







Biomass and abiotic variables change in phytotelmic environment in the tank-bromeliad *Nidularium longiflorum* Ule in tropical forest

Mudanças da biomassa e das variáveis abióticas do ambiente fitotélmico na bromélia-tanque *Nidularium longiflorum* Ule em uma floresta tropical

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Abstract: Aim: Phytotelm plays an important role in plant growth and ecosystem functioning, but this natural aquatic microcosm is poorly known. We evaluated the seasonal (dry and rainy seasons) and spatial variations (forest trail and stream sites) of the phytotelm in *Nidularium longiflorum*, bromeliad that occurs in the Atlantic Forest. **Methods:** Abiotic and biotic variables were measured in tank-bromeliad phytotelms. The biomass was analyzed by ash-free dry mass and chlorophyll-*a* concentration. **Results:** Abiotic variables measured in the phytotelmic environment of bromeliads varied between sampling sites and seasons. Temperature, electrical conductivity and total nitrogen values were significantly different between seasons and sites. Chlorophyll-*a* and ash-free dry mass (organic matter) in phytotelm were significantly different between sampling sites. Eleven genera of algae in the phytotelm were identified. PCA axis 1 ordination evidenced the seasonal variation of environmental conditions. **Conclusions:** Our findings suggest that environmental and micro-environmental conditions do not favor the development of algal community in the phytotelm. Biomass and abiotic variables in phytotelm of *Nidularium longiflorum* change seasonally, however biomass accumulation was strongly influenced by site characteristics.

Keywords: algae; Atlantic Forest; Bromeliaceae; phytotelmata; temporary habitats.

Resumo: Objetivo: Fitotelma desempenha um papel importante no crescimento das plantas e funcionamento do ecossistema, mas este microcosmo aquático natural é pouco investigado. O objetivo do presente estudo foi avaliar as variações sazonais (estações seca e chuvosa) e espaciais (trilha florestal e córrego) do fitotelma de *Nidularium longiflorum*, bromélia que ocorre na Mata Atlântica. **Métodos:** As variáveis bióticas e abióticas foram analisadas em fitotelmas de bromélias-tanque. A biomassa foi analisada pela massa seca livre de cinzas e concentração de clorofila-*a*. **Resultados:** As variáveis abióticas no ambiente fitotélmico das bromélias variaram entre locais de amostragem e estações. Os valores de temperatura, condutividade elétrica e de nitrogênio total foram significativamente diferentes entre períodos climáticos e locais. A clorofila-*a* e a massa seca livre de cinzas (matéria orgânica) no fitotelma foram significativamente diferentes entre os locais de amostragem. Foram identificados 11 gêneros de algas no fitotelma. A ordenação unidades amostrais no eixo 1 da PCA evidenciou a variação sazonal das condições ambientais. **Conclusões:** Nossos resultados indicam que as condições ambientais e



microambientais não favorecem o desenvolvimento da comunidade de algas no fitotelma. Biomassa e variáveis abióticas no fitotelma de *Nidularium longiflorum* mudaram sazonalmente, porém a acumulação de biomassa foi fortemente influenciada pelas características locais.

Palavras-chave: algas; Mata Atlântica; Bromeliaceae; fitotelmata; habitat temporário.

1. Introduction

Plants can accumulate water or water-filled tree holes forming a natural aquatic microcosm termed phytotelmata (Kitching, 2000). Bromeliads present morphological and ecophysiological characteristics that permit the accumulation of water and organic matter, thus allowing the development of phytotelmic communities (Kitching, 2000). An important physical characteristic of bromeliads is their tank which functions as a rainwater reservoir, or “plant-held water”. This structure is formed by a spiral-like arrangement of leaves forming a rosette and a base shaped like an inverted cone (Kitching, 2000; Dias & Brescovit, 2004; Wanderley & Tavares, 2011). Bromeliads present a high degree of endemism, have expressive ecological value, and promote interaction with fauna in tropical ecosystems (Martinelli et al., 2008). These tanks provide a favorable environment for a wide variety of organisms, such as small invertebrates and vertebrates (insects, arachnids, frogs and snakes), algae, fungi and bryophytes (Kitching, 2000).

