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Anaerobic decomposition of submerged macrophytes in semiarid aquatic systems under different trophic states, Paraíba State, Brazil

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The macrophytes play an important role in the regulation of biological and chemical processes in aquatic ecosystems, particularly in shallow lakes. They also play an important role in storage and nutrient cycling, serving as a source of organic matter to native environments. The aim of this study was to describe the kinetic aspects of the nutrients released during the anaerobic decomposition process of *Egeria densa* Planch and *Chara braunii* Gmnel macrophytes in waters with different trophic states. The study was conducted *in vitro* and under anaerobic conditions for determination of both particulate and dissolved fractions of nitrogenous, phosphorus and carbon, in predetermined days in oligotrophic and eutrophic water. Mathematical models were applied to describe the macrophytes decomposition process. Both species showed the same biphasic decay pattern of organic matter and carbon mineralization. The phosphorus, nitrogen and carbon released were high for both species, regardless of the trophic water state. The loss of mass was similar for both species and the nutrients concentration in the dam water did not represent a limiting factor for the mathematical model.

Key words: *Chara braunii*, *Egeria densa,* mathematical modeling, nitrogen, phosphorus.

INTRODUCTION

The submerged plants have a key role in biochemical processes regulation in aquatic ecosystems, especially in shallow lentic systems (Scheffer and Van Nes, 2007). They function as an autochthonous organic matter source and exert a relevant ecological role in nutrient and carbon storage as in their cycling (Palma-Silva et al., 2012). Submerged macrophytes species, *Egeria densa* Planch and *Chara braunii* Gmel are from northeastern Australia and the extreme south of South America (Sampaio and Oliveira, 2005). Once these plants present high ecological plasticity, they colonize aquatic systems in semi-arid regions of Brazil (Macêdo et al., 2012). *C.*

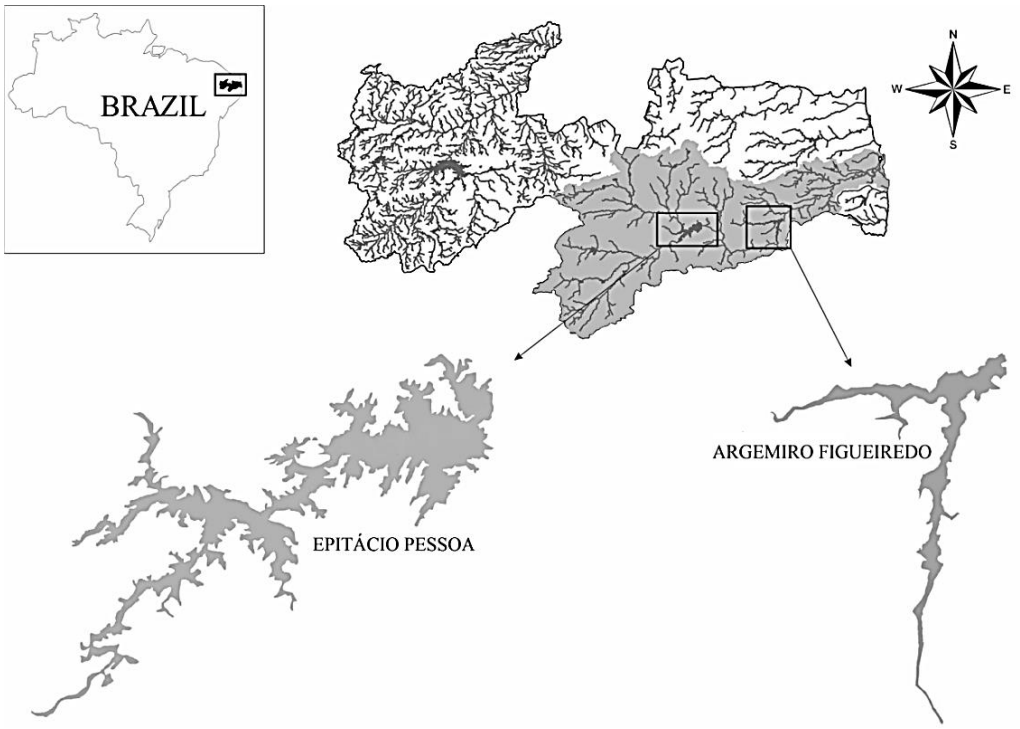

Figure 1. Localisation of sampling points, Epitácio Pessoa and Argemiro Figueiredo dams.

braunii belongs to the *Characeae* family and *Egeria densa* to the Hydrocharitaceae family, both rooted submerged macrophytes. *E. densa*, has a more fibrous plant structure (Rodrigues and Thomaz, 2007) as compared to *Chara braunii*, which like other macroalgae, has a protein-rich structure (Patarra et al., 2011).

