



## Environmental Microbiology

# *Eichhornia azurea* decomposition and the bacterial dynamic: an experimental research



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## ABSTRACT

Organic decomposition is a complex interaction between chemical, physical and biological processes, where the variety of aquatic vascular plants is essential for the trophic dynamics of freshwater ecosystems. The goal of this study was to determine the aquatic macrophyte *Eichhornia azurea* (Sw.) Kunth decomposition rate, the time relation with the limnological parameters, and whether this relationship is a result of decomposition processes. To that end, we collected water and leaves of *E. azurea* in Surf Leopoldo, PR. The experiment consisted of two treatments: 25 containers with 450 mL of water and 0.8 g of biomass dry weight were used with or without the addition of macrophytes. Samples were collected in triplicate at times 0, 3 h, 6 h, 12 h, 24 h, 72 h, 120 h, 168 h and 240 h. When the container was removed, the plant material was dried in an oven. After 48 h, the material was measured to obtain the final dry weight. Analyses of pH, conductivity, dissolved oxygen, total phosphorus N-ammonia (NH<sub>4</sub>), soluble reactive phosphorus (PO<sub>4</sub>) and dissolved organic carbon were performed, and the decomposition rate was calculated. The results showed significant temporal variation of limnological parameters in the study. Additionally, dissolved oxygen, conductivity, dissolved organic carbon and total phosphorus were correlated with the dry weight of the biomass, suggesting that *E. azurea* decomposition significantly interferes with the dynamics of these variables.

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## Introduction

Aquatic macrophytes are important components of ecosystems that provide flood pulse. They have spatial and temporal characteristics that make them interesting for the study of decomposition in aquatic plants.<sup>1–3</sup> Macrophytes are often the

primary producers, especially in lentic environments. They have a major role in nutrient cycling and in debris formation, being an abundant source of organic matter.<sup>4,5</sup> Additionally, they are a mixed stands, which influences the physical and chemical characteristics of water, altering the turbulence, temperature, sunlight penetration, concentration and distribution of dissolved oxygen and nutrients.<sup>6</sup>

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*Eichhornia azurea* (Sw.) Kunth is a major macrophyte in constrained, flooded environments. It is a floating fixing species, perennial and rhizomatous.<sup>7,8</sup> It is distributed in natural and artificial reservoirs from south of the United States to Argentina and in all of the regions and ecosystems of Brazil.<sup>9</sup> It serves as food for capybaras, pigs and other herbivores and as a habitat for many fish, insect larvae, snails and their eggs, etc.<sup>10</sup> Aquatic macrophytes reach high biomass values, making them an important source of organic material to be decomposed.<sup>11</sup> Several studies have hypothesized about the relationship of this macrophyte with others species, such as ephemeropteras,<sup>12</sup> fishes,<sup>13–17</sup> insects<sup>10,18,19</sup> and the endophytic fungal community.<sup>20</sup>

Plant material decomposition releases much dissolved organic matter into aquatic environments. This produces a quantity of debris capable of regulating the nutrient flow in the ecosystem both spatially and temporally.<sup>21</sup> There is a strong link between primary production, decomposition and nutrient cycling.<sup>22</sup> Thus, the compounds released during the aquatic macrophyte decomposition can be responsible for most of the energy flow in aquatic ecosystems.<sup>23</sup> One way to monitor the mass loss over time is by calculating the decomposition rate. It affects the nutrient release, the accumulation of decomposing material in the sediment and the quality of the detritus,<sup>22</sup> and it is usually expressed by the weight loss in a certain period of time.