Bromeliad phytotelma can play an important role in tropical ecosystems. These structures offer a source of nutrients and water for biota and serve as a refuge for protection and reproduction of several species (Oliveira, 2004; Cogliatti-Carvalho et al., 2010). Despite the importance of its ecological role, little is known about the structure and function of this natural aquatic microcosm, particularly in tropical ecosystems. In addition, the phytotelm has an important role on bromeliad development since it provides a supply of nutrients and water, as well as protection against strong solar radiation (Benzing, 2000; Wanderley & Tavares, 2011).

Several factors can directly or indirectly influence the characteristics of the phytochemical environment. For example, tank size is a determinant of both autotrophic and heterotrophic biomass accumulation. Also, its geometry determines the volume of accumulated water, which, in turn, influences reserve efficiency and evaporation rates (Benzing, 1980; Zotz & Thomas, 1999; Cogliatti-Carvalho et al., 2010). In addition, the volume of water in the tank can increase or decrease, depending on climatic conditions, mainly those associated with temperature variations

(Cogliatti-Carvalho et al., 2010). Climatic conditions, such as air temperature, rainfall and wind speed, as well as the type of arboreal canopy, can determine the amount of organic matter in the tank (Schneider & Frost, 1996).

Studies on phytotelm dynamics have mainly focused on macro-organisms, mainly mosquito larvae (Carrias et al., 2014). Thus, we find many gaps in knowledge about the relationship between biomass changes and abiotic conditions in the phytotelm, as well as its association with the occurrence of algae. In tropical regions, studies evidenced the changes of biomass and species composition of algal communities in the tank bromeliads (Sophia et al., 2004; Marino et al., 2011; Ramos et al., 2011). Therefore, considering the important role of the phytotelm plays in promoting tropical biodiversity and food web (Kitching, 2000, 2001), we evaluated the changes in the biomass and nutrient availability, as well as the occurrence of algal genera, in the phytotelm of *Nidularium longiflorum*, near a forest trail and stream in dry and rainy seasons in Atlantic Forest.

2. Material and Methods

2.1. Study site

The study was performed in the Atlantic Forest at the Biological Reserve of Alto da Serra de Paranapiacaba (23°46'S; 48°18'W) located in Santo André, São Paulo State, Brazil (Figure 1). The Biological Reserve has an area of 336 ha, elevation between 750-891 m, and a tropical, mesothermic and superhumid climate, with relative humidity of 95% (Lopes & Kirizawa, 2009). The climate is characterized by a rainy season from October to March (summer) with high temperature and rainfall (annual average 22.4 °C and 192 mm, respectively), and a dry season from April to September (winter) with lower temperatures and less rainfall (annual average 17.5 °C and 59 mm, respectively) (SEMASA, 2014; USP, 2014). The study was carried out near a forest trail (23°46'10.5"S, 46°20'30.4"W) and near a stream (23°46'19.1"S, 46°20'31.2"W). Bromeliads near the forest trail and the stream were located at 47 and 19.5 m.n.m in elevation, respectively.