With the senescence of macrophytes, the plant detritus enters through the carbon and nutrients cycle into the aquatic environment, which is incorporated in plant tissues during primary production (Kim and Rejmánková, 2004). However, depending on the colonization intensity, a relevant contribution of these organisms to eutrophication could occur, since the release of water-soluble compounds during senescence may act either as a nutrition or pollution to the water column (Anesio et al., 2003). During the biomass decomposition of submerged macrophytes, significant changes can occur in water, that is, increase of dissolved and particulate organic matter, aquatic system acidification, increased electrical conductivity (Bianchini and Cunha-Santino, 2010) and nutrient release (Wang and Fan, 2013). Such physical and chemical changes may be affected either by extrinsic factors like intrinsic ones, such as the species chemical composition (Bianchini and Cunha- Santino, 2008) and the nutrient concentration in the water which can accelerate the organic matter degradation or make it slower (Xie et al., 2004) due to the heterotrophic activity dynamics during the degradation process.

In situ and *in vitro* studies (Fonseca et al., 2014) on these kinetic aspects of macrophytes degradation, focus on the Brazilian Savanna (Cerrado) region and data on the Brazilian semiarid region decomposition kinetics of submerged macrophytes are still lacking. Therefore, the aim of this study was to describe the kinetic aspects of anaerobic decomposition of submerged macrophytes, *E. densa* and C. *braunii* in waters with different nutrients concentration. The hypotheses of this study is that the decomposition process occurs faster in eutrophic than in oligotrophic waters. Among the submerged species studied, *C. braunii* is expected to decompose rapidly, since it is an alga.

MATERIALS AND METHODS

The water samples used for the decomposition experiment were collected in two dams located in the Paraiba River basin in the Brazilian State of Paraiba, with varying trophic degrees. The “Epitácio Pessoa” dam (7° 30'41 "S 36° 11'52" W) is an oligotrophic dam (Vasconcelos et al., 2013). The “Argemiro Figueiredo” dam (07° 27'43 "S 35° 35'6" W), and its waters are characterized as eutrophic-hypertrophic status (Vasconcelos et al., 2013) (Figure 1).

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Macrophytes decomposition and mass balance experiment

The macrophytes were collected in the Epitácio Pessoa dam using a dredge collector for particles excess removal and dried in low temperature (40°C) to achieve the constant mass and to avoid loss of volatile compounds. Anaerobic incubations were performed according to Bianchini et al. (2010). Macrophytes fragments (size average = 0.5mm; s = 0.14) from both species were incubated in glass bottles in a proportion of 10 g/L^{-1}, kept in the dark under anaerobic conditions (oxygen ≤ 1.00 mg/L) at 27°C (ranging from 26 to 27ºC), which is the average temperature of the studied dam. Different treatments (species × trophic level) were analyzed in triplicate using *E. densa* fragments placed in glass bottles with eutrophic (eutrophic environment) and oligotrophic waters (oligotrophic environment). The same procedure was performed with *C. braunii*. In the respective sampling days (1, 3, 5, 15, 30, 60 and 90 days), three incubations of each treatment had contents fractionated in particulate organic matter (POM) and dissolved organic matter (DOM) by pre-filtration fiber filter glass (Φ pore = 0.8 µm) to remove the coarser material and then by pore membrane filtration (0.45 µm) (Bianchini et al., 2010). The control treatment consisted of only incubations with water from each dam.

The particulate mass was determined by gravimetry (Wetzel, 2001) in an analytical scale and converted into carbon-based particulate organic carbon (POC). For the conversion of particulate organic matter into carbon, it was assumed that the macrophytes carbon content is 47% ash-free (Wetzel, 2001). The organic matter content of particulate detritus was obtained by muffle incineration of the samples at 550°C for two hours (Blindow et al., 2006).

In a filtered dissolved organic matter fraction, the dissolved organic and inorganic carbon concentrations were determined using a carbon analyzer (Shimadzu TOC-5000A). In the dissolved fraction of the incubations, the pH and the electrical conductivity were determined with a multiparameter probe (HORIBA U-50). The concentrations of total nitrogen and total phosphorus were determined spectrophotometrically according to APHA (1998).

