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DECOTAB: a multipurpose standard substrate to assess effects of litter quality on microbial decomposition and invertebrate consumption

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Abstract. Currently available tools for studying plant litter decomposition and invertebrate consumption in aquatic ecosystems have at least 2 major limitations: 1) the difficulty of manipulating litter chemical composition to provide mechanistic insights into attributes of litter quality controlling decomposition rate, and 2) lack of a standardized litter that hampers comparisons of results among studies. These limitations point to a need for a standard litter surrogate with adjustable chemical composition. We propose using a decomposition and consumption tablet (DECOTAB) consisting of cellulose powder embedded in an agar matrix to evaluate decomposition and consumption rates in aquatic environments. We describe the preparation of DECOTABs and demonstrate some applications in laboratory microcosms and outdoor mesocosms. A leaf shredder, the isopod *Asellus aquaticus*, and a collector-gatherer, the nonbiting midge larva *Chironomus riparius*, readily consumed DECOTABs, leading to massive mass loss of the tablets within 21 d (~90%). The isopod also consumed DECOTABs amended with extracts of riparian plants and soil to create a chemically complex source of organic matter. Our results highlight the potential utility of DECOTABs to assess invertebrate contributions to organic matter decomposition in aquatic systems. In the absence of invertebrates, exposure of basic and complex DECOTABs to microorganisms resulted in significant mass loss within 21 d (10–25%), and addition of an antibiotic and fungicide suppressed microbial decomposition, suggesting that the tablets are useful for studying microbial processes. Complex tablets decomposed faster than the basic tablets, a result illustrating the importance of chemical composition of organic material for microbial decomposers. DECOTABs are a novel, versatile tool for addressing long-standing questions in aquatic ecology and environmental assessment.

Key words: litter decomposition, cellulose degradation, microorganisms, detritivores, benthos, functional ecosystem assessment, aquatic ecosystem health, methodology, standardization.

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Decomposition of plant litter is a vital ecosystem process driven by both microorganisms and detritivores (Webster and Benfield 1986, Graça 2001, Gessner et al. 2010, Tank et al. 2010). The standard approach to studying decomposition in the field is to measure leaf mass loss from litter bags made of coarse or fine mesh that controls access of invertebrates differing in body size (Boulton and Boon 1991). Although not perfect, partly because of risks of hypoxia in fine mesh bags or litter fragmentation by

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turbulent flow in coarse mesh bags (Boulton and Boon 1991), this approach adequately mimics natural conditions in many situations and represents the influences of both litter quality and environmental factors on decomposition dynamics. Litter bags are the method of choice in comparative field studies (comparisons of litter types, locations, etc.) and often yield realistic estimates of local decomposition rates (Webster and Benfield 1986, Boulton and Boon 1991). Laboratory studies in microbial microcosms (Suberkropp 1991, Dang et al. 2009) and feeding trials with detritivores (Cameron and La Point 1978, González and Graça 2003) complement the litterbag approach by providing important insight into the decomposition process. However, rigorous tests of the mechanisms responsible for differences in decomposition are difficult with approaches relying on natural litter that varies in many quality attributes.

Standardized ways to measure decomposition rates that exclude confounding effects of varying litter quality are required to allow comparisons on large geographical and temporal scales, e.g., for studies on wide-ranging impacts of human activities (pollution, habitat modification, etc.) on ecosystem functioning in natural environments (Gessner and Chauvet 2002, Young and Collier 2009, Woodward et al. 2012). Selecting individual plant litter species for large-scale studies (Boyero et al. 2011) is only a partial remedy because litter quality can vary widely even within a given plant species (Lecerf and Chauvet 2008). Filter paper and cotton strips have long been used as substitutes for natural plant litter (Egglishaw 1964, Hildrew et al. 1984, Tiegs et al. 2007, Imberger et al. 2010). Cotton strips, in particular, are used as a standardized substrate for this purpose (e.g., Boulton and Quinn 2000, Young et al. 2008, Young and Collier 2009) because they are essentially composed of cellulose, a major component of plant litter, and have much greater tensile strength than filter paper.

