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Effects of drying regime on microbial colonization and shredder preference in seasonal woodland wetlands

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SUMMARY

1. Energy budgets of wetlands in temperate deciduous forests are dominated by terrestrially derived leaf litter that decays under different drying conditions depending on autumn precipitation. We compared decay rates and microbial colonization of maple leaves under different inundation schedules in a field experiment, and then conducted a laboratory study on shredder preference. In the field, litter bags either remained submerged (permanent), were moved to a dried part of the basin once and then returned (semi-permanent), or were alternated between wet and dry conditions for 8 weeks (temporary).
2. There was no difference in decay rates among treatments, but leaves incubated under permanent and semi-permanent conditions had higher fungal and bacterial biomass, and lower C : N ratios than those incubated under alternating drying and wetting conditions.
3. To determine the effects of these differences in litter nutritional quality on shredder preference, we conducted a laboratory preference test with larvae of leaf-shredding caddisflies that inhabit the wetland. Caddisflies spent twice as much time foraging on permanent and semi-permanent litter than on litter incubated under temporary conditions.
4. There is considerable variation among previous studies in how basin drying affects litter breakdown in wetlands, and no previous information on shredder preference. We found that frequent drying in a shallow wetland reduces the nutritional quality of leaf litter (lower microbial biomass and nitrogen content), and therefore preference by invertebrate shredders. These results suggest that inter-annual shifts in drying regime should alter detritus processing rates, and hence the mobilization of the energy and nutrients in leaf litter to the wetland food web.

Keywords: caddisfly, drying, litter, microbial processing, shredders, wetlands

Introduction

Leaf litter inputs dominate the energy budgets of forested wetlands (e.g. deepwater swamps, riparian forests, shallow woodland pools), and there has been considerable research on input schedules and decay rates (Brinson, Lugo & Brown, 1981; Boon, 2006; Palik, Batzer & Kern, 2006). However, there is a striking lack of information about the biological pathways by which this allochthonous energy is transferred to the aquatic food web (Oertli, 1993; Batzer & Palik, 2007), especially compared to the extensive research on head water streams in forested landscapes (Webster & Benfield, 1986; Wallace *et al*., 1997; Webster & Meyer, 1997). The 'stream paradigm' for leaf litter decay emphasizes the interdependence of microbial and invertebrate processing, and the multiple trophic pathways by which this energy is incorporated into stream food webs (reviews by Wallace *et al*., 1997; Graça, 2001; Hershey *et al*., 2006). Although it is assumed that these processes are similarly important for litter processing in wetlands, the absence of

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physical fragmentation and differences in dissolved oxygen levels and community composition between running and standing waters make that assumption tenuous (Batzer & Wissinger, 1996; Wissinger, 1999).

In forested headwater streams, one of the key steps in litter processing is colonization by fungi and bacteria that 'condition' leaf litter for primary animal consumers, especially aquatic insects and other invertebrates collectively known as shredders (Anderson & Sedell, 1979; Cummins *et al*., 1989; Benfield, 2006; Merritt & Cummins, 2006). Stream shredders preferentially colonize conditioned leaf litter, and their growth, development, and secondary production depends mainly on the nutritional value of the microbes rather than on the structural carbon (e.g. cellulose, lignin) of the litter *per se* (Bärlocher, 1985; Graça, Maltby & Calow, 1993; Friberg & Jacobsen, 1999) Stream shredders are an important direct trophic link between litter and predatory invertebrates and fish, and mobilize dissolved and fine particulate organic matter used by other primary consumers (Hershey *et al*., 2006; Merritt & Cummins, 2006). Comparably little is known about the role of shredders in wetlands. Because some wetlands do not have readily identifiable shredders, it is has been suggested that animal processing of leaf litter is relatively unimportant compared to streams (Brinson *et al*., 1981; Batzer & Wissinger, 1996). In other types of wetlands such as temperate woodland pools, invertebrate shredders abound (e.g. tipulid fly larvae, amphipods, isopods, caddisflies; see Bärlocher, Mackay & Wiggins, 1978; Higgins & Merritt, 1999; Schneider, 1999; Wissinger, Brown & Jannot, 2003; Batzer *et al*., 2005), but their role in litter breakdown and the degree to which they are dependent on microbial processing has been largely unstudied.

