Running head: Cellulose filter breakdown in streams

An assessment of cellulose filters as a standardized material for measuring litter breakdown in headwater streams

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ABSTRACT

The decay rate of cellulose filters and associated chemical and biological characteristics were compared to those of white oak (*Quercus alba*) leaves to determine if cellulose filters could be a suitable standardized material for assessing deciduous leaf breakdown in headwater streams. The comparison was done across reaches draining mixed deciduous forest and post-coal mining catchments, in natural and constructed channels, and ranged in flow duration from ephemeral to perennial. Decay rates of leaves and filters were predicted to differ at a given site, but the decay rates and associated characteristics of leaf and filter litterbags would be positively related. Filter decay rates did not differ across channel type or flow permanence class. Oak leaves decayed ca. 2.5X faster than cellulose filters and there was no relationship between decay rates (R^2 = 0.02). Ergosterol concentration, total invertebrate density, shredder density, total invertebrate biomass, and taxa richness were significantly higher in oak litterbags than in filter litterbags across four sampling dates over 366 d. The biomass of invertebrate shredders colonizing litterbags did not differ between the substrate types. The C:N content was higher for filters than for oak leaves, but the mean difference between substrates decreased by ~10-fold over the 306 d study. In contrast, mean differences in ergosterol concentration between substrates increased 3-fold over the study. Although characteristics associated with filter litterbags were positively related to those of oak leaf litterbags, most relationships had low explanatory power ($R^2 \leq 0.3$); however, stronger relationships existed for total invertebrate density, shredder density, and taxa richness (R^2 = 0.78). Although a standardized material would be useful for incorporating litter breakdown in stream assessments, because of the strong differences in decay rate and associated characteristics we cannot recommend cellulose filters as a suitable substrate to represent the natural breakdown of leaf material.

KEY WORDS: litter breakdown; cellulose decomposition; streams; functional assessment; standardization

INTRODUCTION

Leaf breakdown is a critical function in forested streams and has been extensively studied (e.g., Webster and Benfield, 1986; Gessner et al., 1999; Cummins, 2002). More specifically, leaf litter is a major energy source to forested streams and provides physical habitat, therefore the breakdown of this material is an integrative measure linking riparian vegetation, physical forces, and biological activity. For these reasons, litter breakdown has been endorsed as an ecosystem process for assessing the ecological health or condition of stream ecosystems (Gessner and Chauvet, 2002; Young et al., 2008). Although stream assessments have traditionally relied on structural indicators (e.g., water chemistry, habitat characteristics, macroinvertebrate assemblages) to characterize ecological health, there has been a recent movement to incorporate both structural and functional measures for regulatory purposes (e.g., Davies and Jackson, 2006).

Among the concerns with using functional measures like leaf breakdown for regulatory purposes have been within-treatment variability (i.e., leaf quality) and time investment needed, including the collection of abscised leaves (seasonally restricted in temperate regions). Standardization of carbon source addresses both of these problems. Cotton-strip assays have been used extensively in terrestrial systems (e.g., Latter and Howson, 1977; Harrison et al., 1988) and more recently in streams (e.g., Hildrew et al, 1984; Boulton and Quinn, 2000; Claret et al., 2001; Tiegs et al., 2007). Although previous studies have demonstrated that the cotton strip assay is a useful technique for estimating potential litter decay, particularly in reducing within site variation (Boulton and Quinn, 2000; Tiegs et al., 2007), the manufacturer (Shirley Soil Burial Test Fabric, Shirley Institute, Manchester, UK) no longer produces the standard cotton strips.

We chose to assess cellulose filters as a possible standardized alternative to cotton strips for estimating litter breakdown in headwater streams. Cellulose filters are a logical choice for a standard material because cellulose represents much (30-50%) of the plant material entering streams (Egglishaw, 1972). In forest soils, decay of cellulose filters decreased with increasing soil acidification and filter decay was comparable to natural organic matter decay under laboratory conditions (Bieńkowski, 1990). The goal of the present study was to determine if cellulose filters could be a standardized material for measuring deciduous leaf breakdown in headwater streams. Specifically, we compared decay rates and associated chemical and biological characteristics of cellulose filters and white oak (*Quercus alba*) leaves across sites with varying land cover (forest and post-coal mined) and flow duration. In a previous paper we reported how oak leaf breakdown varied across these sites (Fritz et al., *in review*). Here our primary focus was the paired comparisons of cellulose filter and white oak leaf breakdown at these sites. However, we also compared filter breakdown rates across land cover and flow duration to determine if differences were comparable to those we detected using white oak leaves. Because the filters are composed almost entirely of cellulose, we hypothesized that breakdown rates of leaves and filters would differ. However, we expected similar patterns across study sites, therefore we predicted there would be positive relationships for decay rate and associated attributes between filters and oak leaves.