The metabolic activity of heterotrophic bacteria has important implications for the function of aquatic ecosystems.<sup>24</sup> Bacteria and fungi are essential for organic matter decomposition.<sup>25</sup> They use a variety of organic compounds under different environmental conditions, extracting energy from these compounds by fermentation and aerobic and anaerobic respiration.<sup>26</sup> They convert large amounts of matter into inorganic nutrients. The factors that affect the composition of the bacterial community and its activity have been the basis for many studies in recent years.<sup>1,2,27–29</sup> With no evaluation of the mechanisms that regulate microbial food webs and given the area covered by aquatic ecosystems, the functioning of aquatic ecosystems has been only partly described.<sup>30</sup>

This study investigated the existence of temporal fluctuations of limnological parameters during *E. azurea* decomposition that simulates the flood pulse because in this period, there is an organic matter input from aquatic macrophytes.

## Materials and methods

### Collection and assembly of the experiment

Samples of water and *E. azurea* leaves were gathered in the Ressaco Leopoldo (22°45'24" S, 53°16'7" W), located in Puerto Rico, in the Flood Plain of the Alto Paraná River.

The species *E. azurea* was chosen because it is more common in areas subjected to flooding and because it has high biomass levels.<sup>11</sup> In addition, many decomposition studies have been performed with this macrophyte in floodplains.<sup>30,31</sup>

In the laboratory, the water collected was kept under aeration until the experimental assembly. The macrophytes were dried in an oven for seven days in order to obtain the dry weight.

For the experimental assembly, 51 bottles of polyethylene were used (500 mL). An aeration system was used in each individual container. The experiment occurred in an insulated environment in order to maintain a stable temperature. In the experiment, 450 mL of water and 0.8 g of macrophyte dry weight were added to the bottles.

The mass was based on the values of macrophyte biomass obtained in the environments of the Upper Paraná River Floodplain<sup>30</sup> and simulated the decomposition events during the flood pulse.

The increase in *E. azurea* biomass can simulate the nutrient input effect characteristic of the flood pulse process. In this process, a large biomass concentration decomposes, leading to an increase in nutrient cycling and affecting the microbial loop.<sup>32</sup>

A control was also performed, in which the same volume of water was added without the addition of the macrophyte dry weight. Samples were taken at 0 (experiment initiation), 3 h, 6 h, 12 h, 24 h, 72 h, 120 h, 168 h and 240 h. These sampling times represent the leaching period of organic matter decomposition. At each time point, three containers from each treatment were randomly removed, and the plant material contained within was sent to the oven for drying. After 48 h, the material was weighed to obtain the final dry weight. Furthermore, 5 mL of water was separated for the bacteriological analysis. The remaining volume was used in physical and chemical analyses.

### Bacterial density and biomass

The density and bacterial biomass were estimated by filtering 0.1 mL of water from the experiment. Black polycarbonate filters (Nucleopore®) with pore openings of 0.2 µm, stained with 1 mL of the fluorochrome DAPI (4,6-diamidino-2-phenylindole), were used for 5 min in the dark. The filters were mounted on slides and stored in the freezer. Bacteria were quantified using an epifluorescence microscope (1000×). The biovolume was determined using the equation proposed by Fry (1990):  $v = (p/4) \cdot w2(l \times w/3)$ , where  $v$  = cell volume,  $l$  = length,  $w$  = width. For the conversion of biovolume to biomass, it was considered that  $1 \mu\text{m}^3 = 3.5 \times 10^{-13} \text{ gC}$ .<sup>13</sup>

### Abiotic analysis

The dissolved oxygen ( $\text{mgL}^{-1}$ ) levels and the water temperature were determined directly in the bottles using a portable digital oxymeter (YSI-550A). The electrical conductivity and pH values were determined using portable digital potentiometers. An aliquot of 50 mL was used for total dissolved organic carbon (DOC) determination, which was carried out by catalytic oxidation at a high temperature (720 °C) using the Shimadzu TOC analyzer V-CSN. The remaining water was filtered through a fiber glass filter (Whatman® GF/C) to determine the soluble phosphorus (P- $\text{PO}_4^{3-}$ ), ammonia ( $\text{NH}_4^+$ ), and total phosphorus concentration (TP).<sup>33–36</sup>

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