Seasonal woodland wetlands in northeastern North America (also referred to as vernal pools, seasonal woodland ponds, seasonal forest pools and autumnal ponds; see Colburn, 2004; Brooks, 2005; Williams, 2005) are often inhabited by cased caddisflies (Trichoptera) in the families Limnephilidae and Phryganeidae (Wiggins, Mackay & Smith, 1980; Oertli, 1993; Batzer, Palik & Buech, 2004; Batzer *et al*., 2005). Larvae of these caddisflies use leaf litter for the construction of their portable cases and as a primary source of energy, at least during early larval instars (Winterbourn, 1971; Berté & Pritchard, 1986; Wissinger, Eldermire & Whissel, 2004b). Dietary data at our study sites reveal that some caddisfly species feed primarily on leaf litter throughout larval development (e.g. *Limnephilus indivisus* (Walker), *Nemotaulius hostilis* (Hagen), whereas others shift ontogenetically towards increasing carnivory [e.g. *Banksiola dossuaria*(Say), *Ptilostomis ocellifera*(Walker)] (S. Wissinger, M. Mumford & S. Angelo, unpubl. data). The life cycles of caddisflies that live in woodland wetlands appear to be timed to the seasonal pattern of filling and drying, as well as the flush of leaf litter inputs in autumn (Wiggins, 1973; Wiggins *et al*., 1980). At our study site, pupation and adult emergence occur in spring, and females enter an ovarian diapause until late summer when they deposit desiccation-tolerant egg masses in dried wetland basins (S. Wissinger, M. Mumford & S. Angelo, unpubl. data). When basins fill in autumn, larvae emerge and begin to forage on the flush of food that accompanies deciduous leaf fall. Although cased caddisflies are assumed to play an important role in detrital processing in standing water habitats (Bärlocher *et al*., 1978, Whiles, Goldowitz & Charlton, 1999), nearly all of the research on their foraging ecology, food preferences, and role(s) as primary detrital consumers has been conducted in streams (e.g. Sinsabaugh, Linkins & Benfield 1984; Jacobsen & Sand-Jensen, 1994; Graça *et al*., 2001; Eggert & Wallace, 2003).

The purpose of this study was to determine how differences in drying regime affect (1) microbial colonization and decay of leaf litter and (2) preference by caddisfly shredders that inhabit seasonal woodland wetlands. Historical filling and drying records at our study site combined with life history and dietary data for the caddisflies were used to design ecologically relevant experimental treatments (see Methods below). We were particularly interested in determining how drying affects leaf litter breakdown rates, microbial colonization and preference by shredders. We first conducted a field experiment in which we manipulated the drying regime of red maple (*Acer rubrum*) leaf litter in a natural wooded wetland and compared how drying/rewetting affected decay rates and several measures of nutritional value to shredders (C : N ratio, fungal biomass and bacterial biomass; Anderson & Cargill, 1987; Graça *et al*., 2001). We then conducted a laboratory experiment in which we determined whether caddisfly larvae (*Nemotaulius hostilis*) exhibited preferences for detritus incubated under the different drying regimes in the field

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experiment. We could not easily make *a priori* predictions about the effect of drying, given the variable results in the literature; i.e. previous studies have found that drying can increase, decrease, or have no effect on decay rates and leaf litter palatability across a range of wetland habitat types (see discussion by Brinson *et al.*, 1981; Boon, 2006). Our results provide evidence for how inter-annual variation in drying regime should affect litter decay, microbial colonization, and processing by invertebrate shredders in seasonal woodland wetlands.

Methods

Study Site

The field component of this study was conducted in a small (basin filled area = 150 m^2; maximum depth = 0.5 m) woodland wetland at the Bousson Environmental Research Reserve of Allegheny College in northwestern Pennsylvania (see Wissinger & Gallagher, 1999 for more detail). This is one of eight woodland pools that we have been studying since 1995 in a mixed deciduous (maple-beech-ash) and conifer (hemlock-white pine) old growth forest. Hydroperiods in these wetlands are driven mainly by fluctuations in precipitation and shallow groundwater perched on till and glaciofluvial deposits (see Brooks 2005). This particular basin has dried in nine of the past 11 years during late summer (S.A. Wissinger, unpubl. data). Filling typically occurs in autumn after deciduous leaf fall. In some years, the basin remains filled until the following summer, but in other years, drying occurs one or more times before it freezes in winter. Caddisfly larvae survive these short drying periods by burrowing in the moist leaf litter (S. Wissinger, unpubl. data). Surface water in the basin is typically circumneutral to slightly acidic (6.0–6.8) with relatively high conductivity (100 μs cm^{-1}) and alkalinity (110 mg L^{-1}) as a result of sedimentary glacial soils (for additional habitat and water chemistry details for adjacent wetlands see Wissinger & Gallagher, 1999).

