the current study made these prolonged observations inefficient. Instead, we recorded presence or absence of macroconsumers during spot checks of all replicates on all sampling dates, as well as every 5 days when fence charger batteries were changed (n = 80 spot checks in summer, 100 in autumn). In addition, any macroconsumers seen during leaf pack removal (i.e. that were hiding under leaf packs or cobbles during spot checks but were disturbed during sampling) were noted, and we conducted four spot checks of all replicates at night.

Leaf packs were processed within 24 h of sampling. Leaves were rinsed to remove invertebrates and sediment. Invertebrates were live-picked from the rinsed material and preserved in 70% ethanol. We chose to focus on insect shredders and predators because they were the functional feeding groups most likely to affect rhododendron decomposition (shredders directly through leaf consumption, predators indirectly through consumption of shredders). Insects classified as shredders or predators by Merritt & Cummins (1996) were later identified to the lowest practical level (usually family or genus) using a dissecting microscope (10× magnification), and measured to the nearest 0.5 mm using 1 mm grid paper. Shredder and predator biomasses were calculated using family-specific length-mass regressions from Benke et al. (1999). Organisms < 1.5 mm were identified to order and were not included in shredder or predator biomass values (typically they contributed < 0.01% of total invertebrate biomass). Shredders and predators from days 20, 32 and 44 (summer experiment) and days 20, 35 and 56 (autumn experiment) samples were identified.

After invertebrates were rinsed from leaf packs, 100 leaf discs were randomly removed from each pack using a hole punch (6 mm diameter). Fifty discs were preserved in methanol for fungal biomass analysis via ergosterol extraction (Newell, Arsuffi & Fallon, 1988; with slight modifications after Paul & Meyer, 1996). Ergosterol was extracted from days 0, 10, 20 and 32 (summer) to days 0, 8, 20 and 35 (autumn) samples. By day 44 of the summer experiment, only two control and two exclusion treatments had enough leaf material remaining for ergosterol analysis; by day 56 of the autumn experiment, no packs had enough leaf material remaining. Fungal biomass was estimated from ergosterol concentration using a conversion factor of 5 μg ergosterol (mg mycelial dry mass)⁻¹ (Gessner & Chauvet, 1993; Paul & Meyer, 1996). The remaining 50 discs from each pack underwent the same drying and ashing process as the leaf packs. Packs were dried at 70 °C for 3 days, weighed, then burned at 500 °C for 6 h and reweighed. Total ash-free dry mass (AFDM) remaining was calculated by summing AFDM of each leaf pack and 2 × AFDM of the 50 leaf discs.

To quantify the availability of rhododendron and non-rhododendron leaves at the end of each experiment, we randomly selected 10 cross-stream transects (two near each treatment pair, each 1 m wide) and collected all leaves within each transect. These leaf
collections were returned to the laboratory, rinsed free
of macro-invertebrates, and sorted into rhododendron
and non-rhododendron leaves. Leaves were dried at
70 °C for approximately 1 week, then weighed to
determine dry weight (g m$^{-2}$) for the two leaf types.

To assess crayfish density, we sampled a 50-m
transect within the experimental reach on 7 August
and again on 4 November (no treatments were located
inside the 50 m transect). On each date, 15 randomly
located samples were taken using a quadrat sampler,
which blocked off 1 m$^2$ of the stream bottom (i.e. total
area sampled, 15 m$^2$). Crayfish from each sample
were counted, identified and measured before being
returned to the stream.