Despite their potential utility in decomposition studies, cotton strips have limitations (Imberger et al. 2010). First, the previously used standard material (Shirley Soil Burial Test Fabric) is no longer available, so comparisons between present-day studies and earlier studies are complicated, although results with Shirley Soil Burial Fabric and replacement materials, such as 'calico' are correlated (Imberger et al. 2010). However, calicoes are produced from natural cotton and, therefore, have an uncertain and variable chemical composition. Second, the chemical composition of cotton strips is extremely simple (cellulose content $> 95\%$) compared to natural plant litter, such that numerous leaf constituents (N, lignin, tannins, fatty acids, etc.) that are potentially important factors in decomposer activity and decomposition rate are lacking. Third, the composition of cotton strips is difficult to manipulate experimentally to test for effects of specific chemical plant constituents or of compounds inhibiting microbial or detritivore activity (e.g., antibiotics, fungicides, insecticides; see Rader et al. 1994). These limitations highlight the need to identify a standardized substrate whose composition can be altered according to the needs defined by the question posed.

The objective of our study was to develop and test a standardized proxy material whose chemical composition could be adjusted easily for experimental purposes. Inclusion of specific plant constituents and other compounds at desired concentrations in polycarbohydrate gels (e.g., agar or phytagel) has been useful in studies of allelochemical interactions (Hay et al. 1998), colonization dynamics of sessile invertebrates (Henrikson and Pawlik 1995, Hunting et al. 2010b), and feeding preferences of aquatic herbivores (Pavia and Toth 2000). We assessed whether this matrix could be modified to serve as surrogate plant material whose chemical composition can be controlled and whose texture broadly resembles that of natural litter. However, agar has a low nutritional value for invertebrates and can be degraded by only a limited number of microorganisms (Bärlocher and Porter 1986, Armisén 1991). Therefore, we developed and tested the performance of a decomposition and consumption tablet (DECOTAB) consisting of a high concentration of cellulose powder (75%) embedded in an agar matrix to evaluate the importance of factors affecting microbial decomposition and invertebrate feeding. We describe the preparation of the proposed DECOTABs and evaluate some potential applications in laboratory microcosms and outdoor mesocosms.

Methods

Preparation of DECOTABs

We made basic DECOTABs from a suspension containing 60 g of powdered cellulose (Sigma–Aldrich, St. Louis, Missouri), 20 g of purified agar (Oxoid Ltd., Basingstoke Hampshire, UK), and 60 μmol ascorbic acid/L deionized water (dH_2O) as an antioxidant (Merck GmbH, Darmstadt, Germany) (Niki 1991). We heated the mixture to 100°C to dissolve the agar, allowed it to cool to 50°C with frequent stirring, and poured it into a multiwell polycarbonate mold (15-mm diameter, 5-mm height) to cast tablets with a final volume of 118 mm^3 (Fig. 1A). The tablets initially had a convex surface that quickly flattened during solidification of the agar at 7°C. The tablets could be stored in closed containers

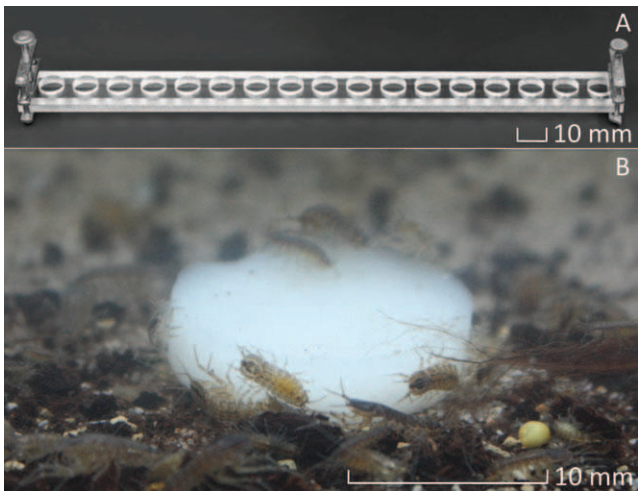

FIG. 1. Polycarbonate mold used to prepare DECOTABs (A), and isopods (*Asellus aquaticus*) rallying around and feeding on a basic cellulose DECOTAB (B).

for up to 3 wk in the refrigerator without noticeable decay or dehydration. We measured the dry mass of freshly prepared tablets (mean ± 1 SD = 81.3 ± 3.1 mg, n =25) by drying them for 3 d at 70°C and then weighing them with an analytical balance (precision = 0.1 mg; Mettler AT261, Mettler-Toledo, Tiel, The Netherlands).

