



## Structure, functional organization and biological traits of macroinvertebrate assemblages from leaf-bags and benthic samples in a third-order stream of Central Apennines (Italy)



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### ABSTRACT

Information on both structure and functions is essential to evaluate the ecological integrity of stream ecosystems and their response to natural and anthropogenic disturbance. Leaf-bags have been widely employed to assess stream ecosystem processes and the degree of leaf mass consumption has been proposed as one of the most useful functional descriptor in aquatic environments. However, the breakdown rate of leaves has been compared with structural indicators of macroinvertebrate assemblages derived from leaf-bags or from benthic samples, without any direct comparison on the characteristics of communities sampled with the two methods. The main objective of the paper is to conduct a comparative analysis of the structure, functional organization and biological traits of macroinvertebrate assemblages from artificial leaf packs and from benthic samples of a third-order stream in the Central Apennines (Italy).

Of the 43 macroinvertebrate taxa globally found in our survey, 9 showed low ability or scarce attitude to colonize leaf-bags, while 6 rare taxa were exclusively sampled in artificial leaf packs. Both assemblages were characterized by the dominance of Chironomidae, though they were more abundant in leaf-bags (71% of total individuals collected) than in benthic samples (44%). Conversely, the mayfly *Baetis* sp. comprised more than 17% of total individuals collected with Surber nets and only 5% of leaf-bag assemblages. We found that compared to benthic assemblages, leaf-bag communities were less diversified with a lower richness and a lower number of Ephemeroptera, Trichoptera and Plecoptera (EPT) taxa; significant differences also emerged in assemblage composition.

Contrary to what expected, artificial leaf packs resulted not particularly attractive for shredder organisms and were mainly colonized by collectors. Also the biological trait profiles of the leaf-bag community were significantly different from those shared by resident benthic taxa.

Our findings could have profound implications in the assessment of the structural and functional integrity of stream ecosystems and in studies on freshwater biodiversity and ecosystem functioning. In these studies, the two methods (leaf-bags and Surber nets) should be regarded as complementary and not alternative.

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### 1. Introduction

Information on both structure and functions of stream ecosystems is essential to evaluate their ecological integrity and their response to natural and anthropogenic disturbance (Bunn and

Davies, 2000; Castela et al., 2008; Riipinen et al., 2008; Young et al., 2008; Death et al., 2009; Sandin and Solimini, 2009). While structural indicators and relative metrics are mainly based on the quantitative analysis of richness, diversity and composition of biological assemblages, the functional characteristics of stream ecosystems (i.e. energy flows, nutrient cycling, primary and secondary production) are more difficult to measure and often involve large efforts or sophisticated and expensive techniques (Matthews et al., 1982).

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However, since in most streams and rivers the allochthonous organic detritus is the main energy input, the evaluation of the in-stream detritus processing may represent a good indicator of ecosystem functionality (Webster and Benfield, 1986), being influenced by the activity of invertebrates and microbial organisms and by natural and human-induced variations in a wide range of environmental factors (Bunn et al., 1999; Gessner and Chauvet, 2002; Danger and Robson, 2004; Pinna and Basset, 2004; Niyogi et al., 2013).

An indirect method to assess the breakdown rate of detritus in aquatic systems is the leaf-bag assay (Petersen and Cummins, 1974; Boulton and Boon, 1991). Artificial leaf packs are used to simulate the colonization–utilization pattern of natural leaf-pack accumulations by microorganisms and macroinvertebrates (Webster and Benfield, 1986; Boulton and Boon, 1991; Abelho, 2001), and the degree of leaf mass consumption has been proposed as a cost-effective and synthetic indicator of ecosystem functioning in aquatic environments (Gessner and Chauvet, 2002; Dangles et al., 2004; Lecerf et al., 2006; Young et al., 2008; Niyogi et al., 2013).

Notwithstanding some shortcomings (Tiegs et al., 2013; Imberger et al., 2010), the leaf-bag technique has been also frequently utilized in studies on biodiversity and ecosystem functioning in freshwater habitats (Jonsson and Malmqvist, 2000; Ruesink and Srivastava, 2001; Dangles and Malmqvist, 2004; Giller et al., 2004) and was recently proposed as an important tool to integrate results from biomonitoring programs which are mainly based on the analysis of structural indices (Sandin and Solimini, 2009).

However, recent research aimed at highlighting the role and the relative importance of structural and functional indicators in describing the ecological integrity of streams has given contrasting results: from redundancy (Pascoal et al., 2003; Lecerf et al., 2006) to complementarity (Castela et al., 2008; McKie and Malmqvist, 2009; Young and Collier, 2009; Silva-Junior and Moulton, 2011). In these studies the breakdown rate of leaves has been compared with structural descriptors of macroinvertebrate assemblages derived either from artificial leaf packs (Hury et al., 2002; Pascoal et al., 2003; Silva-Junior and Moulton, 2011) or from Surber net benthic samples (Lecerf et al., 2006; Castela et al., 2008; McKie and Malmqvist, 2009; Young and Collier, 2009), without any direct comparison on the characteristics of communities sampled with the two methods.