**Statistical analysis**

Initial physical parameters for each replicate were
compared using a two-factor MANOVA (treatment
and season), with water velocity, water depth,
% canopy cover and shear stress as response vari-
bles. If MANOVA showed a significant effect, separ-
ate univariate two-factor ANOVAs were run for each
physical parameter. Availability of rhododendron and
non-rhododendron leaves was compared using a two-
factor ANOVA (leaf type and season); if ANOVA
showed a significant interaction between factors,
separate paired t-tests were run for each season. To
calculate leaf breakdown rate ($k$), we regressed the
natural log of % AFDM remaining against day or
degree day (where $k$ is the slope of the regression).
Breakdown rate was calculated for each replicate,
then compared using two-factor ANOVA (treatment
and season); separate ANOVAs were run for day and
degree day calculations. A two-factor MANOVA
(treatment and season), with predator and shredder
biomass (average over three sample dates) as
response variables, was run to test for any season or
treatment differences in insect biomass. If significant
effects were detected with MANOVA, univariate two-
factor ANOVAs were run for each response variable.
To examine whether insect shredders were affecting
leaf pack mass, we regressed AFDM remaining
against shredder biomass for the summer and autumn
experiments. Fungal biomass (average over three
sample dates) was compared using a two-factor
ANOVA (treatment and season). Prior to all statistical
analyses, Levene's test was used to determine whe-
ther variances were equal; where necessary, data were
transformed using a natural log or inverse transfor-
mation. For all analyses, $\alpha = 0.05$, and all were
conducted in SAS® System for Windows™, Release
9.2.

**Results**

Mean daily water temperature during the summer
experiment was 17.9 °C (range 16.9–19.2 °C) and
11.2 °C (range 6.4–14.9 °C) during the autumn experi-
ment. Peak daily discharge was greater and more
variable in autumn (mean ± SE, 2175 L s$^{-1}$ ± 54.7)
than in summer (99.1 L s$^{-1}$ ± 52), largely because of
the occurrence of three distinct discharge peaks
during the autumn experiment (Fig. 1). Water
conductivity was similar in summer and autumn (mean ± SE, 12.5 μS cm⁻¹ ± 0.26 in summer, 12.0 μS cm⁻¹ ± 0.25 in autumn). Nutrient concentrations were relatively low in both summer and autumn, although concentrations were higher in summer: mean NO₃⁻-N, NH₄⁺-N, and soluble reactive phosphorus concentrations were 0.057, 0.004 and 0.009 mg L⁻¹, respectively, during the summer experiment, versus 0.004, 0.003 and 0.003 mg L⁻¹ during the autumn experiment.

Initial physical parameters for treatment replicates are presented in Table 1. In the autumn experiment, one treatment pair differed significantly from the remaining four pairs in terms of these physical parameters. This pair was excluded from all analyses, leaving four replicate pairs for the autumn experiment and five replicate pairs for the summer experiment. Control and exclusion treatments did not differ in the measured parameters (MANOVA: Pillai’s trace = 0.045, Fᵣ,₄₁ = 0.131, P = 0.968), but there were significant seasonal (i.e. summer versus autumn) differences (MANOVA: Pillai’s trace = 0.953, F₄₁,₁₁ = 55.8, P < 0.0001). Univariate analyses for each parameter indicated that water velocity (ANOVA: F₁,₁₄ = 21.5, P = 0.0004), water depth (ANOVA: F₁,₁₄ = 6.25, P = 0.0254), and percentage canopy cover (ANOVA: F₁,₁₄ = 145, P < 0.0001) contributed to this significant season effect: initial water velocities and depths were lower in autumn than in summer, while percentage canopy cover was greater (Table 1).

No macroconsumers were observed in the electrified frames, indicating that the exclusion technique was effective. Crayfish and sculpins occasionally entered the exclusion treatment while fence chargers were turned off briefly for sampling, but they left immediately when chargers were reactivated. During the summer experiment, a total of 11 crayfish were observed in control replicates (40 spot checks). Crayfish densities within the experimental reach were slightly higher in summer (mean ± SE, 2.33 m⁻² ± 0.69) than in autumn (1.87 m⁻² ± 0.50), although this difference was not significant.

Rhododendron leaves comprised more than 50% of the leaf material found in Lower Ball Creek at the end of the summer experiment (Table 2). Standing crop of rhododendron was similar in summer and autumn, while standing crop of non-rhododendron leaves (e.g. maple, birch, sycamore, dogwood) was nearly four times greater in autumn than in summer (Table 2). The ANOVA showed a significant leaf type-season interaction (ANOVA: F₁,₁₆ = 13.8, P = 0.0007); subsequent paired t-tests indicated that there were significantly more non-rhododendron than rhododendron leaves in autumn (t-test: t₁₄ = -5.13, P = 0.0003), but no significant difference in summer (t-test: t₁₄ = 1.34, P = 0.106).

