



## Review

## Small leaf breakdown in a Savannah headwater stream



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## ABSTRACT

The chemical nature and nutritional quality of leaves influence microbial colonization, microbial activity and consequently leaf breakdown rates. In the present study, we compared the decomposition of *Baccharis concinna* and *Baccharis dracunculifolia* leaves and the influence of leaf quality on the microbial activity during the decomposition process. This investigation was conducted in a Brazilian savanna headwater stream with a riparian zone composed predominantly of herbaceous and shrubs. The breakdown coefficient was higher in *B. dracunculifolia* than in *B. concinna*; for both species, increases in leaf mass were observed after the 60th day. The secondary compounds were quickly leached in the first seven days, but the structural compounds persisted longer and served as the main carbon source for the detritus-associated microorganisms. The highest values of ergosterol were observed in the final stages of leaf breakdown and indicated the difficulty of colonization on the detritus; these values were related to the increase in leaf mass. The ATP content increased without corresponding increase in ergosterol content, suggesting a biofilm formation during leaf breakdown. These results indicated that the total microbial biomass can assimilate organic compounds released from detritus by the enzymatic action of fungi, demonstrating the importance of this group for releasing the energy stored in small leaves.

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## Introduction

The functioning of fluvial ecosystems is determined by the relationships between organisms and the environment, as well as by local physical and chemical processes (Allan and Castillo, 2007). In headwaters and small streams, the functioning depends on the metabolic activity of organisms and the flow of energy between the terrestrial and aquatic systems (Gomi et al., 2002).

Several investigators have noted that in headwater streams, where light can be a limiting factor for primary producers (as a consequence of the dense forest cover), the main source of energy and organic matter is leaves from riparian vegetation (Vannote et al., 1980; Pozo et al., 1997; França et al., 2009). In streams where the riparian canopy is poorly developed, light is not a limiting factor for the growth and development of the algal community, and consequently, high autochthonous production becomes the main source of energy in aquatic food webs (Bunn et al., 2003; Esteves and Gonçalves, 2011).

Decomposition is an ecological process essential for the flow of energy and organic matter cycling in headwater streams. This process is responsible for mineralization, aiding mineral remobilization to the trophic web (Allan and Castillo, 2007), and the colonization and conditioning of the detritus by microorganisms is one of the most important factors in this remobilization (Gessner et al., 1999). Microorganisms (fungi and bacteria) colonize the detritus, producing extracellular enzymes that are able to degrade plant structural polymers such as cellulose, hemicelluloses and pectin, making detritus more palatable for consumption by shredders (Canhoto and Graça, 1999). Bacteria usually colonize in the early stages, using easily assimilated molecules (Komínková et al., 2000). Fungi reach a higher biomass and act during the advanced stages of decomposition (Gulis and Suberkropp, 2003b; Nikolcheva and Bärlocher, 2005).

According to Gessner and Chauvet (1994), studies of leaf breakdown must consider the chemical nature and nutritional quality of leaves, which directly influence the breakdown rates. Plant species with low contents of structural macromolecules (lignin) and defense compounds (polyphenols and tannins) and high concentrations of nitrogen and phosphorus are more susceptible to microbial colonization, leading to higher decay rates (Gessner and Chauvet, 1994; Ostrofsky, 1997; Das et al., 2008). Leaf toughness, which may be related to high concentrations of lignin (a compound with a complex molecular structure and low nutrient content) and waxes, which provide rigidity to the plant tissue and form layers of preventive barriers to water loss, can also influence microbial activity, reducing leaf breakdown (Canhoto and Graça, 1999; Raven et al., 2007; Li et al., 2009).