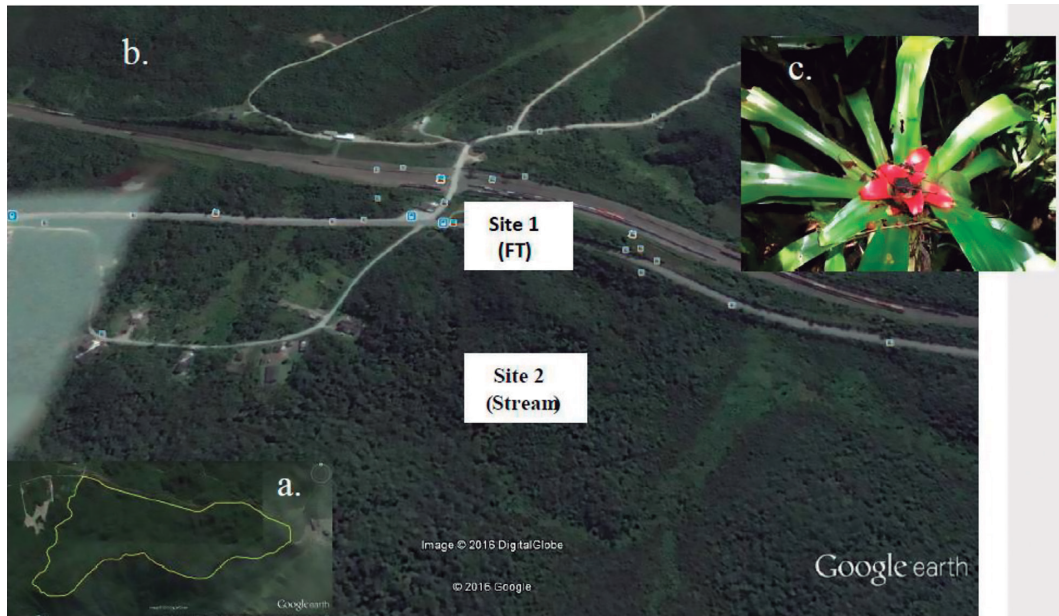


Figure 1. (a) Biological Reserve of Alto da Serra de Paranapiacaba (Santo André, São Paulo State, Brazil (Google Earth, 2014); (b) Localization of sampling sites (Image from Google Earth, 2014); (c) Bromeliad *Nidularium longiflorum* Ule (Photo: Neves, K.).

The bromeliad chosen for the study was *Nidularium longiflorum* Ule, which has a tank with the capacity to store water, about 170 mL, and is one of the most abundant species, as observed in the forest. The species is a rupicolous or epiphytic bromeliad endemic to Brazil, occurring in São Paulo and Rio de Janeiro States. The aspect is one of rosette with broad and contiguous sheaths with leaves between 30 and 60 cm in length. Flowering occurs from February to October (Wanderley & Martins, 2007).

2.2. Sampling design

Phytotelm samples were collected from bromeliads growing in the forest trail and stream sites during the rainy (March–April 2014) and dry (September 2014) seasons. We randomly sampled 12 bromeliads near the forest trail and 12 bromeliads near the stream in each climatic season.

The phytotelmata in both tanks and bromeliad leaf axils were completely removed using Pasteur pipettes. In the laboratory, phytotelm total volume was measured, and aliquots were separated for chemical and biological analyses.

2.3. Analyzed variables

Air temperature (mercury thermometer) and solar radiation (LICOR, model LI-250) were measured at the sampling sites. Rainfall volume was provided by SEMASA and Estação Meteorológica

do IAG/USP. The measures were chosen to verify the variables that exerted influence on the chosen plants.

Temperature (mercury thermometer) and pH (universal indicator paper pH 0–14 Merck) were measured in each tank-bromeliad. Total nitrogen (TN) and total phosphorus (TP) concentrations in the unfiltered water samples of the phytotelm were analyzed (Valderrama, 1981).

Phytotelm chlorophyll-*a* (corrected for phaeophytin) was determined from a subsample filtered on glass-fiber filters (GF/F Whatman, Maidstone, UK), following 24 h extraction with 90% ethanol in the dark (Marker et al., 1980; Sartory & Grobbelaar, 1984). Samples were filtered on glass-fiber filters, which were precalcined and weighed to determine dry mass (DM, particulate matter) and AFDM (particulate organic matter). Subsequently, samples were weighed every 24 h until obtaining a constant mass to determine dry mass. Samples were calcined (500 °C, 1 h) and weighed to determine AFDM (APHA, 2005). We used photosynthetic biomass (chlorophyll-*a*) and ash-free dry matter (organic matter) to examine biomass variation in the phytotelma.