Leaf breakdown

During both summer and autumn, exclusion of macroconsumers led to a decrease in rhododendron breakdown rate (Fig. 2). We estimated the amount of decay attributable to macroconsumers in each experiment by dividing the difference between breakdown rates in control and exclusion treatments by the breakdown rate in the control treatment. Macroconsumers were responsible for approximately 33% of rhododendron decay during the summer experiment.

Table 1. Initial physical parameters for control and macroconsumer exclusion treatments, summer and autumn experiments. Values are mean of five (summer) or four (autumn) replicates, ± 1 SE

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water velocity (m s⁻¹)</td>
<td>0.20 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Water depth (cm)</td>
<td>16.8 ± 0.1</td>
<td>12.1 ± 3.5</td>
</tr>
<tr>
<td>Canopy cover (%)</td>
<td>88.3 ± 0.9</td>
<td>97.7 ± 0.3</td>
</tr>
<tr>
<td>Shear stress (dyn cm⁻²)</td>
<td>160 ± 26</td>
<td>145 ± 28</td>
</tr>
</tbody>
</table>

Table 2. Dry weight of rhododendron and non-rhododendron leaves (g m⁻²) collected from in-stream transects at the end of summer and autumn experiments. Values represent mean of 10 transects, ± 1 SE

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhododendron</td>
<td>3.17 ± 0.49</td>
<td>2.43 ± 0.59</td>
</tr>
<tr>
<td>Non-rhododendron</td>
<td>2.27 ± 0.69</td>
<td>9.78 ± 1.97</td>
</tr>
</tbody>
</table>
while this percentage increased to 54 in the autumn experiment. Comparison of individual replicate breakdown rates \((k, \text{day}^{-1})\) by ANOVA showed that there were significant treatment \((P = 0.011)\) and season \((P = 0.016, \text{Table 3})\) effects: rhododendron breakdown was more rapid in autumn than in summer, and more rapid in control than in exclusion treatments (Fig. 2). No significant treatment-season interaction was found \((P = 0.136, \text{Table 3})\), although the difference between control and exclusion breakdown rates was much greater in autumn than in summer (Fig. 2).

To account for the shorter duration of the summer experiment (44 versus 56 days) we examined breakdown rates in two additional ways. When rates were calculated through day 32 (summer experiment) and day 35 (autumn experiment) no significant treatment or season effects were detected \((P \geq 0.121, \text{Table 3})\), indicating that treatment and season differences became pronounced only after more than a month of

**Table 3** Results of two-factor ANOVAs for leaf breakdown rates calculated by day. Breakdown rates were also calculated through day 32 (summer) and day 35 (autumn) for comparison; these data were transformed \((1/X)\) prior to analysis to correct for unequal variances. Each factor had two levels (control and macroconsumer exclusion for treatment, summer and autumn for season)

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k (\text{day}^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>7.38 \times 10^{-4}</td>
<td>8.47</td>
<td>0.011</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>6.48 \times 10^{-4}</td>
<td>7.44</td>
<td>0.016</td>
</tr>
<tr>
<td>Treatment \times season</td>
<td>1</td>
<td>2.19 \times 10^{-4}</td>
<td>2.51</td>
<td>0.136</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>1.22 \times 10^{-3}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(k (\text{day}^{-1})) through day 32 or day 35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>1</td>
<td>13652</td>
<td>2.73</td>
<td>0.121</td>
</tr>
<tr>
<td>Season</td>
<td>1</td>
<td>4026</td>
<td>0.80</td>
<td>0.385</td>
</tr>
<tr>
<td>Treatment \times season</td>
<td>1</td>
<td>308</td>
<td>0.06</td>
<td>0.898</td>
</tr>
<tr>
<td>Error</td>
<td>14</td>
<td>70071</td>
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exclusion. We also calculated breakdown rates based on degree days. Although the autumn experiment lasted longer than the summer experiment, water temperatures were significantly lower. As a result, leaf packs in the summer experiment experienced more degree days during both pre-conditioning (1574 versus 1302 degree days) and the experimental period (786 versus 621 degree days), although the summer experiment was of shorter duration. Comparison of individual replicate breakdown rates showed similar results whether \( k \) was calculated by day or by degree day (i.e. significant treatment and season effects, with no significant treatment–season interaction).