Plants of the Cerrado present higher concentrations of secondary and structural compounds, which protect against herbivores and pathogens in these ecosystems and may directly affect leaf breakdown. In addition, the riparian zone of the rocky field system is composed predominantly of herbaceous and shrubs and does not form a true canopy over the water body, allowing the high light incidence on the stream (Oliveira-Filho and Ratter, 2002). We propose the following hypotheses: (i) the small leaves associated with the open canopy increase the leaf breakdown due to the facilitation of microbial colonization due to its negative correlation with leaf size when compared to other Cerrado streams; (ii) the higher concentrations of secondary and structural compounds in detritus will negatively affect microbial colonization and thus leaf breakdown rates. Our objectives were as follows: (1) to compare the decomposition rates of leaves from two typical and abundant species in high-altitude areas of the Cerrado biome, including the riparian zones of headwater streams; (2) to compare changes in the concentrations of secondary and structural compounds during leaf breakdown; and (3) to analyze changes in the reproduction and

biomass of fungi and total microbial community correlating with the dynamics of chemical compounds of detritus. We chose *Baccharis dracunculifolia* DC. and *Baccharis concinna* G.M. Barroso because they are common in the riparian zone, and the *Baccharis* genus is important for honey production and produces many compounds of pharmacological interest. The study of these species may help to demonstrate the importance of the conservation of this genus; thus, any human exploitation should be minimal and consider that these species contribute to the energy stability of ecosystems.

## Methods

### Study site

This study was conducted in a 2nd order stream (Geraldinho Stream) in Serra do Cipó, Minas Gerais, Brazil, within the São Francisco Basin (19°16'55.51"S, 43°35'34.46"W; altitude 1135 m). The riparian zone is composed predominantly of herbaceous and shrubs. The experiment was conducted in the dry season from May to September 2009. The mean annual temperature in the region varies between 17.0 and 18.5 °C, and the mean annual precipitation varies between 1450 and 1800 mm (Gonçalves et al., 2006).

### Water parameters

In each sampling period, we measured current speed with a Fluxometer (model SWOFFER 2100 series), temperature and dissolved oxygen (YSI 55 dissolved oxygen meter), pH (Digimed MD 20), and electrical conductivity (Minipa MCD-2000) in the field. We collected 1 L of water in each period to analyze total alkalinity using the Gran method (Carmouze, 1994). Nitrate and soluble reactive phosphorus concentrations were measured using the cadmium column reduction method and the ascorbic acid method, respectively (APHA, 2005).

### Experimental procedure

Senescent leaves of the shrubs *B. dracunculifolia* and *B. concinna* were collected from various individuals around the stream with nets before they fell to the ground; samples were collected 6 months before the experiment. Leaves were air-dried and incubated separately in fine-mesh litterbags (0.5 mm mesh size), with each bag containing  $1.5 \pm 0.1$  g of dried leaves. Sixty-four litterbags were placed in rows in the stream just above the streambed and tied to steel rods and submerged rocks.

The decomposition rate was measured by the weight loss of the detritus incubated in the stream for a period of 120 days (with partial removal after 3, 7, 15, 21, 30, 60, 90, 120 d). On each sampling date, four litterbags with each leaf species were removed, numbered sequentially, placed in individual plastic bags, and transported to the laboratory on ice in an insulated container. In addition, four replicates per species were prepared, corresponding to day zero, which were used to assess the weight loss resulting from preparation, handling, and transporting the sample to the field, i.e., to correct for weight loss that did not result from decomposition. These replicates were also used to determine the initial chemical composition (structural and secondary compounds).

In the laboratory, the leaf material was carefully rinsed with distilled water, and 20 leaves of the same size were removed from each litterbag and divided into four groups of five leaves each. Each group of leaves was used to determine: (1) ash free dry mass (AFDM) of leaves; (2) fungal biomass, estimated from the ergosterol concentration; (3) sporulation rate of aquatic hyphomycetes; and (4) microbial biomass, estimated from the ATP concentration. For AFDM analysis, groups of leaves were dried at 60 °C for 72 h and then placed in the muffle furnace for 4 h at 550 °C to combust the

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