Phytotelm samples were preserved with a 4% formalin solution for qualitative analysis of algae community. Algae were examined under a Zeiss Axioskop 2 and identified to genus level (Bicudo & Menezes, 2017) and the nomenclature was updated using AlgaeBase (2017).

2.4. Statistical analysis

Analysis of variance (two-way ANOVA) was used to compare means of abiotic variables and biomass (Chlorophyll-*a*, DM, AFDM) in the phytotelma ($\alpha = 0.05$; logarithmic data). Data were logarithmic when necessary to fulfill the assumptions of the analysis (homogeneity of variance and normality). Univariate analysis was performed using the statistical software PAST 3.01 (Hammer et al., 2001). Spearman correlation was used to test the association between phytotelm volume and biotic variables ($\alpha = 0.05$).

To reduce dimensionality of abiotic and biotic data, Principal Components Analysis (PCA) was performed, using covariance matrix with log-transformed data $[(\log(x + 1))]$, except to pH. The PC-ORD 6.0 program was used (McCune &

Mefford, 2011). Pearson correlation (r) between the ordination scores and the individual variables was used to assist in the interpretation of the ordination ($\alpha < 0.5$).

3. Results

The climatic data are shown in Table 1. Solar radiation was significantly different between sites and seasons (two-way ANOVA: $p < 0.05$). The highest value of solar radiation was recorded at the forest trail site. Air temperature was higher in the rainy season compared to the dry season. The highest rainfall was found in forest trail sites during the rainy season.

Abiotic variables measured in the phytotelm environment varied between sampling sites and seasons (Figure 2). However, only temperature,

Table 1. Medium values (n = 8) and standard deviation of climatic variables in the study area. The values of rainfall refer to accumulated.

Seasons	Site	Solar radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Temperature ($^{\circ}\text{C}$)	Rainfall (mm)
Rainy	Forest trail	433.9	20.7	316.5
	Stream	336.5	23.2	107.5
Dry	Forest trail	557.0	18.2	109.7
	Stream	353.4	17.4	109.7

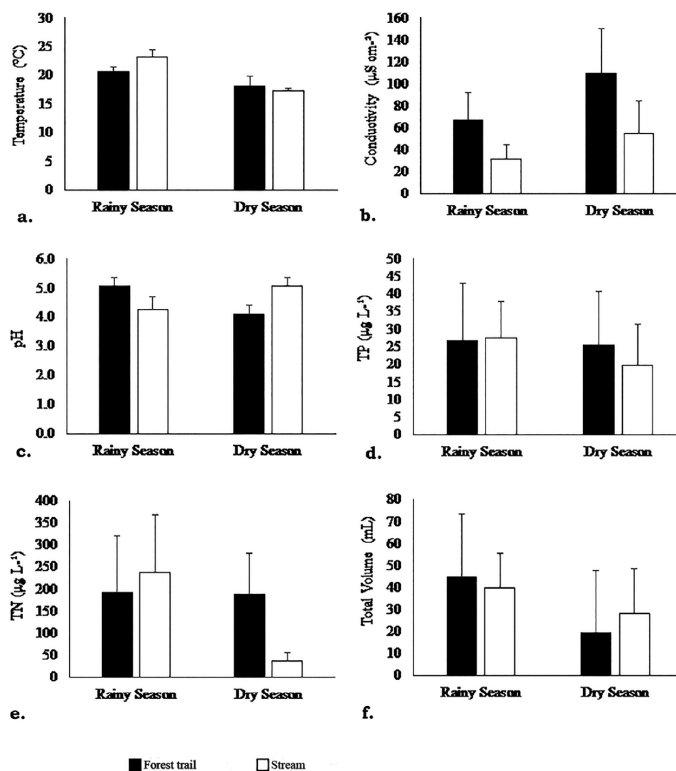


Figure 2. Temperature (a), electric conductivity (b), pH (c), total phosphorus (d) and nitrogen (e) concentrations and total volume (f) (Means; \pm SD; N = 12) in the phytotelmata of *Nidularium longiflorum* at the forest trail and stream in rainy and dry seasons.