**Insect shredder and predator biomass**

In each season, four taxa dominated the assemblage of insect shredders (> 90% biomass), although these taxa differed between seasons. The stoneflies *Tallaperla* and *Pteronarcys* were among the dominant taxa in both summer and autumn; in addition, the stonefly *Leuctra* and the caddisfly *Lepidostoma* contributed to summer shredder biomass, while the stonefly *Taeniopteryx* and the cranefly *Leptotarsus* contributed to autumn shredder biomass. Similar predator taxa contributed the greatest biomass in both summer and autumn (i.e. perlid and perlodid stoneflies, dipteran predators such as *Atherix*, ceratopogonids and tanypodids).

Because *Pteronarcys* stoneflies can attain sizes comparable with small crayfish, it is possible that they could have been adversely affected by the exclusion treatment. Comparison of *Pteronarcys* biomass in control and exclusion treatments, however, showed no significant differences in either summer or autumn; in fact, the largest *Pteronarcys* individual obtained (length, 27 mm) was found in the exclusion treatment.

The MANOVA (using total predator and shredder biomass per pack as response variables) showed no significant difference between control and exclusion treatments (\( P = 0.332, \text{Table 4} \)). However, there was a significant effect of season (\( P = 0.005 \)), with greater predator and shredder biomass found in summer versus autumn (Fig. 3). Univariate ANOVAs indicated that both insect shredders (\( F_{1,14} = 10.9, P = 0.005 \)) and predators (\( F_{1,14} = 7.70, P = 0.015 \)) demonstrated a significant seasonal effect. Similar results were obtained when biomass was expressed in terms of mg g\(^{-1}\) AFDM rather than mg pack\(^{-1}\). Although no significant treatment differences were detected, there was a tendency toward greater insect predator biomass in control versus exclusion treatments in the autumn experiment (Fig. 3). Higher predator biomass was not accompanied by a significant decrease in shredder biomass in the control treatment.

When AFDM remaining was regressed against insect shredder biomass on each date, a significant relationship \( (r^2 = 0.482, P = 0.006) \) was found in the exclusion treatment during the summer experiment: as shredder biomass increased, AFDM remaining decreased (Fig. 4). This pattern was not observed in the control treatment during the summer \( (r^2 = 0.041, P = 0.467) \), nor was it observed in either control or exclusion treatments in the autumn experiment \( (r^2 < 0.083, P > 0.364) \).

**Fungal biomass**

No fungal biomass was detected on day 0 samples in either the summer or the autumn experiment. Fungal biomass increased throughout each experiment but, by the end of both summer and autumn, most leaf packs did not contain enough material for ergosterol extraction. Therefore, days 44 (summer) and 56 (autumn) were excluded from analyses. Comparison by ANOVA indicated that there was a significant effect of season \( (\text{ANOVA: } F_{1,14} = 78.2, P < 0.0001) \), with greater fungal biomass in summer than autumn (Table 5). In both seasons, however, fungal biomass remained relatively low. There was not a significant effect of treatment \( (\text{ANOVA: } F_{1,14} = 0.04, P = 0.844) \), although there was a significant treatment–season interaction \( (\text{ANOVA: } F_{1,14} = 12.2, P = 0.004) \). In summer, fungal biomass tended to be greater in control than exclusion treatments; in autumn, this trend was reversed (i.e. there was greater biomass in exclusion...