We prepared DECOTABs including an antibiotic and fungicide as described above except that we added chloramphenicol (60 mg/L dH_2O; Sigma–Aldrich) and cycloheximide (60 mg/L dH_2O; Sigma–Aldrich) to the cooled suspension at 50°C. We prepared DECOTABs of more complex composition to enhance their resemblance to natural particulate organic matter (POM). These tablets had the same volume as the basic DECOTABs, but consisted of 46.7 g of cellulose, 20 g of purified agar, and 60 µmol of ascorbic acid/L dH_2O. We extracted plant constituents from bulk standardized garden soil (Baseline, Maxeda DIY, Diemen, The Netherlands), stinging nettle (*Urtica dioica*), and willow leaf extract (*Salix alba)*, both common plants in the riparian zone of temperate rivers in Europe. We used 70% acetone as the extraction solvent and air-dried the extract (Hunting et al. 2010a). We added powdered extracts (6.7 g/L for soil; 3.3 g/L for stinging nettle, and 3.3 g/L for willow leaves) to the cooled suspension at 50°C.

Experiment 1: Microbial vs invertebrate-mediated decomposition

We ran an experiment in laboratory microcosms to evaluate the relative contribution of microorganisms and detritivores to decomposition of DECOTABs. We used 100-mL glass microcosms with sediment containing 50 g quartz sand (0.1–0.5-mm grain size; Dorsilit, Eurogrit, Papendrecht, The Netherlands) and a mixture of standard culture food (7.5 mg) composed of Trouvit® (Trouw, Fontaine-les-Vervins, France) and Tetraphyl® (Tetrawerke, Melle, Germany) at a ratio 20:1 as organic material. The overlying water consisted of 250 mL Dutch Standard Water (Maas et al. 2002). To obtain a natural microbial inoculum, we added 50 mL of filtered (<75 µm) natural surface water from a local shallow lake that typically supports a diverse microbial community (del Giorgio and Gasol 1995). A detailed description of Dutch freshwater bacteria in comparable lakes was published by Zwart et al. (1998, 2002). We aerated the water gently with compressed air throughout the experiment.

We tested 2 invertebrate species: the nonbiting midge larva *Chironomus riparius* (laboratory culture) and the isopod *Asellus aquaticus* (collected from a nearby shallow lake). We used relationships among invertebrate length, fresh mass, and dry mass to standardize invertebrate biomass (Hunting et al. 2012). We added invertebrates based on equal initial dry mass. Thus, we added either 5 *A. aquaticus* (7–9 mm) or 20 *C. riparius* larvae (2nd instar) to each microcosm and placed 1 DECOTAB in each microcosm. Treatments were: 1) basic DECOTAB, no invertebrates; 2) DECOTAB containing an antibiotic and fungicide, no invertebrates; 3) basic DECOTAB and *C. riparius*; and 4) basic DECOTAB and *A. aquaticus*. We did not include a treatment with DECOTAB containing both antibiotics and invertebrates because of the toxicity of both antibiotics to invertebrates (Baliga et al. 1970, Monari et al. 2008). We replicated each treatment 10 times. After 21 d, we removed the DECOTABs with a needle, rinsed and dried (3 d at 70°C) them, and weighed them with an analytical balance (precision = 0.1 mg; Mettler AT261). We subtracted the final dry mass from the estimated initial dry mass to calculate DECOTAB mass loss. We tested for treatment differences with a 1-way nonparametric permutation-based multivariate analysis of variance (PERMANOVA) based on Bray–Curtis distances and 9999 permutations (Anderson 2001), followed by a Bonferroni-corrected PERMANOVA pairwise comparisons in PAST (Hammer et al. 2001).

Experiment 2: Effects of DECOTAB complexity on decomposition and consumption

We ran a 21-d mesocosm experiment in June 2011 to evaluate the performance of DECOTABs under outdoor conditions and to compare basic and complex

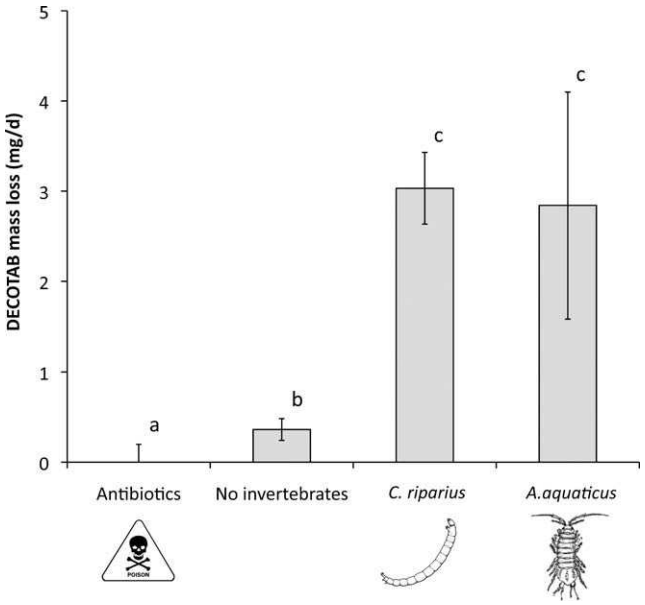

FIG. 2. Mean (±1 SD) mass loss of cellulose DECOTABs in laboratory microcosms inoculated with microorganisms only or additionally stocked with 1 of 2 invertebrate species (*Asellus aquaticus* or *Chironomus riparius*) (t = 21 d). Bars with different letters are significantly different (Bonferroni-corrected permutation-based multivariate analysis of variance [PERMANOVA] pairwise comparisons).