Inconsistencies on the effectiveness of artificial substrates to provide an accurate characterization of macrobenthic communities have been reported for shallow freshwater ponds (Muzaffar and Colbo, 2002) and for transitional waters (Quintino et al., 2011), but information on stream ecosystems is particularly scant and only a few studies have directly addressed this topic (Murphy and Giller, 2000; Peralta-Maraver et al., 2011).

Therefore, our main objective is to conduct a comparative analysis of the structure, functional organization and biological traits of macroinvertebrate assemblages on leaf-bags and Surber net benthic samples of a third-order stream in the Central Apennines (Italy). Assuming that artificial leaf packs are a resource but a rather homogenous microhabitat for macroinvertebrate colonizers, we hypothesized that they may sample only a fraction of the whole community which can be found in more heterogeneous natural benthic substrates.

## 2. Materials and methods

### 2.1. Study area

The study was conducted in a small reach of a third order stream in the Central Apennines: the River Aterno (Abruzzo, Italy). The study area, about 5 km in length (geographical coordinates:

42°12'21.683" N, 13°38'27.895" E; altitude: 507 m a.s.l.) is located 60 km from the spring, in a semipristine and poorly urbanized valley inside the protected area of the "Velino-Sirente" Regional Natural Park. In this zone the River Aterno is characterized by rather homogeneous environmental and hydrological features (Vignini, 2009). It is about 14 m in width and has a mean depth of 16 cm. The substrate is mainly composed by gravel (70%), pebbles (20%) and sand (10%), with patchily distributed organic debris accumulation. The riparian zone is characterized by the presence of a continuous strip of natural vegetation, dominated by *Salix* spp. and *Populus* spp.

### 2.2. Sampling methods

The survey was conducted using the leaf-bag technique (Petersen and Cummins, 1974) and parallel Surber net sampling. Leaf-bags were assembled using leaves of *Phragmites australis* (Cav.) Trin. ex Steudel, collected during autumn, just after the beginning of leaf senescence, air dried and subsequently stored in a dry, dark and well aerated laboratory room. The stored leaves were dried in an oven at 60 °C for 72 h, weighed and organized into  $3.000 \pm 0.005$  g lots, placed inside nylon net bags with 5 mm mesh size and closed with monofilament nylon lines. Benthic samples were collected using a Surber net (0.12 m<sup>2</sup> area, 200 µm mesh size).

We conducted a preliminary sampling survey at the study area (6 Surber nets and 18 leaf-bags), in order to establish the minimum number of samples necessary to characterize the overall structure and composition of macroinvertebrate assemblages at each sampling occasion. Data from this survey, analyzed using sample based rarefaction methods (Colwell, 2013), indicated that 3 Surber samples and 9 leaf-bags were sufficiently representative of the general structure of both assemblages (~90% of total estimated richness and abundance of taxa).

The field experiment started in May 2010; 9 leaf-bags were introduced into the stream and regularly spaced in order to cover the whole river transect and including all possible microhabitats. After about 30 days of exposure, leaf-bags were retrieved, identified using a numbered tag and returned to the laboratory in a cooler. Three Surber net benthic samples of the macroinvertebrate community were collected at the time of leaf-bag retrieval. This sampling procedure was repeated six times, from May through September 2010. In the laboratory, leaves from leaf-bags were gently washed to remove inorganic deposits and invertebrate colonizers. Material from benthic samples was sorted separately. Organisms were preserved in 70% ethanol, identified to family or genus and counted under a stereo-microscope (Leica MZ9.5). The taxa collected were assigned to a Functional Feeding Group (FFG) following Merritt and Cummins (1996) and were also classified according to their biological attributes: following a fuzzy coding procedure (Chevenet et al., 1994), affinity scores (0–5) were assigned to each taxon in relation to 11 biological traits and 63 different modalities (Tachet et al., 2000; Table 1). At each sampling occasion, some hydraulic (river bed width and depth, current velocity, instantaneous discharge) and physico-chemical parameters (water temperature, conductivity, pH, dissolved O<sub>2</sub>) were also recorded using a magnetic Flow Probe (N.01.200) and a multiparameter probe (Hach Lange HQ40D Multi).

### 2.3. Data analysis

Differences in the structure of benthic and leaf-bag assemblages were tested using taxa richness, Shannon's diversity ( $H'$ ) (Shannon and Weaver, 1963) and number of EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa (Lenat, 1988) as dependent variables. At each sampling event (numbered from 1 to 6) the values of the variables were calculated by cumulating data from 3 Surber samples and 9 leaf-bags, respectively. Given the low spatio-temporal

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