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Pillai's trace</th>
<th>( F )</th>
<th>( P )</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>2, 13</td>
<td>0.156</td>
<td>1.20</td>
<td>0.332</td>
</tr>
<tr>
<td>Season</td>
<td>2, 13</td>
<td>0.563</td>
<td>8.38</td>
<td>0.005</td>
</tr>
<tr>
<td>Treatment x season</td>
<td>2, 13</td>
<td>0.039</td>
<td>0.26</td>
<td>0.773</td>
</tr>
</tbody>
</table>

versus control treatments), and the difference between treatments was more pronounced (Table 5).

Discussion

Do macroconsumers influence rhododendron breakdown in both summer and autumn?

In both summer and autumn, breakdown rates (day$^{-1}$ or degree day$^{-1}$) were slower when macroconsumers were excluded (Fig. 2), indicating that macroconsumers contribute to rhododendron breakdown. We attribute this macroconsumer effect to shredding by crayfish. In summer, when visibility was suitable for observations, crayfish were the only macroconsumers detected in control replicates. Although mottled sculpin are common in Lower Ball Creek, they are insectivorous, feeding primarily on aquatic insect larvae. In the study area, mottled sculpin feed predominantly on chironomids, heptageniid mayflies and hydropsychid caddisflies (i.e. taxa that are not shredders or predators); many of the dominant shredders found in rhododendron leaf packs (e.g. peltoperlid and taeniopterygid stoneflies, Lepidostoma caddisflies) make up < 5% of mottled sculpin diets (Stouder, 1990).

While sculpins (as well as crayfish) could have indirectly affected rhododendron breakdown via effects on insect shredders or predators (Short & Holomuzki, 1992; Malmqvist, 1993), there were no significant differences in insect biomass (mg pack$^{-1}$ or mg g$^{-1}$ AFDM) between control and exclusion treatments. Crayfish, however, have been shown to consume large quantities of detritus and to increase leaf breakdown (e.g. Huryn & Wallace, 1987; Parkyn et al., 1997; Whitledge & Rabeni, 1997; Usio, 2000). Crayfish density is relatively low in Lower Ball Creek (approximately 2 m$^{-2}$), but even so they are able to influence an ecosystem process such as leaf decay.

Based on the predictions of Huryn & Wallace (1987), we expected that the effect of macroconsumer exclusion on rhododendron breakdown would be
Fig. 4 AFDM remaining (g) versus shredder biomass (mg pack$^{-1}$) in (a) control and (b) macroconsumer exclusion treatments during the summer experiment. Each point represents one replicate on a given day; one day 44 exclusion replicate was omitted from the regression because it was considered an outlier (it contained a single 156 mg Pteronarcy).
summer and autumn experiments were run for different lengths of time (44 versus 56 days), and effects of macroconsumer exclusion became pronounced only after 32—35 days. It is possible that, had we extended our summer experiment for an additional 12 days, we would have observed greater treatment differences.

Total biomass of insect shredders and predators was more than fourfold greater in summer than in autumn leaf packs (Fig. 3), probably because summer leaf packs served as ‘resource islands’ in a relatively leaf-poor environment (Table 2). This phenomenon has been noted in several other studies (e.g. Webster & Waide, 1982; Benfield & Webster, 1985; Benfield et al., 1991). When natural leaf litter is unavailable (e.g. because of season, disturbance, etc.), even leaf species that were previously ignored may be colonized (Webster & Waide, 1982). Thus, rhododendron may be an especially important resource during summer, when other leaves are relatively unavailable. Rhododendron has refractory leaves that can persist for long periods in streams (Monk et al., 1985; Huryn & Wallace, 1987; Whiles et al., 1993) and can comprise a large proportion of the leaves available in forested southern Appalachian streams (Stout, Benfield & Webster, 1993). Shredders that are active in spring and summer (e.g. Lepidostoma) may be especially reliant on slow-decaying leaf species such as rhododendron (Cummins et al., 1989).