METHODS

Study area

The six study catchments were located in the Central Appalachian level III ecoregion (Woods et al., 2002) of eastern Kentucky, U.S.A. within Breathitt County. The area is characterized by having a mixed mesophytic forest and a sandstone, siltstone, shale, and coal geology. The

dominant tree species on lower slopes of forested catchments were *Quercus alba*, *Liriodendron tulipifera*, *Tsuga canadensis*, and *Fagus grandifolia*, whereas *Q. velutina* and *Q. prinus* dominate the upper slopes (Phillippi and Boebinger, 1986). Two catchments (Falling Rock Branch and Little Millseat Branch) drained intact forest, whereas the other four catchments (Bee Branch, Guy Cove, Wharton Branch, and Williams Fork) drained reclaimed surface coal mines. Specifically, these catchments were mined using a combination of surface coal mining methods, including mountaintop removal (MTR), where the overburden or spoil overlying coal seams is removed and typically deposited into adjacent valleys creating valley fills (VFs). Often headwater streams are permanently buried under VFs. To meet regulatory standards, these VFs must be physically stable. In addition to compaction and terracing of VFs, constructed channels (i.e., groin drains) are built on top of VFs to carry surface runoff and prevent destabilization of the VFs. The bed and banks of constructed channels are built with durable, nonacid-, nontoxic-forming boulders (>1 m diameter). Since 2004, coal mine operators have received mitigation credit through the U.S. Army Corps of Engineers Clean Water Act Section 404 permitting program for the creation of these constructed channels to compensate for the permanent loss of the natural headwater channel buried under VFs.

There were a total of 19 study reaches (30 m long) across the six catchments. The reaches were positioned longitudinally along the six catchments, such that a range of flow permanence was captured within and among catchments (Table 1). Perennial streams have continuous surface flow, except during drought, and have streambeds that are always positioned below the groundwater table. Intermittent streams dry for part of the year, coinciding with the dry season(s), when the groundwater table drops below the streambed elevation. Ephemeral streams flow for short periods of time following heavy precipitation or snowmelt runoff, and

have streambeds always above the groundwater table. Electrical resistance loggers (Fritz et al. 2006) were used to measure the duration and frequency of dry periods at each reach. Briefly, these loggers record timing of binary state (dry or wet) changes at contact ends of a cable within stilling wells positioned at the streambed surface. Temperature was measured at 4-h intervals using StowAway TidbiT® temperature loggers (Onset® Computer Corp., Bourne, MA) also positioned at the streambed surface. Two VF catchments (Guy Cove and Williams Fork) had natural ephemeral channels upslope of the VFs, whereas the remaining intermittent and ephemeral channels in the VF catchments had constructed channels (Table 1). The perennial reaches within the MTR/VF catchments were located downstream of the VFs and all except for one (Williams Fork Pc) had natural channels. Williams Fork Pc was constructed immediately downstream from the VF and 130 m upstream from Williams Fork P (natural perennial reach). See Fritz et al. (*in review*) for more detail on the study reaches.

Litter decay and invertebrate colonization

We used a standard litter bag technique (Boulton and Boon, 1991) to measure breakdown rates of *Quercus alba* (white oak) and cellulose filters (Whatman® 1002-090, 90 mm Ø). White oak leaves were collected in aerial littertraps during September – October 2005, sorted from other species, combined among traps, and air dried (~20°C) for ca. 30 d in the laboratory. The cellulose filters used were not pre-treated with antimicrobial agents (Whatman technical personnel, personal communication). Nylon bags (30 x 35 cm) had 6-mm openings and were filled with either ~5.0 g of *Q. alba* leaves (4.17 g AFDM) or ~8.6 g of cellulose filters (8.58 g AFDM).

At the end of October, pairs of bags (oak and filter) were staked to the streambed surface in pool habitat throughout the study reaches. We chose pools because this was a common habitat

that retained surface water longer than other habitats in our study reaches. Three pairs of litterbags were randomly collected from each study reach at time 0 (to estimate handling loss), 21 (November), 82 (January), 166 (April), and 306 d (August). Litter bags were placed individually into resealable bags, stored on ice, and returned to the laboratory.

In the laboratory, litter bag contents were rinsed with tap water into a 250-µm sieve to separate leaves and filters from invertebrates. Invertebrates were placed into Whirl-Pak® bags and preserved with 75% ethanol prior to identification, measurement, and enumeration. We identified most aquatic taxa to genus (except chironomids to tribe, mites and oligochaetes to family, and meiofauna to suborder, order, or phylum), whereas terrestrial insects, snails, and spiders were identified to family and other terrestrial taxa to order or suborder (e.g., Diplopoda, Pseudoscorpiones, Oribatida). Invertebrate biomass was estimated using published allometric equations (e.g., Edwards, 1967; Sample et al., 1993; Benke et al., 1999).

Ergosterol concentration was used as a measure of fungal biomass within leaves and filters. Leaves and filters were subsampled for ergosterol concentration using a cork borer (9.5 mm Ø), subsamples were placed in methanol, and stored in a freezer until analysis (Montgomery et al., 2000). Ergosterol concentration was measured using an HP series 1100 HPLC with a Varian Microsorb MV (100 Angstroms) HPLC column and expressed on a µg/g AFDM basis. The remaining leaf and filter material was dried at 70 °C for at least 48 h, weighed, and ground to a fine powder using a mill. Separate subsamples of the ground material were weighed and used to determine percent AFDM remaining and carbon and nitrogen content (C:N). Subsamples for AFDM were ashed at 550 °C for 2 h to determine % AFDM. Total C (organic and inorganic) and N contents of subsamples were determined by dry combustion using a LECO CHN 2000 analyzer. The initial soluble, cellulose, and lignin fractions of filters and oak leaves were also

measured following the procedure outlined by Moorhead and Reynolds (1993) and Li et al. (2009). The samples were subjected to a sequential extraction procedure to determine the quantity that was (1) soluble in water and ethanol, (2) soluble in hot sulfuric acid, and (3) insoluble in hot sulfuric acid. Although this method has been recognized as not being as precise as other standard methods for cellulose and lignin analysis, these fractions measured by this method have been described as the soluble, cellulose and lignin fraction, respectively (Moorhead and Reynolds, 1993; Li et al., 2009).