Litter bag field experiment

To determine the effects of drying regime on microbial colonization and litter breakdown rates, we manipulated drying by moving litter bags in and out of the wetted portions of the basin. We used red maple (*Acer rubrum*) leaves because of their high abundance in the litter of the wetland basin, and in the diet of the caddisflies (S. Wissinger, unpubl. data). Previous work on leaf decay rates in a nearby pond suggested red maple leaves would provide a suitable substrate for observing appreciable litter decomposition before winter (Ostrofsky, 1997). Newly abscised autumn-shed red maple leaves were collected from the dried wetland basin in late September 2006 and air-dried for 1 week. This drying procedure was ecologically relevant for our study site; i.e. leaves often fall into dry basins, and then are subsequently inundated (see Discussion by Boon, 2006). We constructed litter bags by placing 10 + 0.05 (SE) g of dried maple leaves in 15 × 15 cm litter bags constructed of fibreglass window screen (1 mm^2mesh) (after Ostrofsky, 1997). We used small mesh to reduce/eliminate detritivory by newly hatched cased caddisfly larvae (mainly *Limnephilus indivisus*, *Ptilostomis ocellifera* and *Nemotaulius hostilis*) and other detritivorous invertebrates. The litter bags were randomly assigned to one of three inundation schedules (Fig. 1) that reflected historical inter-annual variation in hydroperiod in this basin. Initial oven-dried leaf mass [9.13 + 0.23 (SE) g] was obtained from drying and weighing six 10 g air-dried litter packets (drying methods below) identical to those used to assemble the litter bags.

Prior to the experiment, the wetland had been dry from late July until early October when it filled with autumn rains. Inundation schedule was manipulated

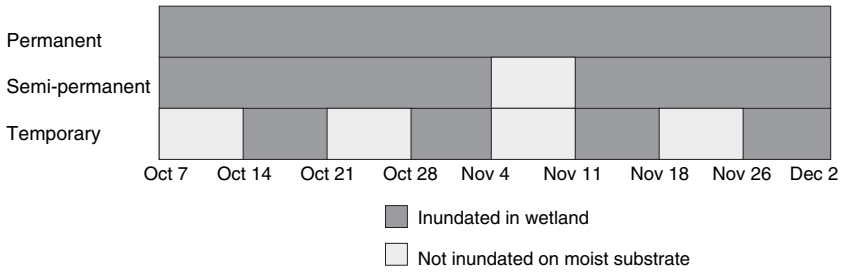

Fig. 1 Schematic representation of the three inundation treatments for incubating autumn-shed leaves in a small woodland wetland.

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by placing litter bags in the centre of the basin and then moving them to and from marginal dried areas on the periphery of the basin (Fig. 1). The three inundation schedules were (1) permanent = lifted off the bottom, but retained below waterline, and then replaced, (2) semi-permanent = dried once for 1 week, and (3) temporary = weekly alternating wet-dry. These treatments mimicked the range of observed inter-annual variation in drying after leaf fall in this basin (S. Wissinger unpubl. data).

After 8 weeks, the litter bags were removed from the wetland and 18 packs (6 replicates × 3 treatments) were immediately weighed to estimate litter decay rates. The litter was dried at 50 °C and weighed to the nearest 0.001 g on a Denver Instruments TR 102 balance (Denver, CO, U.S.A.). We compared average final mass among treatments using a one-way ANOVA after testing for departures from normality and homoscedasticity. All statistics were conducted using Statview (SAS, 1999). We calculated decay constants (k) assuming that leaf decay followed the general form $\ln(W_t/W_o)/t = -k$, where W_t and W_o were final and initial leaf masses, respectively, and t was time in days.