Mathematical modeling

To describe the macrophytes decomposition kinetics, the double exponential model was adopted, which considers that the detritus is composed by two fractions as described in Equation 1 (Lousier and Parkinson, 1976).

$$COP = COPLS \times e^{(-kT*t)} + COPR \times e^{(-kR*t)} \quad (1)$$

Where: COP_{LS} = soluble labile carbon (%); COP_R = refractory organic carbon (%);$k_T = k_1 + k_2$; overall coefficient of mass loss (day^{-1}); k_1 = the coefficient of mineralization of the labile fraction (day^{-1}); k_2 = leaching coefficient (day^{-1}); k_R = mass loss coefficient of the refractory fraction (day^{-1}).

The formation and mineralization of DOC were fitted to the model described in Equation 2 (Cunha-Santino et al., 2010).

$$\frac{dC_{COD}}{dt} = k_T\left(\frac{k_2}{k_T} C_{COPLS}\right) - k_3 C_{COD} \quad (2)$$

Where, dC_{COD} = change per time unit in the DOC concentration; k_T = leaching rate(day^{-1}); k_3 = COD digestion coefficient (day^{-1}).

The half-life ($t_{1/2}$) of the decomposition process was calculated by Equation 3:

$$t^{1}/_{2} = \ln(0.5)/-k \quad (3)$$

Where: k = decay coefficient for each type of plant fraction (K_{LS} and k_R).

Data analysis

Statistical analyzes were conducted to check the differences in the loss of the mass between species and the relationship between macrophytes decomposition coefficients and the nutrients concentration in the water. ANOVA statistic test was used, two-way repeated and measured in the STATISTIC software 7. This test was also used to test the isolated effects and the macrophytes species treatments and the trophic level over the phosphorus concentrations. Analytical approach was used for the data, and the significance was assumed as different at p ≤0.05 level.

RESULTS

Decomposition kinetics and carbon balance

The decomposition kinetics for soluble-labile fraction of the *E. densa* detritus (k_{COPLS} = 0.58, $t_{1/2}$ = 1 day) as well as the refractory fraction (k_{COPR} = 0.009; $t_{1/2}$ = 77.0) were higher in oligotrophic water. The same occurred with the *C. braunii* with both decomposition kinetics (k_{COPLS} = 0.99 ($t_{1/2}$ = 0.77 days) and refractory detritus (k_{COPR} = 0.008 ($t_{1/2}$ = 87 days) being higher in oligotrophic environment. Thus, there was no influence of the decomposition trophic state kinetics on the studied macrophyte ($F_{2,1}$ = 0.003; p = 0.85), disproving the hypothesis.

As for the average levels of the remaining particulate carbon, *E. densa* lost 76.93 and 64.66% of its initial mass, in eutrophic and oligotrophic incubations, respectively, while the *C. braunii* detritus lost 64.58 and 66.47% in eutrophic and oligotrophic waters, respectively (Figure 2). Statistically, the decomposition between the two species was similar, not corroborating our hypothesis ($F_{2,1}$=0.00,p = 0.97).

Regarding the carbon balance, it was observed that decrease in particulate carbon occurred concomitantly with the leaching of dissolved organic carbon (DOC) (Figure 3). Regarding dissolved carbon formation, the content observed in the *E. densa* decomposition was 6.0% over the initial carbon content of 3.5% in the incubations with *C. braunii* detritus. The dissolved carbon values increased until the 30th day in incubations with *E. densa* and as a result, there was a decrease at the end of the experiment which is up to 2% lower than in eutrophic and 4% in oligotrophic waters. In the *C. braunii* incubations, the dissolved carbon increase occurred until the 15th day, then, decreased for concentrations less than 0.5% in both trophic states tested. The dissolved carbon content generated from the species decomposition were different ($F_{2.1}$ = 29.68; p≤0.05), the organic carbon dissolved formed in *E. densa* decay was higher than that from *C. braunii* from the beginning to the end of the experiment. For the treatments, however, there was no significant difference between the dissolved carbon content ($F_{2.1}$ = 0.38, p = 0.53).