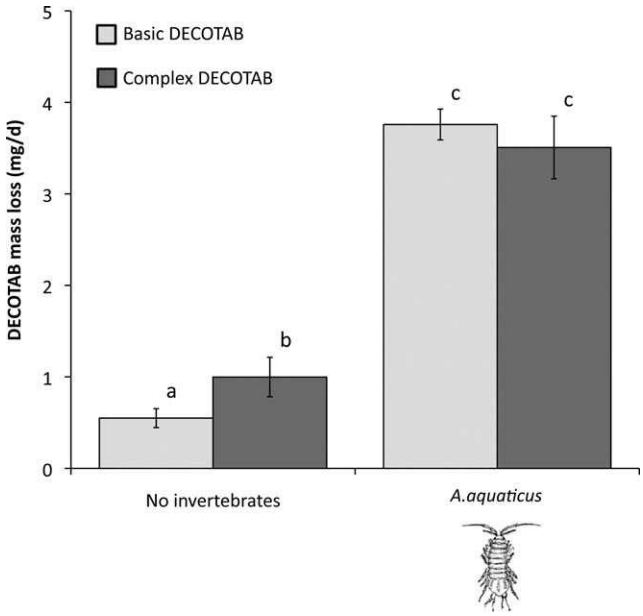

FIG. 3. Mean (±1 SD) mass loss of basic cellulose DECOTABs and complex DECOTABs containing plant and soil extracts mediated by *Asellus aquaticus* or microorganisms in outdoor mesocosms (t = 21 d). Bars with different letters are significantly different (Bonferroni-corrected permutation-based multivariate analysis of variance [PERMANOVA] pairwise comparisons).

DECOTABs containing organic matter extracted from plants and soil. Mesocosms consisted of rectangular (66 cm long × 34 cm wide × 30 cm high) 90-L plastic tubs. They contained ~40 L of rainwater and 18.5 L of sediment made of standardized garden soil (Baseline) and quartz sand (0.1–0.5 mm; Dorsilit, Eurogrit, Papendrecht, The Netherlands) mixed in a ratio of 5 L soil/25 kg sand. We placed the mesocosms in concrete containers filled with water to buffer temperature fluctuations. We pulled a gauze screen (mesh size = 1 mm) over the concrete containers to reduce colonization by allochthonous fauna. Before starting the experiment, we allowed the mesocosms to sit for 2 d to allow the sediment to settle. We assumed that microbial communities could sufficiently acclimate during these 2 d. Subsequently, each mesocosm received 3 basic and 3 complex DECOTABs and either no invertebrates or *A. aquaticus*. We added the isopods at densities of 402 individuals (ind.)/m^2 (90 ind./mesocosm), which falls within the density range reported for natural populations (67–586 ind./m^2; Adcock 1979). We replicated both treatments 5 times. During the experiment, we gently aerated the overlaying water with a permanently installed air compressor aeration system. After 21 d, we removed the DECOTABs and weighed them as described above. We tested for treatment differences with a 2-way factorial PERMANOVA based on Bray–Curtis distances and 9999 permutations (Anderson 2001), followed by Bonferroni-corrected PERMANOVA pairwise comparisons in PAST.

Results

DECOTAB mass loss differed between treatments in the microcosm experiment ($F = 23.61$, $p < 0.0001$; Fig. 2). Both *C. riparius* and *A. aquaticus* fed actively on the basic DECOTABs (Fig. 1B), resulting in 80 to 90% mass loss over the course of the 21-d experiment. In microcosms without invertebrates, mass loss averaged 10 to 25% of the initial mass, and no mass loss occurred when DECOTABs contained an antibiotic and fungicide (Fig. 2).

Mass loss in outdoor mesocosms was higher in the presence of invertebrates than in the microorganism-only treatment ($F = 317.4$, $p = 0.0001$; Fig. 3). Mass loss of complex DECOTABs containing soil and plant extracts was higher than that of basic DECOTABs when only microorganisms were present ($F = 13.0$, $p = 0.002$; pairwise comparison, $p = 0.031$), but this difference was not apparent when isopods were allowed to feed on DECOTABs (pairwise comparison, $p = 0.43$). Mass loss in the presence of invertebrates was

similar in outdoor mesocosms and laboratory microcosms in the presence of *A. aquaticus* (cf. Figs 2, 3).