Laboratory experiment on shredder preference

To determine the effect of the different drying schedules on shredder preference, we conducted a laboratory experiment using 5th (final) instar larvae of the cased caddisfly *Nemotaulius hostilis* (Hagen), one of several detritus-shredding species in wooded wetlands at our study sites (Wissinger & Gallagher, 1999). This species develops rapidly in autumn and dietary analyses indicated that leaf litter composed 89.65 (±11.5%SD; $n = 17$) of the stomach contents of 5th instars during late autumn (S. Wissinger, unpubl. data). The experimental arenas were designed to present the caddisfly larvae with a choice among litter samples incubated under the three drying schedules in the field experiment. Each aquarium (37.85 L) was fitted with a 5 cm thick styrofoam floor with depressions into which 200 mL cups were inserted so that the top edges were flush with the floor. Four of the 12 cups in each aquarium were randomly assigned to one of the three field treatments and filled with 10 (±0.08 SE) g of detritus from the field-incubated leaf litter immediately after retrieval from the wetland. The remaining detritus in each litter bag was frozen (−20 °C) for subsequent microbial assays (see below). A coarse metal screen (mesh size 1 cm^2) was placed on the detritus to prevent it from floating out of the containers, and a 4 cm^2 opening was cut to facilitate access for the caddisfly larvae. Caddisflies could forage on the detritus through the top of the screen or crawl into the cups through the large opening. Aquaria were filled to a depth of 15 cm with filtered water from the study site, housed in a climate-controlled chamber (12 : 12 day–night; 08:00–20:00 hours day; 10 °C), and gently aerated with a single air stone connected to a common air supply.

Six 5th instar *Nemotaulius hostilis* caddisfly larvae were placed in each aquarium. After an initial day for caddisfly acclimation, the location and behaviours of each of these large, conspicuous caddisflies were recorded three times during the 12-daylight period (09:00; 14:00; 19:00 hours) for 6 days. We conducted a short-term experiment to minimize the confounding effects of convergence in leaf-litter quality after removal from the field. The following categories were recorded and assigned a value corresponding to the strength of evidence for caddisfly preference for a particular type of detritus: 3 = grazing in or on detritus within a leaf pack; 2 = grazing on top of the leaf pack through the screen from the edge of the well; 1 = near or on a well, but not currently grazing; and 0 = located on the intervening substrate between detritus wells. A simple index of preference for different leaf litter in each tank was recorded as the sum of the values for the six caddisflies. The maximum value for a treatment within a tank was 18 (six caddisflies each in and grazing on detritus of that treatment) and the minimum was zero (six caddisflies not foraging in or near any of the detritus wells). Preliminary analysis (three-way ANOVA time of day × date × treatment) indicated no time of day effect within or among treatments or days (all $P >> 0.05$); thus, shredder preference in the final analysis was compared among litter incubation treatments across the 6 days using two-way ANOVA (date × treatment).

Fungal biomass

Fungal biomass in the field-incubated leaf litter was used to determine (1) how inundation schedule affected fungal colonization and (2) the degree to which fungal biomass was correlated with caddisfly preferences. Fungal biomass was determined using a standard ergosterol technique (Newell, Arsuffi &

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Fallon, 1988; Gessner & Newell, 2002; Gessner, 2005). Fragments of detritus were lyophilized (SPD 1010 Speed Vac System; GMI, Inc., Minneapolis, MN, U.S.A.) to create 10 replicate 500 mg samples for each of the field treatments. The 30 samples were ground into fine fragments using a Wiley mill to increase the precision of dry mass measurements, to minimize variation within samples, and to improve ergosterol extraction efficiency (no. 20 screen; Thomas Scientific, Swedesboro, NJ, U.S.A.) (Gessner & Newell, 2002). The samples were then individually placed in a continuous solid phase extraction apparatus for 65 min. Each sample was extracted using 75 mL of 1% potassium hydroxide in methanol. Sterols were separated by partitioning into hexane, and the latter removed by 10 min of vacuum condensation (Buchi Rotavapor R-3000; BÜCHI Labortechnik, Flawil, Switzerland). The dried samples were re-dissolved in 3 mL of methanol and filtered through a 0.2 μm pore nylon syringe filter. An aliquot of 50 μL of each sample along with standards were analysed using an Agilent 110 series HPLC machine (Agilent Technologies, Santa Clara, CA, U.S.A.) using a 250 mm by 4.6 mm C18 column (Phenomenex Hyperclone 5 μ ODS C18; Phenomenex, Torrence, CA, U.S.A.). The ergosterol standards (Sigma-Aldrich, St. Louis, MO, U.S.A.) were used to construct a standard curve for the regression of the ergosterol peak against ergosterol concentration ($\mu g\ mL^{-1}$) (peak occurred at 8.4 min; see Gessner, 2005). This regression (ergosterol peak $= 1.265 \times$ [ergosterol] $- 0.872$; $R^2 = 0.99$) was used to estimate the concentration of ergosterol in the samples. Fungal biomass was inferred by using a conversion factor of 5.5 μg of ergosterol mg^{-1} fungal biomass (Gessner & Chauvet, 1993; Gessner, 2005).