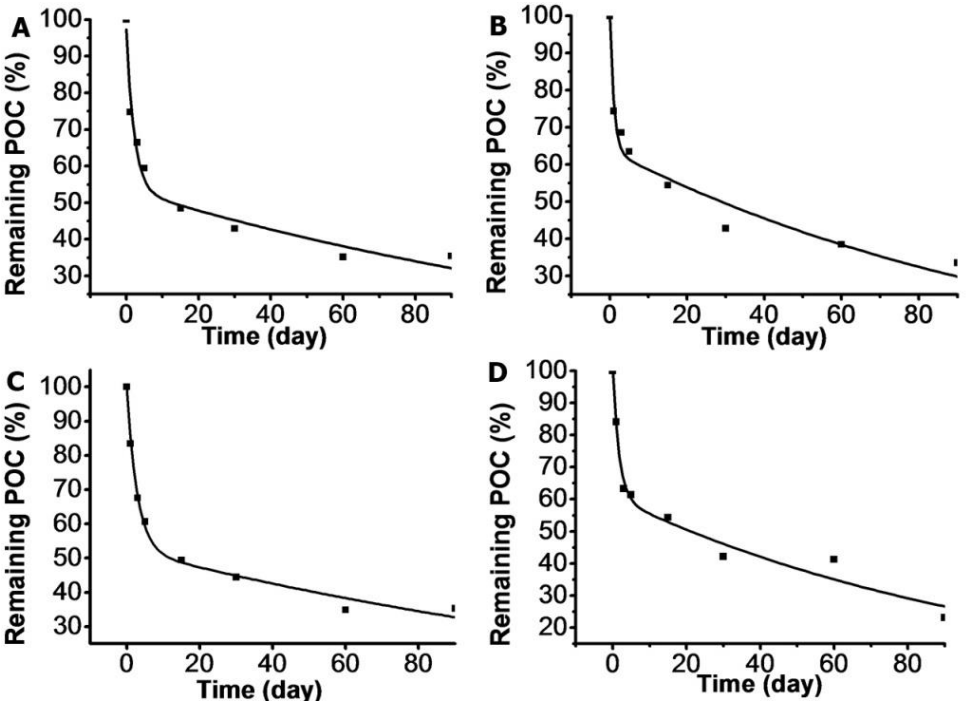

Figure 2. Temporal variation of particulate organic carbon (POC) during the anaerobic decomposition of *C. brauni* and *E. densa* in different states waters. A) Incubating in eutrophic water and *C. braunii;* B) Incubation in oligotrophic water and *C. braunii;* C) Incubation in eutrophic water and *E.densa;* D) incubation in oligotrophic water and *E. densa*.

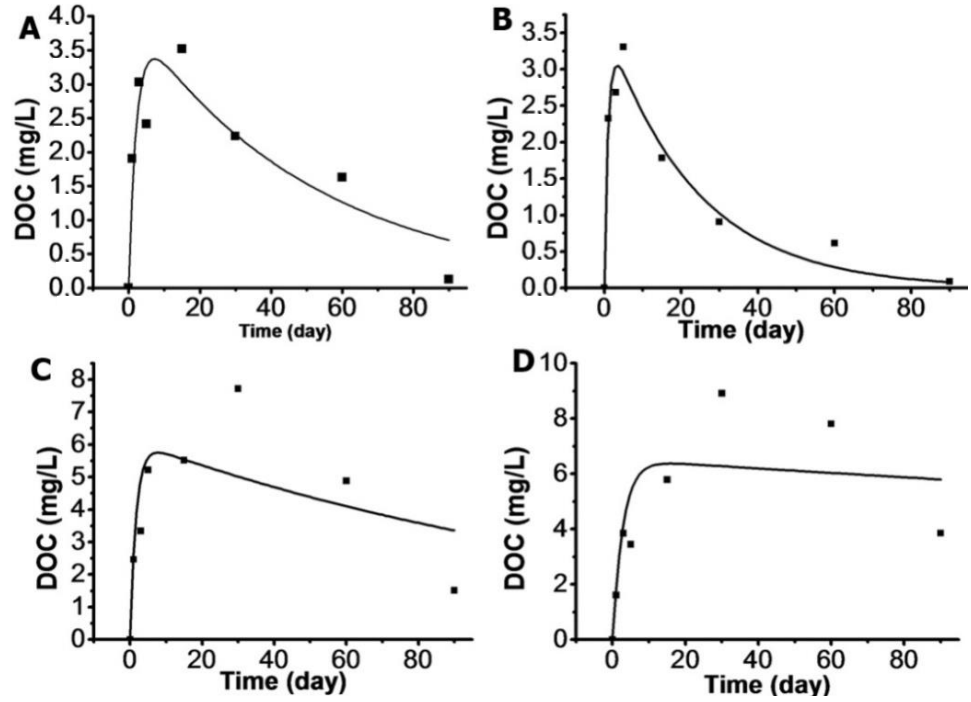

Figure 3. The DOC temporal change for *C. braunii* and *E. densa* decomposition process in oligotrophic and eutrophic media. A) Incubation with eutrophic water and the C. *braunii* B) Incubation with oligotrophic water and *C. braunii;* C) Incubation with eutrophic water and *E. densa* species D) Incubation with oligotrophic water and and *E. densa.*