electrical conductivity and total nitrogen were significantly different between seasons and sites (two-way ANOVA: $p < 0.05$). TP concentration was significantly different between seasons (two-way ANOVA: $p < 0.05$). Phytotelm volume in bromeliad tanks was significantly different between seasons (two-way ANOVA: $p < 0.05$), but no difference was detected between the two sites.

Chlorophyll-*a* and AFDM (particulate organic matter) in the phytotelm were significantly different between sites (two-way ANOVA: $p < 0.02$). On average, chlorophyll-*a* and AFDM were 2.1 to 2.7 times higher in the phytotelm of bromeliads near the forest trail site compared to bromeliads near the stream site (Figure 3). The correlation between AFDM and phytotelm volume in bromeliads was negative and significant (Spearman correlation: $r = -0.4$; $p < 0.004$).

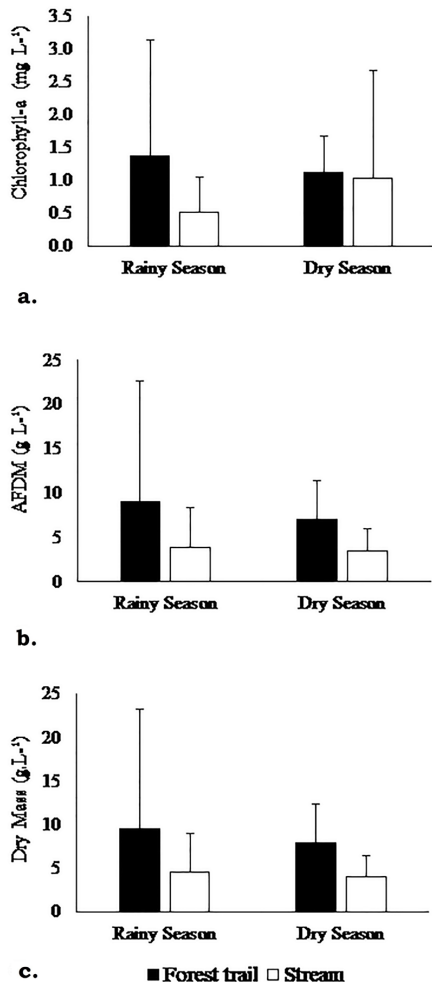


Figure 3. Chlorophyll-*a* (a) ash-free dry mass (b, AFDM) and dry mass (c, DM) (Mean; \pm SD; $n = 12$) in the phytotelmata of *Nidularium longiflorum* at the sampling sites near the forest trail and stream in rainy and dry seasons.

Eleven genera of algae were identified in the phytotelm of *N. longiflorum* during the study period (Table 2). These genera were distributed into the following algal class: Bacillariophyceae, Chlorophyceae, Cyanophyceae, Cryptophyceae, Euglenophyceae, Conjugatophyceae (Zygnematophyceae), and Ulvophyceae.

PCA summarized 67.8% of total data variability on the two first axes (Figure 4). Most samples from the dry season were ordered on the negative side of axis 1 and were correlated with the high values of AFDM, chlorophyll-*a* (Pearson: $r > -0.7$) and electric conductivity (Pearson: $r = -0.6$). Samples from the rainy season were correlated with high TP concentrations and phytotelm volumes (Pearson: $r > 0.8$). On negative side of axis 2, most of samples were correlated with higher TN concentrations (Pearson: $r > 0.9$). PCA axis 1 ordination evidenced the seasonal variation of environmental conditions.