Bacterial biomass

Bacterial biomass in the field-incubated litter was measured using epifluorescence microscopy (following Buesing, 2005). A 5.5 mm cork borer was used to cut three leaf discs that were placed in 10 mL glass vials containing 4 mL of formalin. Ten samples were prepared identically for each treatment. The 30 samples were placed in an ultrasonic bath for 120 s. A filtration manifold with a cellulose filter and aluminium oxide membrane was fitted to each vial. With the filter manifold sealed, 1 mL of distilled sterile water was added to each filter funnel. Each sample was vortexed for 5 s and allowed to settle for 10 s. A 1 mL sample was pipetted from just below the surface and placed on the filters. An additional 1 mL of distilled sterile water was added before vacuum was applied. With the vacuum off, four drops of DAPI stain were added to each filter and incubated in the dark for 15 min. One ml of PBS solution was then added to the filter, and the vacuum applied. Lastly, 500 μL of antifade solution was applied to the filters and the vacuum reapplied. The aluminium oxide membrane filters were mounted on glass slides and observed at 1000× magnification through non-fluorescing immersion oil with a Nikon Eclipse E600 microscope (Nikon Instruments, Inc., Melville, NY, U.S.A.). For each slide, digital photographs were taken from 10 random fields. The resulting 300 photos were edited with PhotoPos Pro software (http://www.photopos.com) to enhance the contrast, reduce the brightness, and remove red and green hues to facilitate counting the bacilli and cocci in each photograph. Bacterial biomass was estimated by multiplying cell numbers by an average biovolume calculated for each cell shape ($n = 20$ per shape).

Detrital carbon : nitrogen

The C : N ratio of each of the 30 (3 treatments × 10 replicates) samples taken from the field experiment was estimated using a CNS analyzer (Leco CNS-2000; Leco Corp., St Joseph, MI, U.S.A.) (after Kuehn *et al.*, 2000). We dried each sample at 50 °C for 48 h. The dried samples were then ground into fine fragments using a Wiley mill (no. 20 screen). Seven 100 mg (±0.36 mg) replicate sub-samples were then assayed for C : N in the analyzer. Fungal and bacterial biomass, and C : N were compared among treatments using ANOVA after testing for departures from homoscedasticity and normality.

Results

Microbial colonization and inundation regime

When the litter bags were removed at the end of the field incubation period, there was no difference between the mean dry mass of leaf litter among the three inundation treatments (one-way ANOVA treatment effect, $F_{2,9} = 1.6$; $P = 0.25$; Fig. 2a.). Average leaf litter loss was 25%(±4%SE) of the initial mass during the 8 weeks of incubation. Across all treatments, the