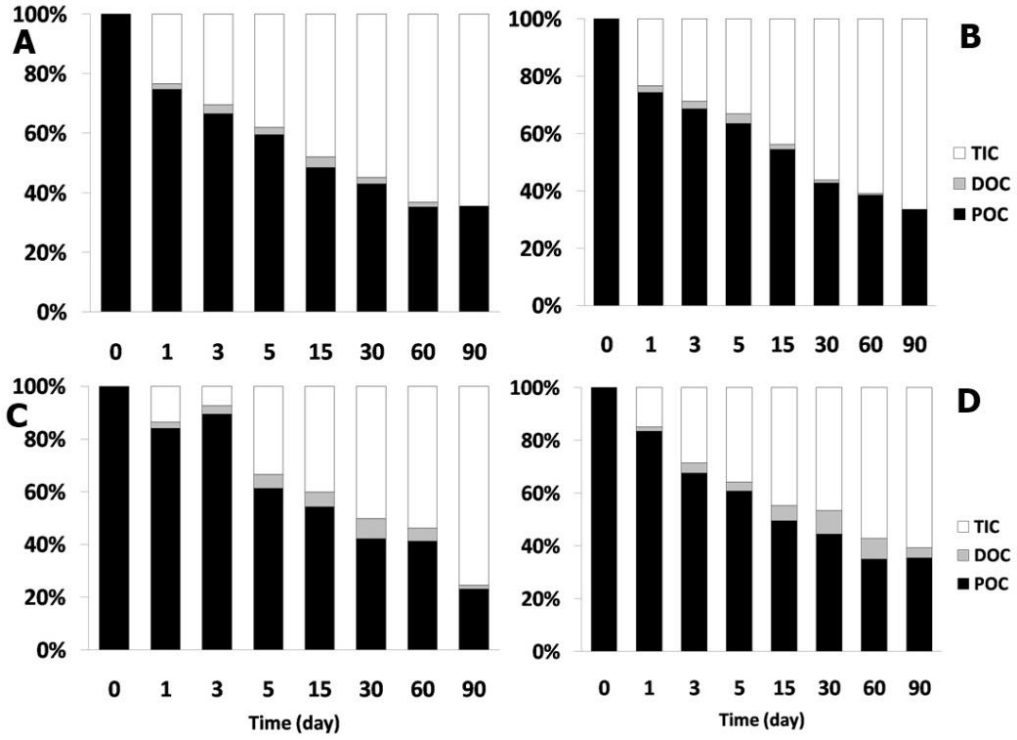

Figure 4. Carbon balance (total inorganic carbon (TIC), dissolved organic carbon (DOC) and particulate organic carbon (POC) (in %) in anaerobic incubations of *C. braunii* and *E. densa* decomposition). Incubation with eutrophic water and the C. *braunii*; B) Incubation with oligotrophic water and *C. braunii*; C) Incubation with eutrophic water and the *E. densa*; D) Incubation with oligotrophic water and *E. densa*.

The formation of dissolved carbon, mineralized carbon (TIC), and the decrease in the particulate carbon is shown in Figure 4. The dissolved carbon half-life was higher for decay of the *E. densa* in oligotrophic water ($t_{1/2}$ = 173.2 days). For both species, the dissolved carbon was mineralized with low coefficients (K_3): *E. densa*/ oligotrophic = 0.004 day^{-1}; *E. densa*/eutrophic = 0.020 day^{-1}; *C. braunii*/oligotrophic = 0.040 day^{-1} and *C. braunii*/eutrophic = 0.010 day^{-1}.

Dynamics of nutrients during decomposition

During the decomposition process, the phosphorus concentrations increased in the dissolved fractions of the decomposition incubations throughout the sampling period for both species (Figure 5). In the eutrophic incubations, the average phosphorus release values were higher than in the oligotrophic incubations, from 0.15 (initial concentration of phosphorus in water) to 7.5 ± 0.45 mg L^{-1} in the *C. braunii* decomposition incubations and 8.53 ± 0.07 mg l^{-1} in the *E. densa* incubations.