Table 2. List of algae genera and their respective groups identified in the phytotelmata of *Nidularium longiflorum*.

Algal class	Genera
Bacillariophyceae	<i>Achnanthydium</i> sp. <i>Diploneis</i> sp.
Chlorophyceae	<i>Chlamydomonas</i> sp.
Cyanophyceae	<i>Aphanothece</i> sp. <i>Hapalosiphon</i> sp.
Cryptophyceae	<i>Cryptomonas</i> sp.
Euglenophyceae	<i>Phacus</i> sp.
Zygnematophyceae	<i>Cylindrocystis</i> sp. <i>Closterium</i> sp.
Ulvophyceae	<i>Binuclearia</i> sp.

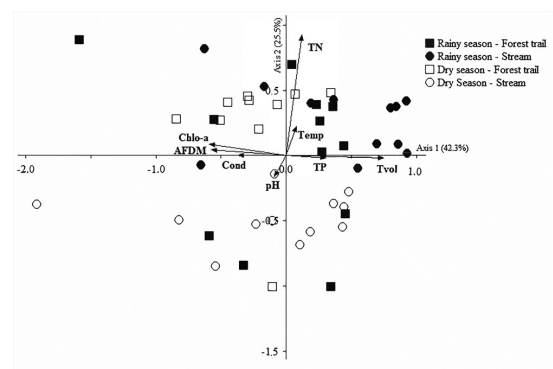


Figure 4. PCA of abiotic and biotic variables in the phytotelmata of *Nidularium longiflorum* at sampling sites near forest trail and stream in rainy and dry seasons. Vectors: Chl-*a* = Chlorophyll-*a*; AFDM = ash-free dry mass; Cond = electrical conductivity; TN = total nitrogen; pH = pH; Temp = temperature; Tvol = total volume; TP = total phosphorus.

4. Discussion

Results showed that biomass and abiotic variables in the phytotelmic environment of *Nidularium longiflorum* varied both seasonally (dry and rainy seasons) and spatially (forest trail and stream). Multivariate ordination analysis (PCA) showed that seasonality could influence characteristics of phytotelmic microenvironment. In contrast with the bromeliad samples near the stream, the phytotelm near the forest trail had high association with the high values of biomass and TN in the dry season. Among the analyzed variables, phytotelm volume, biomass and TN concentrations presented the greatest variability in the tank bromeliad during the study period.

As reported in the literature (Marino et al., 2011; Carrias et al., 2014), significant differences were found in phytotelm volume in bromeliads between seasons. As could be expected, the highest phytotelm volume occurred during the rainy season when the highest rainfall was recorded, giving evidence of the direct influence of rainfall on phytotelm characteristics. According to Pires et al. (2017), rainfall is a determining factor for the environmental characteristics of phytotelma, including changes in the dominance of the algal community and other organisms. Studies reported that the degree of desiccation in bromeliad tanks depends on local conditions, such as temperature and altitude (Cogliatti-Carvalho et al., 2010; Brouard et al., 2011, 2012). Therefore, seasonality, mainly rainfall, exerts influence on phytotelm volume, which may, in turn, directly affect the dynamics of phytotelm microenvironment.

Total nitrogen and phosphorus concentrations in the phytotelma significantly varied between seasons, indicating that seasonality influences nutrient availability in tank bromeliad. The visible dark coloration, high particulate matter, TN concentration and acid pH were indications of the intense decomposition of organic matter in the tank during the study period, mainly in the rainy season. Thus, the results suggest that decomposition was more intense in the rainy season as a consequence of higher temperatures that may have favored the process. Temperature has a direct influence on decomposition rate of organic matter in bromeliad tanks (Lopez et al., 2009). Total nitrogen concentration in the phytotelma were significantly different between sampling sites, indicating that other factors affect nutrient availability in the phytotelma. Nutrient supplies are mainly derived from windborne particles and

captured insects (allochthonous organic matter), but primary producers (autochthonous organic matter) may also contribute to increased nutrient concentrations in the tank (Brouard et al., 2011, 2012). In an experimental study (Ribeiro et al., 2016), the phytotelm in bromeliads changed from neutral to acidic, and ion concentration dropped to very low values close to $10 \mu\text{S m}^{-1}$. These findings suggest that conditions in the microenvironment, such as water chemistry, can be a key aspect toward a better understanding of community structure found inside these natural microcosms.