Data analysis

Decay rates were calculated for 166 d, rather than the full 306 d because of litterbag loss at several sites. Also we were able to compare decay rates at only 18 of the 19 sites because of loss of filter litterbags at Wharton Branch I. Percent AFDM remaining was fitted to an exponential decay model to calculate a decay rate (k) for each reach and litter type based on the formula used by Huryn et al. (2002):

$$\text{decay rate} = [\ln(\text{final AFDM} / \text{initial AFDM})] / \text{cumulative degree days}.$$

The mean decay rate across litterbags of each type (oak and filter) from a reach was treated as a replicate. We compared litter decay rates between leaves and filters using a paired t-test. Next we compared filter decay rates across land use (forest and VF) and flow duration (ephemeral, intermittent and perennial) classes using a 2-way ANOVA (PROC GLM). For both of these analyses the average decay rate for each reach was treated as a replicate.

Variables associated with litter bags included litter C:N, ergosterol concentration, total invertebrate density (number of invertebrates per g of litter remaining), shredder density, total invertebrate biomass, shredder biomass, and taxa richness. The mean value across the bags of each type (oak and filter) from a reach at a time period was treated as the statistical unit for

comparisons of these variables. For each variable, we first compared the differences between litter pairs across the reaches and time periods using a repeated ANOVA to determine if differences were consistent across time. Where the differences between filters and leaves did not vary across time, we proceeded to assess the differences between substrate types with a 2-tailed t-test ($H_O \neq 0$). If differences between filters and leaves did vary across time, we examined the *t* statistic for the time period with the smallest difference. Next we assessed whether or not relationships existed between filters and oak leaves for these seven variables and if such relationships varied over time. To do this we used a repeated two-way general linear model ANOVA with time and filter values as independent variables and leaf values as response variables. Data were transformed where they did not meet assumptions of normal distributions or homogeneity of variances.

RESULTS

Oak leaves were found to exhibit soluble, cellulose, and lignin fractions of 42, 33, and 24.5%, respectively, whereas filters exhibited small soluble (1.9%) and lignin (4.3%) fractions and is largely comprised of cellulose (94%; Table 2). The coefficient of variation for the dry weight of cellulose filters and oak leaves within litterbags prior to deployment were 1.27% and 1.10%, respectively. Dry weight handling loss per litterbag was lower for cellulose filters (0.001 g ± 0.0004, mean ± 1 SE) than for oak leaves (0.654 ± 0.127). Oak leaves decayed ($-5.9 \times 10^{-4} \pm 8.64 \times 10^{-5}$, mean and range) on average ~2.5X faster than cellulose filters ($-2.4 \times 10^{-4} \pm 8.20$; $t = -3.65$, $p = 0.002$, $n = 18$) and there was no linear relationship between decay rates (Fig. 1). Decay rates of filters did not differ across land use and flow duration classes (full model: $F_{5,17} = 1.30$, $p = 0.325$). The discrepancy between decay rates of oak leaves and filters appeared to be

greater at intermittent and perennial reaches draining forested catchments than in ephemeral reaches and reaches draining MTR/VF reaches (Fig.1).

Differences in litter C:N and ergosterol concentration between cellulose filters and oak leaves varied across dates, where C:N differences between filters and leaves became smaller over time and ergosterol difference became larger over time (Fig. 2, Table 3). The patterns of C:N content in the substrates over time were a reflection of %C content of oak leaves declining more rapidly over time for oak leaves (slope ± SE = -0.054 ± 0.005) than for filters (-0.018 ± 0.004; t = 5.25, $p < 0.0001$) and large differences in %N content between substrates throughout the study (Fig. 2b). However, % N content increasing significantly faster in filters ($9.15 \times 10^{-4} \pm 1.15 \times 10^{-4}$) than in oak leaves ($3.30 \times 10^{-4} \pm 1.63 \times 10^{-4}$; $t = 2.90$, $p = 0.004$). All variables, except shredder biomass, differed between filters and leaves; C:N content was higher in filters than in oak leaves and ergosterol, total invertebrate density, shredder density, total invertebrate biomass, and taxa richness were lower in filter than in oak litterbags (Fig. 2 and 3; Table 3).