In oligotrophic treatments, the average P was 6.75 ± 0.35 mg l^{-1} in *C. braunii* decomposition and 7.85 ± 0.12 mg l^{-1} in *E. densa*. In both treatments, the *E. densa* detritus released higher phosphorus concentrations than *C. braunii*, but these differences were not significant ($F_{2,1}$ = 0.23; p = 0.62), the same occurred between treatments ($F_{2.1}$ = 0.00; p = 0.97), and with the combined effect of the species with treatment ($F_{2.1}$ = 0.13; p = 0.71).

Similarly, the N release (Figure 5) was not influenced by the species ($F_{2.1}$ = 0.03; p = 0.85). In the eutrophic environment, an increase in nitrogen concentration of 2.32 ± 0.58 mg l^{-1} was observed in the *E. densa* incubations and in the *C. braunii* incubations, the increase was 2.5 ± 0.23 mg l^{-1}. In the oligotrophic environment, the increase was 2.16 ± 0.03 mg l^{-1} (*E. densa*) and 1.4 ± 0.58 mg l^{-1} for *C. braunii*. There was no trophic state influence on the N release ($F_{2.1}$ = 0.02; p = 0.87).

With high nutrient concentrations, changes in the water physical parameters could be observed such as the electrical conductivity reaching higher values in eutrophic incubations, from 1.574 ± 5.18 to 6.346 ± 5.5 μScm^{-1} in *C. braunii* decomposition and 6.346 ± 5.5μScm^{-1} in *E. densa* decomposition. In the oligotrophic treatment, the increase was 1.135 ± 21.45 to 5.793 ± 5.7 μScm^{-1} (*C. braunii*) and 4.050 ± 26.43 μScm^{-1} (*E. densa*). There were significant differences in the electrical conductivity variation during decomposition considering the different