In contrast to abiotic variables, changes in phytotelm biomass (organic matter) were more significant between forest trail and stream sites than between seasons. Thus, seasonality did not have a strong influence on particulate organic matter (AFDM) content or chlorophyll-*a* concentration in the phytotelma of *Nidularium longiflorum*. These significant differences between sampling sites suggest that forest characteristics had a strong influence over the amount of organic contents. Moreover, compared to the stream site, such forest characteristics at the trail site favored a greater accumulation of biomass in the phytotelma.

Considering the algal community in tank bromeliad species of Brazilian ecosystems (e.g., Sophia et al., 2004; Ramos et al., 2011), we observed few genera of algae in the phytotelmic environment of *Nidularium longiflorum* in Atlantic forest. Studies reported the high species richness of algae in bromeliads from different ecosystems, as restinga (Sophia 2004: 33 taxa of desmids). Pires et al. (2017) found algal species in 70% of the bromeliads in the restinga, where high light availability occurred. Ramos et al. (2018) found high abundant and species richness of algae in bromeliad tank growing at 850 m a.s.l. and fully exposed to sun light (73 taxa: *Alcantarea nahoumii* (Leme) J.R. Grant), while few species were found in bromeliads (13 taxa: *Hohenbergia stellata* Schult. & Schult. f.) growing mainly in shaded forest sites in Atlantic Forest. Thus, differences in light incidence due to forest structure may be a determinant factor of the species richness and abundance of algae in the bromeliad tanks, as observed in the present study. To Farjalla et al. (2016) the penetration of light through the canopy can determine algal productivity and, consequently, can influence the web food.

Besides the low light availability in the bromeliad tanks due to the forest closed at sampling sites, we found the large amount of organic matter, which

may have hampered the development of the algal community. The amount of algal biomass in phytotelm may be linked to physical properties of the bromeliad, particularly maximum volume and diameter of the tank (Marino et al., 2011). The tank volume of *Nidularium longiflorum* is relatively small (170 mL) compared to other species, as *Neoregelia cruenta* (R. Graham) L.B. Sm. and *Hohenbergia castelanosii* L.B. Sm. & Read that can contain until 638 mL and 2.866,7 mL, respectively (Cogliatti-Carvalho et al., 2010). The water volume could be a factor that explains the distribution of the algal (Carrias et al., 2014). The nutrient availability also could be another factor because an experimental study reported that bromeliads could control water chemistry in the tank, suggesting the competition between the two primary producers (Lopez et al., 2009).

5. Conclusions

To summarize, the highest accumulation of water, electrical conductivity and total nitrogen and phosphorus concentrations in the phytotelm of *Nidularium longiflorum* occurred in the rainy season, which was also characterized by the highest values of temperatures and rainfall. On the other hand, the variation of photosynthetic biomass and particulate organic matter in the phytotelm was more associated with local environmental characteristics than seasonality. Based on these data, we can conclude that biomass (chlorophyll-*a*, AFDM) and abiotic variables in the phytotelm change seasonally, but that biomass accumulation is strongly influenced by site characteristics. Our findings indicate that environmental and microenvironmental conditions do not favor the development of algal community in the phytotelm of the bromeliad studied. Furthermore, we evidenced that the variation of the water volume in the tank was a determining factor for dynamics of phytotelm microenvironment of *Nidularium longiflorum*. However, further studies are needed to better understand algal community structure within the phytotelmic environment of tank bromeliads in tropical regions.

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