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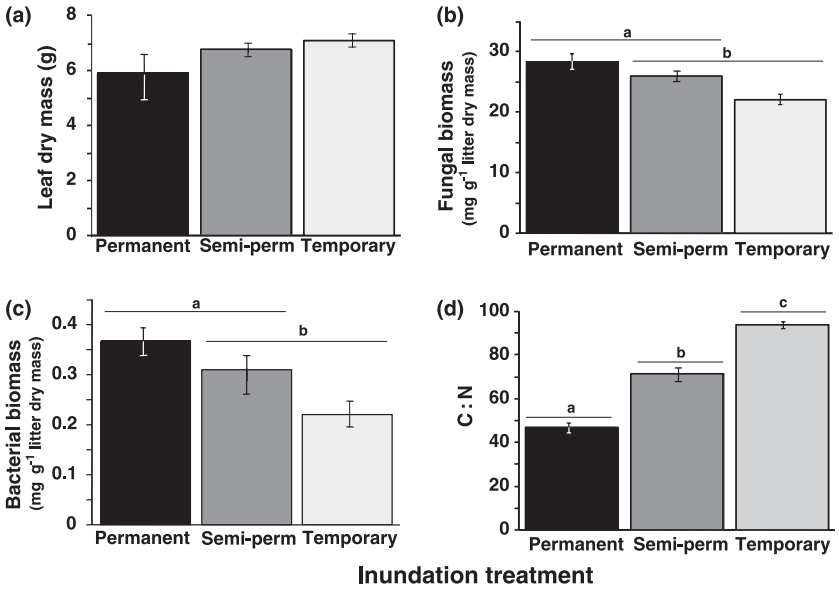

Fig. 2 Mean (±1 SE) (a) dry mass, (b) fungal biomass, (c) bacterial biomass and (d) carbon:nitrogen ratios in autumn-shed red maple leaves incubated for 8 weeks under three different inundation treatments in a small woodland wetland. Small letters and bars group treatments that did not differ ($P < 0.05$) based on Scheffe's multiple contrasts.

average leaf decay rate was 0.00577 day^{-1}. Fungal biomass in the leaf litter differed among treatments at the end of the incubation period ($F_{2,27} = 3.8$; $P = 0.036$) (Fig. 2a). Fungal biomass was significantly lower in the temporary treatment than in the permanent (Scheffe's contrast $P = 0.02$), but neither differed from the semi-permanent treatment (both Scheffe's contrasts $P > 0.05$) (Fig. 2b). Bacterial biomass also differed among inundation treatments (ANOVA bacterial biomass $F_{2,27} = 5.3$; $P = 0.01$) (Fig. 2c), and was greater in the permanent than in the temporary treatment (Scheffe's $P = 0.007$), but neither differed from the semi-permanent treatment (both Scheffe's contrasts $P > 0.05$), which had intermediate bacterial biomass (Fig. 2c).

The carbon to nitrogen ratio in the leaf litter differed substantially among treatments at the end of the field incubation period (one-way ANOVA C : N effect, $F_{2,18} = 480.9$; $P < 0.0001$) (Fig. 2d). The leaf-pack C : N ratio was significantly higher in the litter incubated under temporary conditions than in the semi-permanent treatment (Scheffe's $P < 0.0001$), which was significantly higher than in the permanent treatment (Scheffe's $P < 0.0001$). The C : N values for the temporary treatment were more than twice those in the litter incubated under permanent conditions (Fig. 2d).

Caddisfly litter preference

Caddisflies were observed to be actively foraging in or on litter throughout the experiment, although overall foraging activity declined across the 6 days of observations. Two-way ANOVA revealed that caddisfly foraging preference varied among litter treatments ($F_{2,306} = 27.5$; $P < 0.0001$) and dates of observation (($F_{5,306} = 5.6$; $P < 0.0001$). However, there was no litter treatment by date interaction ($F_{10,306} = 1.76$; 0.07); i.e. foraging declined in general towards the end of the experiment in all treatments (Fig. 3). Because of the absence of a two-way interaction, we could use one-way ANOVA with multiple contrasts to compare all litter treatments across all days. Caddisfly preference was highest for litter incubated under semi-permanent conditions, but marginally not different from that incubated under permanent

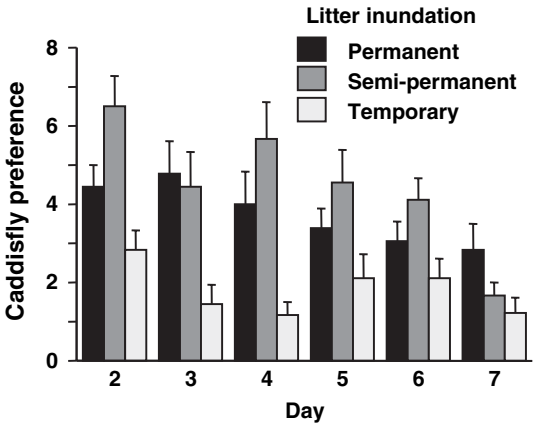

Fig. 3 Mean (±1 SE) preference scores (see text) on days 2–7 for caddisfly larvae foraging on detritus incubated under three different hydroperiods (see Fig. 1).