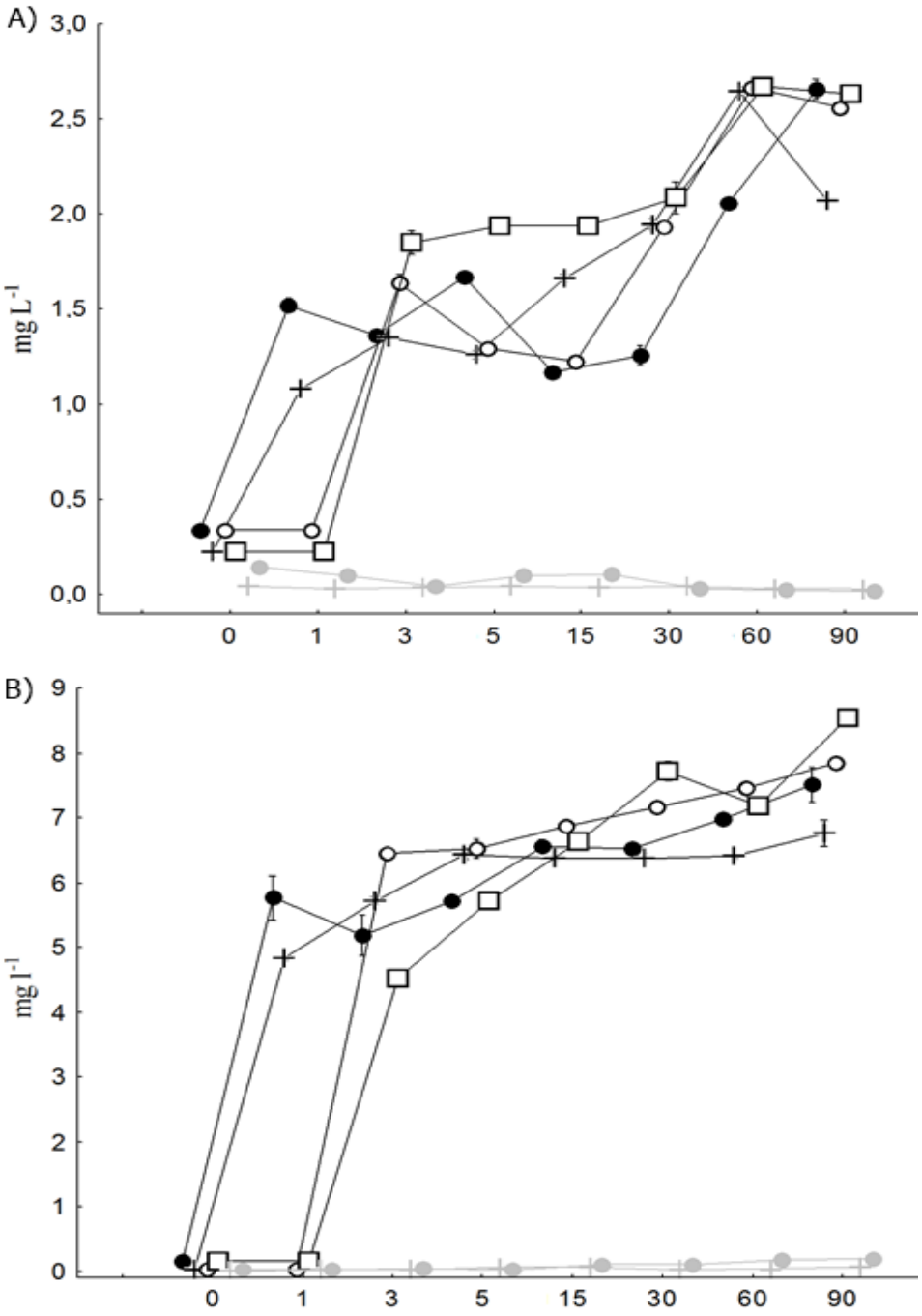

Figure 5. Temporal variation in nitrogen (A) and phosphorus (B) release of *E. densa* and *C. braunii* decomposition incubations. ● Eutrophic *C. braunii* incubation; ┼ Oligotrophic *C. braunii* incubation; ○ Eutrophic *E. densa* incubation; □ Oligotrophic *E. densa* incubation; ● No plant control group in eutrophic water; ┼ No plant control group in oligotrophic water.

species ($F_{2.1}$= 20.35; p ≤0.05) and also in the trophic degree ($F_{2.1}$ = 4.64, p = 0.03).

As in electrical conductivity, pH in the decomposition incubations also changed during the decomposition process, reaching slightly acidic values in the incubation in the first 24 h. In the *C. braunii* decomposition in oligotrophic treatment, the decomposition chamber pH decreased from 9.2 ± 0.40 to 5.7 ± 0.06 on the first day and in the end of the experiment, the pH was 6.3 ± 0 00. In the eutrophic environment, the pH decreased from 8.8 ± 0.48 to pH 6.7 ± 0.11 on day one, reaching the value of 6.4 ± 0.00 at the end of the experiment.

In the *E. densa* decomposition, a higher acidification was observed in the incubations than with *C. braunii* ($F_{2.1}$ = 6.18; p = 0.001). In the eutrophic treatment, the medium pH decreased from 8.8 ± 0.48 to pH 6.4 ± 0.03, with some values below 5.5 ± 0.59 between the 3rd and 15th days. In the oligotrophic incubations, the pH decreased from 9.2 ± 0.40 to pH 5.7 ± 0 on the first day, showing variation of 4.7 ± 0.17 to 5.5 ± 0.0. There were no significant differences between trophic state and pH change ($F_{2.1}$ = 1.66; p = 0.20).

DISCUSSION

Phosphorus and nitrogen-enriched water may accelerate the detritus decomposition (Rejmánkavá and Houdkavá, 2006); however, other studies suggest slower decay rates in eutrophic waters (Sarneel et al., 2010). In this study, the lack of relationship between the trophic state and the mass loss was due to the availability of water nutrients (nitrogen and phosphorus) and does not represent a limiting factor for the macrophytes decomposition and does not influence the chemical immobilization (Xie et al., 2004). The metabolic activities of microorganisms usually occur according to the quantity and especially the quality of the detritus (Cunha-Santino and Bianchini, 2009). In this case, eutrophic waters may have lower decomposition rates if the detritus quality (intrinsic factor) is a predominant constraint on the decomposition process as observed in *C. braunii*.

Overall, there were no differences between the remaining mass content of the two species, although belonging to different groups, both are submerged macrophytes, and have similar habits, with lower content of hard support tissues (Suzuki et al., 2013) than those found in emergent macrophyte. Thus, from the quantitative point of view, the decomposition of these species were similar and fast, since there is a proximity of mineralized carbon in both species (*E. densa* = 87.76% and *C. braunii* = 75.15%).

The k_r coefficients were low in the species, due to the slow degradation and the presence of fibers which can exert a barrier in the anaerobic degradation (Agoston-Szabó and Dinka, 2008). Cellulose fiber in *Chara* species biomass was determined at 9.67% (Muztar et al., 1978), whereas for *E. densa*, it was 29.2% (Little, 1979).