Most slopes of the relationships between characteristics of filter and oak litterbags did not vary across time (Table 4). An exception was total invertebrate density where there was a significant interaction with time and perhaps ergosterol concentration and taxa richess, where there were marginally significant interaction terms (Table 4). Except for C:N content, the slope between characteristics of filter and oak litterbags was significantly different from zero (i.e., Filter effect; Table 3). Although characteristics associated with filter litterbags were related to those for oak litterbags, the relationships were generally weak ($R^2 \leq 0.3$, Table 4). However, there were stronger relationships for total invertebrate density ($R^2 = 0.61$), shredder density ($R^2 = 0.58$), and taxa richness ($R^2 = 0.78$) between filter and leaf litterbags. Moreover, the correlation between taxa richness within filter and oak litterbags approached a 1:1 relationship (Fig. 3e).

DISCUSSION

Our study confirmed one advantage of a standardized material, specifically handling loss was much lower and less variable than natural leaves. Although a standardized surrogate for leaf litter may enhance the utility of decomposition as a measure in stream assessments, based on our findings we cannot recommend using cellulose filters as a suitable substrate in forested headwater streams for several reasons. First, oak leaf decay rates were significantly faster than those of filters. Second, decay rates of oak and filters were not correlated across a range of environmental conditions seen in our stream reaches. Lastly, and perhaps most importantly, we did not detect difference in filter decay between land use and flow duration classes. This contrasts with the differences we detected for oak leaf decay, where decay was faster at perennial and intermittent reaches in forested catchments than at all reaches in post-mined catchments and decay was slower at forested ephemeral than at intermittent and perennial reaches in the forested catchments (Fritz et al., *in review*).

We suspect the difference in decay rates between leaves and filters stemmed from differences in C:N content and this explained differences in ergosterol concentration between the litter types. However, differences in the level of fungal establishment on the litter types at the onset of the study could also have contributed to the differences in ergosterol concentration over the period of study. Although we did not consider bacterial communities in this study, they were likely a major contributor to filter decay and changes in C:N content. Physical differences (i.e., sturdiness) between leaves and filters appeared to be important in determining handling loss; however at sites where litter packs were submerged individual filters were observed to have less tensile strength than leaves. Although the C:N content of cellulose filters declined over time,

oak leaves had significantly lower C:N than cellulose filters throughout the study. Previous studies have shown the chemical composition of organic matter strongly influenced rates of decay (e.g., Petersen and Cummins, 1974; Ostrofsky, 1997) and nutritional quality to consumers (e.g., Quinn et al., 2000; Tuchman et al., 2003). Mass loss of cellulose cloth amended with nitrogen was 50-100% greater than on cellulose cloth without nitrogen (Friberg and Winterbourn, 1997). Although we did not quantify phosphorus content of filters, it was likely to be very low (at least initially) and also may have contributed to lower breakdown rates compared to leaves. Because oak leaves in our study contained higher lignin content than filters and presumably had other chemical components (e.g., tannins, cutin) expected to slowly decay that were lacking or at lower levels in the cellulose filters, C:N may be a more fundamental factor affecting litter decay.

Differences in C:N and ergosterol may have contributed to invertebrate assemblage differences between litter types. Both of these factors contribute to the palatability of leaf litter by shredding invertebrates (Bärlocher and Kendrick, 1975; Enríquez et al., 1993; Graça, 1993) Stream invertebrates use litter packs not only as food source, but as habitat or refuge (e.g., Holomuzki and Hoyle, 1990; Richardson, 1992). Available surface area likely differed between litter types and may explain the differences in total invertebrate density and biomass within litter bags. Filters tended to stick together, perhaps making access between filters more difficult and more inhospitable than between the more irregular shaped oak leaves. This was particularly evident at perennial sites below VFs where the interior filters stacked within litterbags were visibly blackened from anoxic conditions. We did not detect differences in shredder biomass between litter types largely because of three filter bags collected in April that had substantially higher shredder biomass than paired oak bags. All three of these filter bags were collected in

mined reaches and contained large, rare individuals (e.g., tipulid larvae and millipedes) that represented the bulk of shredder biomass.

Although invertebrate taxa richness was higher in oak than in filter litterbags, this measure had the strongest relationship between litter types and was the closet endpoint to a 1:1 relationship between filter and leaf litterbags. This suggests that the diversity of invertebrates colonizing litterbags is more strongly influenced by surrounding habitat and season than litterbag contents. In fact, taxa richness of oak litterbags in perennial and intermittent reaches were higher in forested catchments than in mined catchments (Fritz et al. *in review*). Litterbag deployment, in general, may be a feasible alternative to traditional invertebrate sampling techniques in intermittent and ephemeral forested channels that rely on the presence (e.g., Surber, dipnets) or absence of water (e.g., pitfall traps).

Given the findings of this study, we recommend either a different standard substrate and/or technique (e.g., tensile strength, respiration, enzyme activity) be used for headwater stream assessments. Rather than measuring mass loss, many studies using cotton strips as a standardized material have measured tensile strength as indicator of degradation (Hildrew et al., 1985; Boulton and Quinn, 2000; Tiegs et al., 2007). Egglishaw (1972) demonstrated a positive curvilinear relationship between loss of mass and tensile strength of cotton canvas across six Scottish streams. However, in stream studies using Shirley burial cloth such relationships either did not exist (Boulton and Quinn, 2000) or were weak because of large differences in sensitivity between mass loss and tensile strength (Tiegs et al., 2007). Artist canvas (Fredrix brand 12-ounce duck, style number 548) was assessed recently as a new standard material in marsh soils and was comparable to Shirley burial cloth in loss of tensile strength (Slocum et al., 2009). Slocum et al. (2009) point out that because this material has a consumer base outside of

environmental assessment, the material should be reliably available. Regardless, the ideal standard material and technique should be sensitive to major stressors affecting leaf decay. In our study cellulose filter decay was not correlated with oak leaf decay nor was it as sensitive to gradients of land use and flow duration.