Discussion

The new standardized plant litter substitute developed and tested in our study was useful for measuring rates of microbial decomposition and consumption by invertebrates in sediments. Both invertebrate test species fed actively on the DECOTABs, resulting in a dramatic mass loss, despite the presence of other sources of organic matter in the sediments of our micro- and mesocosms. Moreover, DECOTABs exposed to microorganisms in the absence of invertebrates also lost mass in the 2 experiments, whereas mass loss was suppressed in microcosms with DECOTABs containing an antibiotic and a fungicide. These results suggest that DECOTABs are a useful substrate for studying both microbial decomposition and consumption of organic matter by invertebrates in aquatic systems. However, the extent to which they reflect decomposition of natural plant litter and could be used to develop functional metrics for assessing impacts of anthropogenic stressors on aquatic ecosystems remains to be tested.

DECOTABs were consumed by a shredder (*A. aquaticus*) and by *C. riparius*, which burrows in sediments and feeds on fine particulate organic matter (Cummins and Klug 1979). Chironomids readily consumed DECOTABs, a result suggesting that DECOTABs could be used to study mechanisms affecting use of organic matter by collector-gatherers (in addition to shredders) and to measure their role in organic-matter dynamics of aquatic ecosystems.

Experimental manipulation of substrate composition facilitates mechanistic insights into decomposition processes. One could test whether and which lipids are triggers for detritivory, growth, and reproduction (Cargill et al. 1985). One could assess whether particular phenolics or mixtures of phenolics affect microbial activity or detritivore performance in laboratory or field conditions. We supplemented cellulose DECOTABs with an antibiotic and fungicide to assess whether and to what extent microorganisms vs detritivores contribute to decomposition.

Investigators have used various approaches to assess the contribution of shredders to litter decomposition (Petersen and Cummins 1974, Cuffney et al. 1990, Hieber et al. 2002, González and Graça 2003), but the available information is still scarce (Boulton and Quinn 2000, Young et al. 2008). We did not compare DECOTABs with natural plant litter, so our results are not directly comparable with estimates based on measured litter consumption (Hieber and Gessner 2002, Hunting et al. 2012). Nevertheless, our data corroborate the notion that invertebrates can play dominant roles in organic-matter turnover in aquatic ecosystems (Wallace and Webster 1996). Invertebrates contributed up to 55 to 65% of total DECOTAB mass loss, whereas microorganisms contributed only 10 to 25%.

Plant litter is a complex mixture of structurally diverse compounds. To mimic the composition of natural litter in mesocosms, we created DECOTABs containing extracts of riparian plants and soil and offered them to microorganisms only or to microorganisms in combination with *A. aquaticus*. The differential response of microbes to basic cellulose and complex DECOTABs illustrates the importance of resource composition for microbial decomposers. For invertebrates, a similar distinction was not observed, possibly because resemblance in texture masked differences in chemical composition. However, conclusive answers about the relative importance of texture vs chemistry for DECOTAB palatability require tests with tablets differing more widely in chemical composition.

Standardized measures to determine decomposition rates are increasingly important for assessing effects of anthropogenic stressors on ecosystem processes (Gessner and Chauvet 2002). However, methods differ among studies, and some are based on using litter from different plant species that decompose at different rates. Even litter of the same species may vary in chemical composition and texture if collected at different locations (Lecerf and Chauvet 2008) or at different times. This variability hampers comparisons of decomposition dynamics over large spatial and temporal scales (Boyero et al. 2011). Use of a standardized substrate would facilitate comparisons among studies, including studies aimed at assessing the response of ecosystem functioning to anthropogenic stressors. The DECOTAB approach could facilitate such standardized experiments at larger scales and increase power of meta-analyses.

In conclusion, our study demonstrated the potential of the newly developed DECOTABs for a variety of applications in aquatic environments. The DECOTABs described here were based on an aqueous matrix, so they shrink upon dehydration. Therefore, they cannot be used readily in terrestrial systems because dehydration will affect shape and texture. However, the basic concept could be applicable to terrestrial studies if tablets were prepared dry. One of the greatest assets of DECOTABs is that they can be prepared in almost any desired size, shape, or composition to suit the needs of the specific question

to be examined. Thus, they have the potential to become a highly standardized and versatile tool to address long-standing issues in aquatic ecology and environmental assessment.

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