As they presented slower decomposition rates, the fibers are generally accumulated in limnic sediments, becoming possible precursors of humic compounds (Bianchini and Cunha-Santino, 2008), which allows us to affirm that the refractory fractions of *E. densa* and *C. braunii*, could act in the ecosystem metabolism as a possible precursor source in the humic substances genesis, due to the low coefficient of mineralization (k_R). The dissolved carbon presented a refractory potential during *E. densa* and *C. braunii* decomposition, with low mineralization coefficients (K_3). In aquatic environments, the dissolved carbon is mostly (up 60%) composed of humic substances (Bianchini et al., 2014). The refractability was primarily due to the type of synthesized by-product by decomposing microorganisms in the specific conditions adopted in this experiment, that is, anaerobic, temperature and substrate type (Cunha-Santino and Bianchini, 2009). In the decomposition process, transformations of plant tissues fractions, by leaching, in dissolved carbon, are very important because these compounds interfere with the organic carbon transfers to the water column organisms, as well as to that held on the particulate detritus (Sala and Gude, 1999).

In this study, P and N releases were observed throughout the *C. braunii* and *E. densa* decomposition process, indicating that these nutrients are part of these plants biomass, being raised in the aquatic environment from the growth phase to senescence. The amount of incorporated nutrients depends on the productivity rate and the particular species can interfere with decomposition, like *Chara* species, storing nutrient for long periods, especially during the low temperature periods, slowing the aging process (Kufel and Kufel, 2002).

The decay of detritus in *E. densa* and *C. braunii* provoked an intense release of P and N in the decomposition chamber and may have an impact during its death on the water column in the environment colonized by these species, especially in the first 15 days in which concentrations of these nutrients were higher. In the leaching phase, release of P was higher than N. In plant biomass decay processes, the fractions of P may be more soluble than the N fractions (Rejmánková and Houdkova, 2006). Throughout the experiment, P was accumulated in the water, and not consumed, unlike the study by Kroger et al. (2007) which reported a decrease of P concentration in water since they were added to the pellet (environmental route) by decay.

With regards to the released concentrations of N and P, the decomposition of these species is a potential source of nutrients, thereby contributing to the eutrophication process, since these nutrients act as one of the limiting factors causing the process (Mattar et al., 2009). As the submerged macrophyte have faster decay rates (Petersen and Cummin, 1974) than other macrophytes (emergent, foil floating and floating), nutrients stock in the biomass of these organisms is of short-term duration.

During decomposition, electrical conductivity increased due to the large accumulation and release of ions present in the leached materials (Mun, 2000). In this decomposition stage, an intense generation of inorganic carbonaceous compounds (e.g. CO_2) emissions from anaerobic mineralization also occurred. From the degradation phase of labile-soluble fraction, the electrical conductivity continued to increase without stabilization due to the ions released from the refractory fraction decomposition of detritus, mainly in *C. braunii* incubations.

Parallel to conductivity, pH decreased rapidly at the beginning of the experiment; this was due to the medium acidification, ammonium, bicarbonates and organic acids formation. Over time, the media pH increased due to the anaerobic ammonium oxidation reactions (Mulder et al., 1995). The frequency means of slightly acidic to softly (mean <4.7 and <6.9) were probably due to the balance between the buffering systems and constant input of intermediates during the process, which would tend to the medium acidification (Weimer and Zeikus, 1977).

The hypothesis of this study was that the decomposition process of the *E. densa* and *C.* braunii occurs faster in eutrophic than in oligotrophic waters, surprisingly, it was found in this study that there was no significant difference in mass loss of *E. densa* and *C. braunii* detritus in oligotrophic and eutrophic environment. Although, they are species with distinct chemical and structural evolution from a quantitative point of view of carbon, these species have similar ecosystem metabolic responses. The differences in the contents of labile and refractory-soluble compounds in the same species are due to the use of different proportions of the plant parts (stem and leaf) in the decomposition incubations.

Conclusion

The mass loss kinetics between *E. densa* and *C. braunii* was not significantly different in the present study, which rejects the hypothesis that these species represent distinct divisions (Chlorophyta x Spermatophyta) and present distinct kinetics of mass loss. The trophic state (eutrophic and oligotrophic media) of semiarid Paraiba did not represent a limiting or stimulatory factor for the decay of *E. densa* and *C. braunii* detritus. The decomposition of both species is a potential source of nutrients release that can cause eutrophication in water bodies where such weeds live. The accumulation of these refractory compounds (such as fibers) due to decomposition, occurred in long-term, which could generate accumulation of humic substances in the aquatic environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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