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Table 1. Characteristics of the 19 study reaches in east-central Kentucky. P = perennial, I = intermittent, and E = ephemeral. The overall period of record for dry periods was 346-349 days (electric resistivity loggers were deployed ~42 d before litter bags). Conductivity and pH are mean values from in situ measurements (Quanta HydroLab®, Hach Company, Loveland, CO) taken during site visits. Values in parentheses represent number of in situ measurements taken. VF = valley fill. na = not available.

Study reach	Latitude (˚N)	Longitude (˚W)	Area (ha)	Catchment treatment	Channel	% of period dry	# dry periods	Conductivity (mS/cm)	pH
Bee Branch P	37.43614	83.17154	45.8	VF	natural below VF	0	0	2.150 (6)	6.90
Bee Branch I	37.43484	88.17332	12.2	VF	constructed	95.5	48	0.678 (1)	6.1
Bee Branch E	37.43459	88.17455	10.2	VF	constructed	99.8	7	na	na
Falling Rock Branch P	37.47469	83.13525	88.0	Forest	natural	0	0	0.054 (6)	6.52
Falling Rock Branch I	37.47374	83.12942	19.7	Forest	natural	1.8	12	0.063 (6)	6.55
Falling Rock Branch E	37.47369	83.12644	2.1	Forest	natural	98.6	9	na	na
Guy Cove P	37.41833	83.17157	42.9	VF	natural below VF	0	0	2.330 (6)	6.45
Guy Cove I	37.41705	83.17245	19.0	VF	constructed	57.8	59	0.547 (2)	6.70
Guy Cove E	37.41012	83.17498	1.7	Forest	natural	54.3	62	0.876 (1)	7.22
Little Millseat Branch P	37.47417	83.15398	75.7	Forest	natural	0.5	3	0.059 (6)	6.71
Little Millseat Branch I	37.47825	83.16584	10.1	Forest	natural	19.7	11	0.050 (4)	6.62
Little Millseat Branch E	37.47690	83.16787	1.8	Forest	natural	98.1	17	0.047 (1)	6.99
Wharton Branch P	37.42505	83.17520	44.1	VF	natural below VF	0	0	3.093 (6)	6.69
Wharton Branch I	37.42590	83.17527	20.4	VF	constructed	34.4	35	0.621 (1)	6.4
Wharton Branch E	37.42887	83.16892	0.1	VF	constructed	100	1	na	na
Williams Fork P	37.42350	83.15816	37.3	VF	natural below VF	0	0	2.389 (6)	6.64
Williams Fork Pc	37.42220	83.15839	33.5	VF	constructed below VF	0	0	2.548 (6)	6.53
Williams Fork I	37.42142	83.15817	15.6	VF	constructed	60.8	52	2.580 (1)	5.15
Williams Fork E	37.41600	83.15996	1.9	Forest	natural	98.8	12	0.046 (1)	6.62

Table 2. Mean (SD) chemical fractions of initial *Quercus alba* leaves and cellulose filter paper.

Substrate	Soluble (%)†	Cellulose (%)‡	Lignin (%)*
Q. alba leaves	42.14 (1.11)	33.27 (1.16)	24.58 (1.32)
Cellulose filters	1.89 (0.07)	93.79 (1.15)	4.32 (1.08)

† Soluble (%) in hot water and ethanol.

‡Soluble (%) in sulfuric acid (72%).

*Insoluble (%) in sulfuric acid (72%).

Table 3. Repeated ANOVA results comparing the differences in measures from litterbags with cellulose filter and white oak leaves across time. Adjusted Tukey post-hoc results are shown where differences were significant. N = November (21 d), J = January (82 d), Ap = April (166 d), and Au = August (306 d). * = $p < 0.05$ for 2-tailed t-test for date with lowest estimated difference; ** = $p < 0.05$ for 2-tailed t-test of differences across all dates.

Variable	*F*	*p*	Post-hoc
C:N[1]	10.29 (3, 65) *	<0.0001	N^{a} J^{ab} Ap^{b} Au^{c}
Ergosterol[2]	15.90 (3, 62) *	<0.0001	N^{a} J^{a} Ap^{b} Au^{b}
Total invertebrate density[1]	0.50 (3, 64) **	0.68	
Shredder density[1]	2.18 (3, 64) **	0.10	
Total invertebrate biomass[1]	0.04 (3, 64)**	0.99	
Shredder biomass[1]	0.12 (3, 64)	0.95	
Taxa richness	1.84 (3, 64) **	0.15	

[1] cube root transformed, [2] log transformed

Table 4. General linear model with repeated measures predicting characteristics of oak leaf litterbags (response) from characteristics of cellulose filter litterbags over time.