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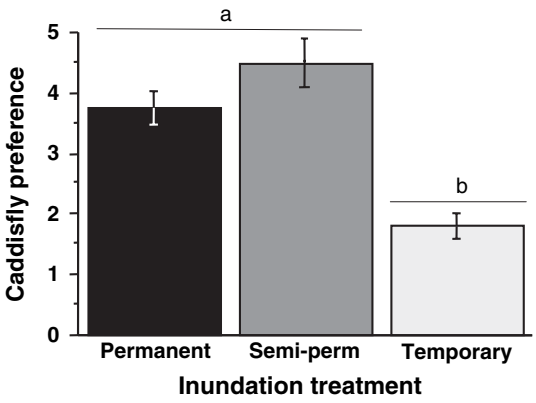

Fig. 4 Mean (±1 SE) preference scores (see text) across all days for caddisfly larvae foraging on detritus incubated under three different hydroperiods (see Fig. 1). Small letters and bars group treatments that did not differ ($P < 0.05$) based on Scheffe's multiple contrasts.

conditions (Scheffe's $P = 0.06$). However, both were much higher than for litter incubated under temporary conditions (Scheffe's contrast $P < 0.001$; Fig. 4). Across all dates, caddisfly preference for litter incubated under permanent and semi-permanent conditions was more than twice that of detritus incubated under temporary conditions (Fig. 4).

Discussion

Leaf decay rates and microbial colonization in the field

Across all treatments, the range of decay rates in our study (0.003–0.007 day $^{-1}$) are at the high end of the wide range of values previously reported for red maple leaves in both running and standing water habitats (Brinson *et al.*, 1981; Webster & Benfield, 1986; Ostrofsky, 1997). The rapid weight loss that we observed is surprising given that leaf breakdown rates are typically faster in streams than in ponds and wetlands (Webster & Benfield, 1986). Most previous studies of leaf decay in lentic habitats have been conducted in relatively deepwater settings (e.g. swamps, ponds, lake littoral zone; see Brinson *et al.*, 1981; Boon, 2006), where benthic substrates are more likely to be anaerobic than under the conditions in which we measured leaf decay (0.2–0.4 m depth). This difference in oxygen levels might explain why decay rates in our study were 2–3 times that measured in a nearby pond (1–2 m depth) in an experiment using identical litter bags and the same pre-treatment procedures (air-dried, autumn-shed leaves) (Ostrofsky, 1997; red maple $k = 0.002$).

The range of values for fungal and bacterial biomass at the end of the field incubation period were comparable to those obtained using similar techniques for marsh plants (Kuehn *et al.*, 2000; Verma, Robarts & Headley, 2003; Buesing & Gessner, 2006), but lower than those observed in stream leaf litter studies (see reviews by Suberkropp, 1997; Hieber & Gessner, 2002; Gulis & Suberkropp, 2003). As has been reported in nearly all leaf decay studies in streams, and macrophyte decay studies in marshes, fungal biomass was much greater than bacterial biomass and accounted for >90% of microbial growth on the leaf litter (Baker & Bradnam, 1976; Benner *et al.*, 1984; Findlay, Howe & Austin, 1990; Keuhn & Suberkropp, 1998; Kominkova *et al.*, 2000; Kuehn *et al.*, 2000).

Literature reviews on the impacts of wetting and drying on leaf-litter breakdown in wetlands (including red maple leaves; see Day, 1983) depict a remarkably inconsistent picture, with some studies reporting faster breakdown rates, some with slower breakdown rates, many with no effect, and others with time-variable effects (e.g. accelerated breakdown early, but no or negative effects later) as compared to permanent inundation (Brinson *et al.*, 1981; Conner & Day, 1991; Boon, 2006). In one of the few previous studies that actually measured how drying influences microbial processing of leaf litter in wooded wetlands, Battle & Golladay (2001) reported that drying 'accelerated decomposition by promoting microbial activity through aeration'. However, fungal biomass did not differ among treatments in their study, and the authors suggested that selective foraging on microbes by invertebrates during inundation was the most likely explanation for the low fungal biomass under permanent conditions.