This ‘resource island’ effect and subsequent concentration of insects on summer leaf packs may have led to the significant relationship observed between insect shredder biomass and AFDM remaining. There was a significant inverse relationship between shredders and leaf pack mass in the summer macroconsumer exclusion treatment (Fig. 4). Insects were the only macro-organisms eating leaves in this treatment and, as shredder biomass increased, AFDM remaining decreased. In the control treatment, however, we did not see this relationship, and we speculate that the effect of insect shredders was swamped by crayfish impacts.

During autumn, natural leaf litter availability was much higher (Table 2), and rhododendron leaf packs were relatively less important. Despite the fact that the biomass of insect shredders was much less on autumn leaf packs, the decay rate of rhododendron was faster. Physical fragmentation may have played a larger role in autumn, given that peak daily discharges were much greater. Paul & Meyer (1996) found that rhododendron decay was greatly enhanced following a flood. It seems likely that abiotic fragmentation (both in control and exclusion treatments) and crayfish effects (in the control treatment) were responsible for the majority of rhododendron breakdown in the autumn experiment. Thus, no significant relationships between insect shredder biomass and leaf pack mass were found in control or exclusion treatments in the autumn.

How do these results compare with other rhododendron breakdown experiments?

Many studies have examined rhododendron breakdown in southern Appalachian streams (Table 6). Our breakdown rates were similar to those reported in Hutchens (2000) and Paul & Meyer (1996), whether calculated by day or by degree day. For example, when the data in Hutchens (2000) are recalculated to obtain breakdown rates by degree day, a value of 0.003 is obtained (J.J. Hutchens, personal communication), while the data in Paul & Meyer (1996) yield a decay rate of 0.002. In comparison, our rhododendron decay rates calculated by degree day ranged from 0.001 to 0.003.

Most other studies, however, found much slower rhododendron breakdown rates (Table 6). With one exception (Paul & Meyer, 1996), previous studies were conducted in much smaller streams (first- and second- rather than fourth-order). Physical fragmentation by high flow may have been reduced in these headwater streams. In addition, Pteronarcy stoneflies are frequently absent from these headwater reaches (e.g. Grubaugh, Wallace & Houston, 1996). These large-bodied shredders are present in Lower Ball Creek, and their presence may have contributed to the faster breakdown rates when observed. Most previous studies also used leaf packs made from 5 mm or smaller mesh. This may have limited access by larger crayfish, which are thought to be more detritivorous than smaller individuals (Momot, 1995; Whitedge & Rabeni, 1997). Webster & Waide (1982) compared rhododendron breakdown at Coweeta between leaf bags (3 mm mesh) and packs (loosely tied with fishing line), and found that decay rates more than doubled when packs were used. Finally, our breakdown rates were accelerated by using
Table 6 Summary of other rhododendron breakdown studies conducted at or near Coweeta. All studies were conducted in first- or second-order streams unless otherwise noted. Treatment refers to any manipulation or disturbance of the study stream.