Variable	Effect	*F*	*p*	R^2
C:N[1]	Filter	1.44 (1, 59)	0.23	0.26
	Time	1.55 (3, 59)	0.21	
	Filter x time	0.96 (3, 59)	0.42	
Ergosterol[2]	Filter	5.59 (1, 57)	0.02	0.26
	Time	1.62 (3, 57)	0.20	
	Filter x time	2.61 (3, 57)	0.06	
Total invertebrate density[1]	Filter	63.70 (1, 60)	<0.0001	0.61
	Time	2.93 (3, 60)	0.04	
	Filter x time	3.20 (3, 60)	0.03	
Shredder density[2]	Filter	79.30 (1, 60)	<0.0001	0.58
	Time	1.08 (3, 60)	0.36	
	Filter x time	0.24 (3, 60)	0.87	
Total invertebrate biomass[2]	Filter	16.22 (1, 60)	0.0002	0.28
	Time	1.15 (3, 60)	0.34	
	Filter x time	1.12 (3, 60)	0.35	
Shredder biomass[2]	Filter	25.51 (1, 60)	<0.0001	0.30
	Time	1.07 (3, 60)	0.37	
	Filter x time	1.72 (3, 60)	0.17	
Taxa richness[3]	Filter	186.08 (1, 60)	<0.0001	0.78
	Time	3.16 (3, 60)	0.03	

	Filter x time	2.61 (3, 60)	0.06

[1] = log transformed, [2] = $y^{0.25}$ transformed, [3] = square root transformed

Figure headings

Figure 1. Mean decay rates for cellulose filters and *Quercus alba* leaves across 18 headwater stream reaches of different flow duration classes (ephemeral, intermittent and perennial) and draining forested and mined catchments. Solid line represents 1:1 relationship.

Figure 2. Mean (±1 SE) C:N content (a), %C (open) and %N (closed) content (b), and ergosterol concentration (c) of cellulose filters (squares) and *Quercus alba* leaves (circles) from 19 headwater stream reaches collected over 4 time periods. C:N, %C, and %N content at time 0 are also shown.

Figure 3. Mean total invertebrate density (a), mean shredder density (b), mean total invertebrate biomass (c), mean shredder biomass (d), and mean taxa richness (e) for litterbags containing cellulose filters and *Quercus alba* leaves across 19 headwater stream reaches over the four collection periods. Solid line represents 1:1 relationship. Note value in parentheses for associated point in (a).