In our study, drying reduced fungal and bacterial biomass, and increased C : N ratios, especially in leaf litter that alternated weekly between wetting and drying (Fig. 2b,c,d). This is consistent with the observation that different microbes thrive under dried vs. inundated conditions. Fungi that colonize leaf litter on dried substrates (forest floors, dried wetland basins), as well as those on the above-waterline parts of dead macrophytes, decline after inundation and are replaced by species adapted to aquatic conditions (Brinson *et al.*, 1981; Wong *et al.*, 1998; Kuehn *et al.*,

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2000; Boon, 2006). When wetland basins dry, the aquatic species of fungi and bacteria die, with a resulting flush of nutrients released from the dead microbes during re-inundation (Boon, 2006). That release, while important for the overall nutrient budget of the wetland, appears to decrease the nutritional value of leaf litter for detritivorous invertebrates (see below).

Microbial biomass and C : N ratios in leaf litter are often correlated, and together are used as evidence for the extent of litter decay (Hieber and Gessner 2002, unpubl. data; Suberkropp, 2003). Thus, it was surprising that the overall loss of mass did not differ among the leaf litter in different treatments (Fig. 2a). One explanation for relatively small differences among treatments is that, at least during the early stages of litter decay, the loss of leaf tissue is partially offset by the rapid accrual of microbial biomass. Regardless, our results emphasize that the nutritional quality of decaying litter for invertebrate shredders cannot always be assessed from decay rates alone.

Shredder preference and microbial biomass

Microbial biomass and the nitrogen content of decomposing leaf litter are strong covariates of nutritional value that should influence the choice of foraging substrates by invertebrate shredders. The strong preference by *Nemotaulius hostilis* larvae for leaf litter with high microbial biomass and nitrogen content is consistent with previous studies conducted in streams (Arsuffi & Suberkropp, 1989; Jacobsen & Friberg, 1995; Graça *et al*., 2001; Suberkropp, 2003). There was no difference in preference for detritus incubated under permanent and semi-permanent conditions, even though microbial biomass and nitrogen content were slightly lower in the semi-permanent treatment. However, the avoidance of leaf litter incubated under temporary conditions suggests this species can detect a lower threshold of nutritional value below which there should be negative effects on growth and development (Smock & Harlowe, 1983; Jacobsen & Sand-Jensen, 1994; Hutchens, Benfield & Webster, 1997; Friberg & Jacobsen, 1999; Eggert & Wallace, 2003). During development, *N. hostilis* larvae increasingly supplement their diet with animal material (S. Wissinger, unpubl. data), which has much higher caloric and protein content than even the most nutritious (i.e. microbially conditioned) leaf litter (Bowen, Lutz & Ahlgren, 1995). A next step for understanding how drying affects shredders in woodland wetlands will be to compare the effects of litter incubated under different drying regimes on (1) the degree to which larvae supplement their diet with animal material, (2) winter survival, (3) the timing of emergence in spring and (4) adult fitness correlates such as fecundity (Wissinger *et al*., 2004a).

In summary, we found that differences in basin drying can affect microbial colonization and the nutrient content of decaying autumn-shed leaf litter in seasonal woodland wetlands. Detritus-shredding caddisflies that inhabit these wetlands prefer to forage on leaf litter that was incubated under relatively permanent conditions, and had the highest microbial biomass and nitrogen content. These results, combined with dietary data on the foraging preferences of resident caddisflies, suggest that invertebrate shredders have the potential to play an important role in the processing of terrestrially derived leaf litter in these wetlands. The small size and discrete nature of woodland pools will facilitate the field manipulation of shredder densities to directly test that hypothesis.

Drying regime (i.e. schedule of filling and drying), is among the most important physical factors that affects the community composition and ecosystem function of wetlands, especially shallow, temporary habitats like seasonal woodland pools (Wissinger, 1999; Batzer *et al*., 2004; Williams, 2006). The drying regime of these habitats should be especially sensitive to changes in precipitation and/or evapotranspiration associated with global climate change (Brooks 2005). Our results indicate that climate change-induced shifts in drying regime should affect the processing rates of the leaf litter that dominates the energy budget of these shallow wetlands.

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