<table>
<thead>
<tr>
<th>Mesh size (mm)</th>
<th>Initial dry weight (g)</th>
<th>Duration</th>
<th>Treatment</th>
<th>Breakdown rate (day⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5</td>
<td>July-Aug</td>
<td>None</td>
<td>0.018</td>
<td>This study</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>Oct-Nov</td>
<td>None</td>
<td>0.037</td>
<td>This study</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>July-Aug</td>
<td>Electric exclusion</td>
<td>0.013</td>
<td>This study</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>Oct-Nov</td>
<td>Electric exclusion</td>
<td>0.017</td>
<td>This study</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>Dec-Aug</td>
<td>None</td>
<td>0.019</td>
<td>Hutchens (2000)</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>Dec-Aug</td>
<td>None</td>
<td>0.010</td>
<td>Hutchens (2000)</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>Oct-June</td>
<td>None</td>
<td>0.007</td>
<td>Paul &amp; Meyer (1996)</td>
</tr>
<tr>
<td>12</td>
<td>15</td>
<td>Oct-June</td>
<td>None</td>
<td>0.017*</td>
<td>Paul &amp; Meyer (1996)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Dec-Dec</td>
<td>None</td>
<td>0.005*</td>
<td>Chung, Wallace &amp; Grubaugh (1993)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Dec-Dec</td>
<td>Insecticide</td>
<td>0.002</td>
<td>Chung et al. (1993)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Dec-Dec</td>
<td>Insecticide recovery</td>
<td>0.006*</td>
<td>Chung et al. (1993)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Nov-June</td>
<td>None</td>
<td>0.002</td>
<td>Benfield et al. (1991)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>Nov-June</td>
<td>Forest disturbance</td>
<td>0.006*</td>
<td>Benfield et al. (1991)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Dec-Dec</td>
<td>None</td>
<td>0.004*</td>
<td>Cuffney, Wallace &amp; Lustgart (1990)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Dec-Dec</td>
<td>Insecticide</td>
<td>0.002</td>
<td>Cuffney et al. (1990)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Dec-Dec</td>
<td>Insecticide recovery</td>
<td>0.005*</td>
<td>Cuffney et al. (1990)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Feb-Feb</td>
<td>None</td>
<td>0.006</td>
<td>Wallace, Vogel &amp; Cuffney (1986)</td>
</tr>
<tr>
<td>3</td>
<td>2-4</td>
<td>Oct-Oct</td>
<td>None</td>
<td>0.004*</td>
<td>Webster &amp; Waide (1982)</td>
</tr>
<tr>
<td>3</td>
<td>2-4</td>
<td>Oct-May</td>
<td>Logging</td>
<td>0.001</td>
<td>Webster &amp; Waide (1982)</td>
</tr>
<tr>
<td>3</td>
<td>2-4</td>
<td>Oct-May</td>
<td>Logging recovery</td>
<td>0.011*</td>
<td>Webster &amp; Waide (1982)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Feb-Nov</td>
<td>None</td>
<td>0.005</td>
<td>Wallace, Webster &amp; Cuffney (1982)</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>Feb-Nov</td>
<td>Insecticide</td>
<td>0.001</td>
<td>Wallace et al. (1982)</td>
</tr>
</tbody>
</table>

*study conducted in a fourth-order stream; ¹-year (1985, 1988-1990) average in reference stream; ²-average from three streams draining disturbed catchments; ³-year (1984-1987) average in reference stream; ⁴-average from three sites within 800 m reach.

We attempted to account for this by comparing our decay rates with those obtained by Hutchens (2000) and Paul & Meyer (1996) for similar degree day periods (i.e. after their leaves had experienced 1300 degree days). In both studies, however, < 35% of rhododendron AFDM remained by the time 1300 degree days accumulated.

It is interesting to compare our results with other experimental manipulations. For example, Wallace et al. (1982) and Cuffney, Wallace & Lusthart (1990) found that rhododendron breakdown rates decreased by 62-78% in a headwater stream when shredders were greatly reduced by insecticide (Table 6). Our findings were similar, although the magnitude of change was not so great: when crayfish were excluded (here, by electricity), rhododendron breakdown was slowed by 33% in summer and by 54% in autumn. Whereas Wallace et al. (1982) and Cuffney et al. (1990) eliminated both insects and crayfish, our manipulation excluded only crayfish. Thus, our results suggest that a significant portion of decay rate decreases may be attributable to reductions in crayfish density.

In conclusion, crayfish play a significant role in the breakdown of rhododendron leaves during both summer and autumn, although rhododendron is considered a low quality food. The influence of other factors (e.g. shredding insects, abiotic fragmentation) varies between seasons. Crayfish exert a direct impact, increasing rhododendron decay via shredding rather than by altering biomass of insect shredders and/or predators. Even at the relatively low density found in Lower Ball Creek (2 m⁻²), crayfish are able to affect an ecosystem process such as leaf decay. Given the threatened status of many crayfish species in the United States, this finding is especially relevant. Even small alterations in crayfish assemblages, whether via loss of native species and/or introduction of exotic species, may have significant repercussions for ecosystem function.

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