Figure 1

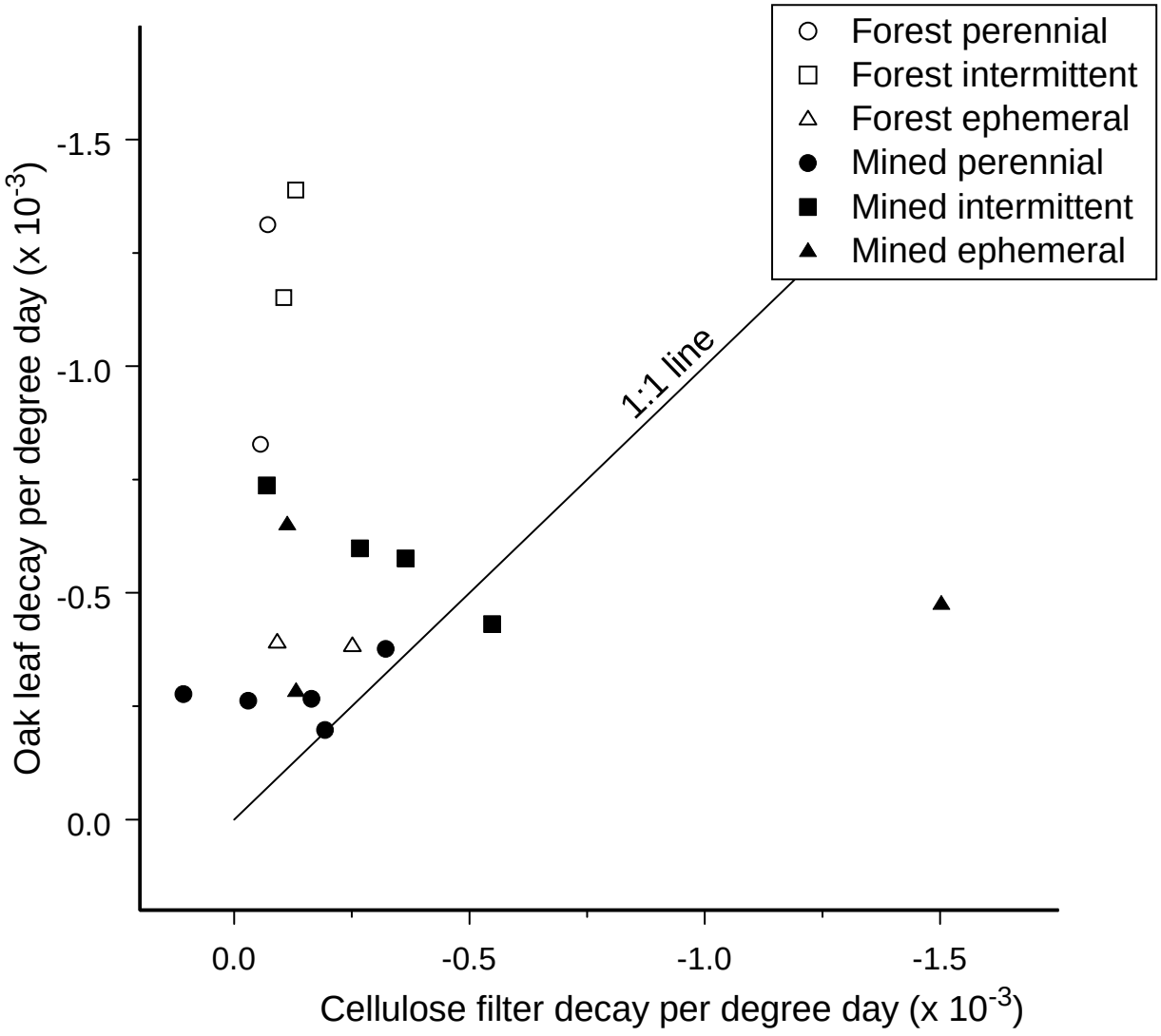

Forest perennial
Forest intermittent
Forest ephemeral
Mined perennial
Mined intermittent
Mined ephemeral
1:1 line
Oak leaf decay per degree day (x 10-3)
Cellulose filter decay per degree day (x 10-3)
-1.5
-1.0
-0.5
0.0
0.0
-0.5
-1.0
-1.5

Figure 2

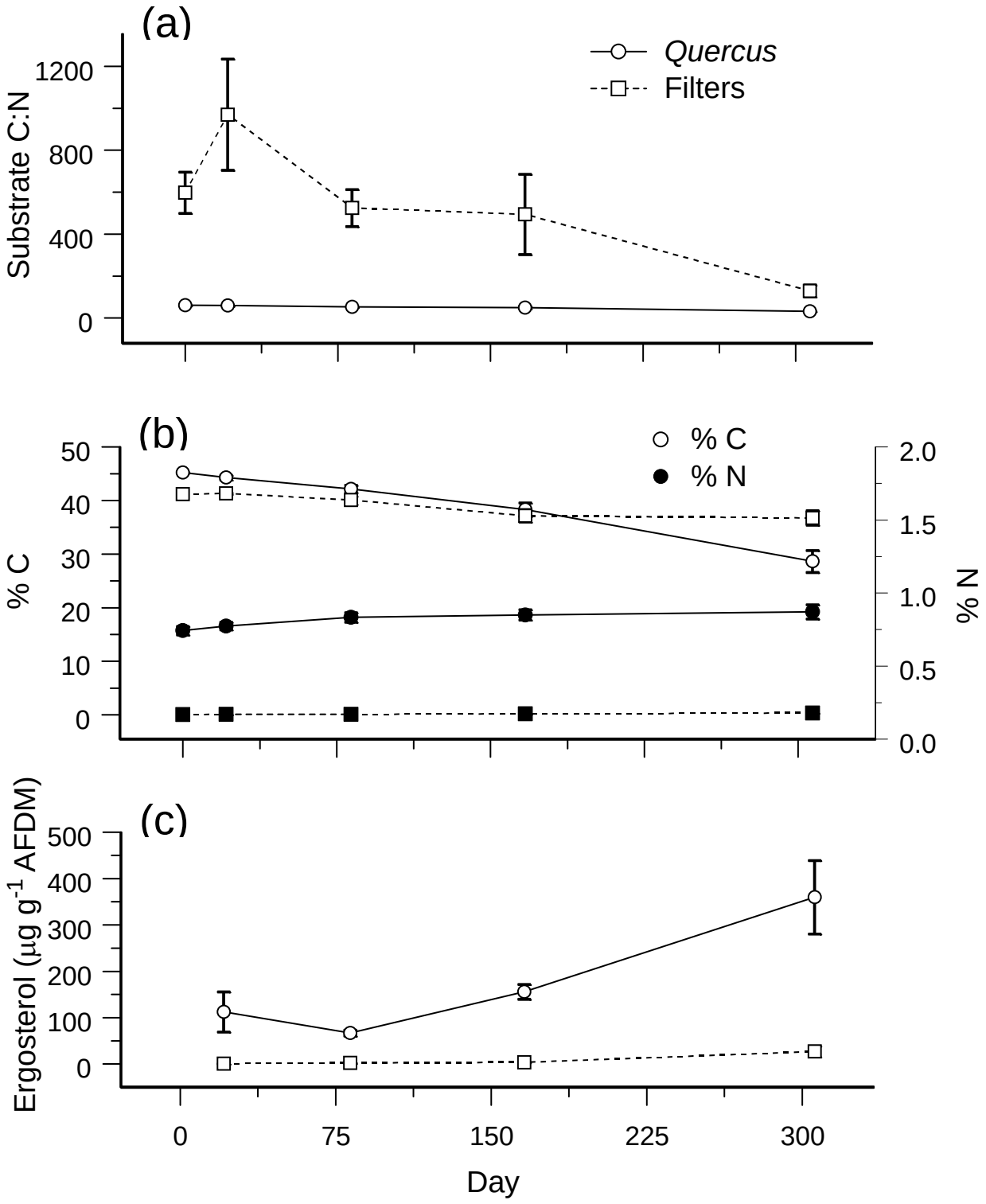
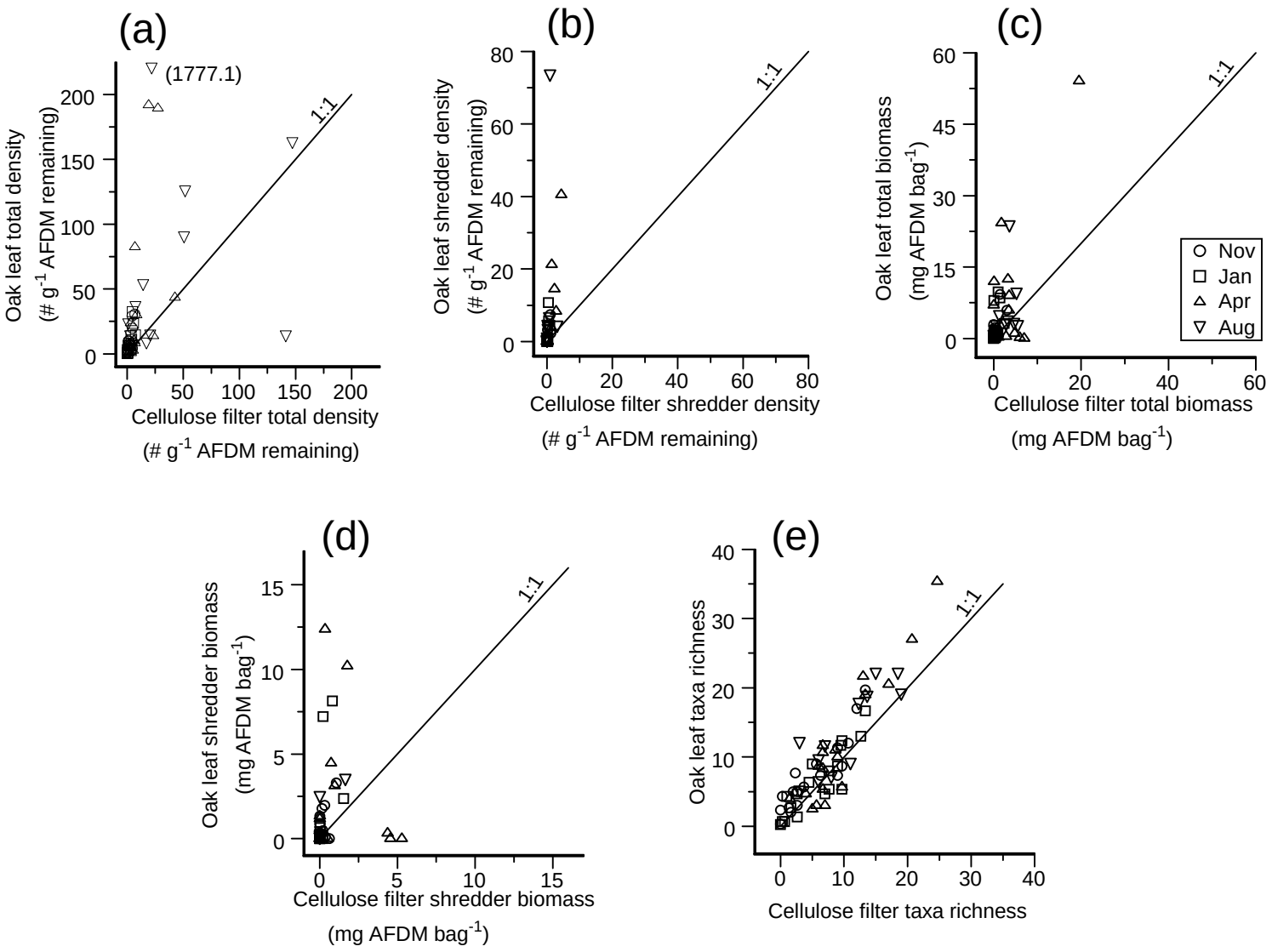

(a)
Substrate C:N
1200
800
400
0
Quercus
Filters
(b)
% C
% N
50
40
30
20
10
0
2.0
1.5
1.0
0.5
0.0
(c)
Ergosterol (µg g-1 AFDM)
500
400
300
200
100
0
0
75
150
225
300
Day

Figure 3


(a)
(1777.1)
1:1
Oak leaf total density
(# g-1 AFDM remaining)
Cellulose filter total density
(# g-1 AFDM remaining)
(b)
1:1
Oak leaf shredder density
(# g-1 AFDM remaining)
Cellulose filter shredder density
(# g-1 AFDM remaining)
(c)
1:1
Oak leaf total biomass
(mg AFDM bag-1)
Cellulose filter total biomass
(mg AFDM bag-1)
Nov
Jan
Apr
Aug
(d)
1:1
Oak leaf shredder biomass
(mg AFDM bag-1)
Cellulose filter shredder biomass
(mg AFDM bag-1)
(e)
1:1
Oak leaf taxa richness
Cellulose filter taxa richness